Neuroendocrine Tumours

Diagnosis and Management

Suayib Yalcin
Kjell Öberg
Editors

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Introduction to Neuroendocrine Tumours

1

Suayib Yalcin

1.1 Introduction

Including a wide range of clinicopathological entities, neuroendocrine tumours (NET) are a group of diverse neoplasms arising from cells of neuroendocrine origin. Recently, significant progress has been observed not only in our understanding of the biology and genetics of NET but also in the diagnosis and treatment [1, 2]. Besides, newer classification and staging system have been adapted and guidelines published to help to establish a more standardised approach [3, 4].

The incidence of NET is increasing in the last few years. The estimated incidence of 5.25/100,000 in 2004 is expected to reach to 8/100,000 today [5]. Although some series report slightly higher incidence in men compared to women, there appears no significant difference in terms of gender [6]. The prevalence of the disease is however estimated to be much higher and ranks only second after colorectal cancers among gastrointestinal (GI) tumours in the USA [5, 7]. Although NETs may occur at any age, it is more common after the age of 50 [6]. NETs may be associated with familial genetic neuroendocrine tumour syndromes such as multiple endocrine neoplasia (MEN) syndromes (MEN-1 and MEN-2), neurofibromatosis type 1, von Hippel-Lindau (VHL) disease, tuberous sclerosis and Carney complex. In patients with these syndromes, the age of diagnosis is 15–20 years lower than those with sporadic NETs [5–7].

NETs are usually slow-growing tumours. They can arise from many organs but commonly from GI tract and pancreas, lung, thymus and other endocrine organs. NETs may synthesise and secrete peptides and/or amines. These secreted peptides/amines can be used as tumour markers, and they may lead to clinical symptoms.

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About 67 % of NETs are located in the GI system; these NETS are sometimes also called carcinoids or GI NETs. Carcinoids secrete numerous peptides, including serotonin (5-HT) and tachykinins. About 10 % GI NETs metastasize to liver and release 5-HT into the blood resulting in carcinoid syndrome characterised by cutaneous flushing, diarrhoea and abdominal pain. Pancreatic NETs comprise the second common group of NETs. These tumours may be functional (~40 %) or non-functional (~60 %). Functional pancreatic NETs are usually defined by the predominant, clinically relevant hormone secretion such as insulin, gastrin, glucagon, vasoactive intestinal peptide (VIP), etc.

NETs have some common histopathologic characteristics. They show similar immune reactivity to pan-neuroendocrine markers, chromogranin A and synaptophysin [8]. Neuron-specific enolase (NSE), CD56 and CD57 are less specific markers; they can be used to identify poorly differentiated NETs. Immunohistochemical assessment of specific hormone expression is not routine in pathological evaluation, and positive immune reaction for hormone expression in the tumour tissue does not indicate that the tumour is functional [8].

NETs are graded based on mitotic count and Ki-67 index (Table 1.1). Grading should be combined with organ-specific TNM staging system. Primary localization, size and invasion depth of the tumour and status of surgical margin of the excision/resection material are also important [8, 9].

NETs usually express somatostatin receptors; therefore, somatostatin expression can be used both diagnostically and therapeutically [10]. Somatostatin receptor imaging (^{111}In-DTPA-octreotide or preferably ^{68}Ga-DOTATATE) can be used for initial staging, follow-up and selecting patients for peptide receptor radionuclide therapy (PRRT) [11, 12]. According to the localization of the primary tumour, computerised tomography and/or magnetic resonance imaging (MRI) should also be used for diagnosis and staging. Ultrasound and endoscopic ultrasound can be used when necessary as complementary examinations. 5-Fluorodeoxyglucose (FDG)-positron emission tomography (PET) can be used in diagnosis, staging and follow-up of aggressive, poorly differentiated NETs. FDG-PET may also show transformation to aggressive biological behaviour [11, 12]. The role of newer PET tracers warrants further validation for routine clinical practice. Endoscopic evaluation of the patients with esophagogastroduodenoscopy, colonoscopy, double-balloon enteroscopy or capsule endoscopy is critical according to the localization of the primary tumour and also in patients with primary unknown metastatic neuroendocrine tumour or carcinoma.

Table 1.1 Grading system for neuroendocrine tumours

Mitosis index	Ki-67 index (%)	ENETS/WHO definition	
<2	<3	NET	Grade I
2–20	3–20	NET	Grade II
>20	>20	NEC (small cell or large cell)	Grade III
		Mixed adenoneuroendocrine carcinoma (MANEC)	

NET neuroendocrine tumour, *NEC* neuroendocrine carcinoma, *ENETS* European neuroendocrine tumour society, *WHO* World Health Organization

Surgery is the only potentially curative treatment modality. Surgery should be considered for patients with early-stage disease, in patients with locoregional and resectable metastatic disease and in some selected symptomatic patients [13]. Resection of the primary tumour and metastasectomy should be considered for all suitable patients. However, surgery can not be used, because many patients are diagnosed at an advanced stage. Hence, medical treatment is necessary for both symptom and tumour control. Since somatostatin analogues have provided critical symptom control which was once the main reason for significant morbidity and mortality, now tumour bulk-related complications have become the leading cause for death [14]. The main clinical symptoms are pain, obstruction, diarrhoea, hypoglycaemia symptoms, hyperglycaemia, weight loss and carcinoid syndrome findings. Clinical symptoms may vary according to functionality of the tumour and depend on the localization and size of the tumour. The majority of these findings can be eliminated by treatment of the tumour. If carcinoid symptoms are present, somatostatin analogues, interferon, symptomatic treatment for diarrhoea, mammalian target of rapamycin (mTOR) inhibition and appropriate treatments can be used.

More patients are diagnosed at earlier and asymptomatic stage, and more non-functional tumours are detected with the aid of widespread use of improved diagnostic and pathological tools, coupled with increased awareness of the public and medical community. However, this disease is still diagnosed more commonly at advanced stage, making cure difficult to provide. The good side of the story is that the outcome of patients with NET is becoming more favourable with increased survival rates although cure may not be possible [1].

In addition to early diagnosis, improved surgical techniques can be used either aggressively or conservatively according to the presentation of the case [15, 16]. There are reported series of successful liver transplantation in selected cases, although the criteria for liver transplantation have not yet been fully established for NETs. Especially in cases with removed primary tumours, liver-directed therapies, ablation methods such as radio-frequency ablation (RFA), cryotherapy, radiosurgery or blant embolization and chemoembolization or radioembolization can be offered where surgery is not amenable. PRRT can be used in patients with well-differentiated low-grade NETs if positive for somatostatin receptor imaging. PRRT can be considered independently from primary tumour site in both functional and non-functional tumours. ^{177}Lu-DOTA-Tyr3-octreotate is preferred as it has less renal toxicity and higher somatostatin receptor 2 affinity. Response rates are higher in PNETs in comparison to small intestinal NETs [17].

Besides symptom control, somatostatin analogues improve the patient's quality of life and provide the control of tumour progression in NETs [10]. Somatostatin analogues, octreotide LAR and lanreotide, can be used in functional and non-functional, well-differentiated gastrointestinal and pancreatic NETs for anti-proliferative purposes [18, 19].

Interferon (IFN) has been used alone and in combination with chemotherapy and somatostatin analogues for both symptom and tumour control. IFN can be used in selected cases as salvage therapy.

Systemic chemotherapy is more effective in patients with a rapidly progressive disease or a tumour with high proliferation rate and aggressive pathological features. Combination chemotherapy is more effective than single-agent chemotherapy in NETs. The role of chemotherapy is relatively established for pancreatic NETs and for poorly differentiated tumours. There is a strong need for data to define the role of adjuvant treatment in low- or intermediate-degree tumours that are surgically resected [1].

Targeted agents are recently proven to show clinical activity [20, 21]. mTOR is a serine/threonine protein kinase that is a part of the phosphatidylinositol-3′kinase (PI3K)-AKT signalling pathway [20]. Everolimus is an mTOR inhibitor. Sunitinib is a multi-targeted tyrosine kinase inhibitor, inhibiting VEGFR-1, VEGFR-2 and VEGFR-3, as well as platelet-derived growth factor (PDGF), KIT and FLT3 [21]. Both of these agents have shown clinical efficacy in phase 3 randomised studies in pancreatic NETs.

The follow-up of patients who have been completely resected with surgery or endoscopy should be at 3- to 6-month intervals. Response evaluation in patients receiving systemic treatment should be performed at 3-month intervals. The imaging method to be used depends on primary disease site, the course of the disease and the best imaging method used at diagnosis.

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History of Neuroendocrine Tumors

2

Paula Marincola and Eric H. Liu

2.1 Introduction

Neuroendocrine tumors (NETs) are a heterogeneous group of neoplasms that arise in many tissues throughout the body. They share common pathologic features but have a broad spectrum of clinical presentations and malignant behavior [1]. The history of NETs, their discovery and classification, the evolution of their treatment, and the scientists who were responsible for these advancements over the past two centuries are the background upon which we now base our modern understanding of these cancers. This chapter will highlight the contributions of scientists in the field of NETs and the gastrointestinal neuroendocrine system from which they derive.

2.2 Pavlov, Starling, and the Discovery of the Gastrointestinal Neuroendocrine Axis

Ivan Pavlov (1849–1936) first introduced the concept of nervism in 1883, based on research by Ivan Sechenov (1849–1936) in the 1860s and 1870s. Pavlov's subsequent work with dogs supported his theory that the nervous system plays the dominant role in the regulation of all bodily functions. In 1935, he wrote, "the more developed the nervous system becomes in an animal, the more centralized it is and the more its highest division acts as the director and distributor of all functions of the organism… This highest division controls all of the phenomena that originate in the

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body" [2–6]. Although revolutionary, Pavlov's work was incomplete in its failure to recognize the effects of the endocrine system in the regulation of organ systems. As a result of his theory, arguably overemphasized in the medical community, the role of hormones in the regulation of bodily functions was neglected for nearly 20 years.

William Bayliss (1860–1924) and Ernest Starling (1866–1927), English physiologists and later brothers-in-law, were the first to challenge the concept of nervism, in 1902 [7]. In a series of graceful experiments conducted on the small intestines of animals, Bayliss and Starling suggested that blood alone could be responsible for physiologic responses in the gastrointestinal (GI) tract. In January 1902, they skeletonized an isolated loop of jejunum, such that only the mesenteric vessels remained. A dilute solution of hydrochloric acid was introduced into the jejunal lumen, resulting in excretion of pancreatic juices; this contradicted previous theories that a dedicated loop of reflex nerves between the duodenum and pancreas was solely responsible for pancreatic secretions, suggesting instead that blood alone could carry the signal for pancreatic secretion [2, 7–10].

In follow-up experiments, Bayliss and Starling created an extract from scraped jejunal mucosa that they injected intravenously into the pancreatic blood supply. This elicited pancreatic secretion, just as in the previous experiment. A boiled preparation of the jejunal mucosa elicited the same response when delivered intravenously, but a preparation from the distal ileum did not. As a result of these experiments, Starling famously proclaimed, "Then it must be a chemical reflex." The pair presented their findings at the meeting of the Royal Society only six days later, calling their newly discovered chemical agent "secretin" [2, 11]. Support for the chemical messenger theory grew. Even Pavlov had a laboratory assistant repeat the experiment later that year, and after the team watched the experiment unfold as it had for Starling and Bayliss, Pavlov retreated to his study without a word. When he returned half an hour later, he stated, "Of course, they are right. It is clear that we did not take out an exclusive patent for the discovery of truth" [12, 13]. Despite this famous admission, when Pavlov was awarded the Nobel Prize in 1904 for his work on the mechanism of digestion, he made no mention of Bayliss, Starling, secretin, or the possibility that any mechanism other than nerves was responsible for the physiology of digestion. Pavlov is said to have given up his research on the physiology of the digestive tract around this same time [14].

William Hardy (1864–1934) may have been the one to originally suggest the word "hormone" to Starling during a casual dinner conversation in 1905. The Greek word ὁρμάω (*ormao*), meaning to "excite" or "arouse," was proposed as fitting to describe these chemical messengers, and Starling scribbled it into his pocketbook. The word made its début only a few weeks later during a lecture given by Starling at the Royal College of Physicians in London. He stated, "These chemical messengers… or 'hormones'… as we might call them, have to be carried from the organ where they are produced to the organ where they affect…" [15, 16].

In the years that followed, Starling recognized that pancreatic gland secretion was in fact under dual control of both hormones and nerves, and thus came the recognition of the gastroenteropancreatic (GEP) neuroendocrine system [7].

2.3 Langerhans, Kulchitsky, and the Histology of the GEP Neuroendocrine System

Throughout the final years of the nineteenth century while the concept of internal secretion and its relation to the neural system were being studied extensively, debate about the cells that comprise this GEP neuroendocrine system was vigorous. Paul Langerhans (1847–1888) was a German pathologist and physiologist who made multiple histologic discoveries while he was a medical student in Berlin. Perhaps most famously, during the summer of 1867 while working in Rudolf Virchow's (1821–1902) laboratory, he discovered previously unrecognized clusters of pancreatic cells embedded within sheets of acinar cells. In his Doctorate of Medicine thesis that he defended publicly in February 1869, he noted that scattered among the exocrine acinar cells, in the interacinar spaces, were "…small cells of almost perfect homogenous content and of a polygonal form, with round nuclei without nucleoli, most likely lying together in pairs or small groups." Although Langerhans recognized these as novel structures, he did not identify their endocrine function, and again, time would pass before a French pathologist proposed a viable function for these newly discovered cells [17].

Edouard Laguesse (1861–1927), the French pathologist, studied the pancreatic cell clusters discovered by Langerhans and postulated that they produced an internal secretion. When he published his theory in 1893, he named them "les ilots de Langerhans" – or "islets of Langerhans" – after the young pathologist who had first described the clusters nearly three decades before. It was Laguesse's characterization of these clusters as *islets* that led to the hormone they secrete, described 30 years later by Nobel Prize winners Frederick Banting (1891–1941) and Charles Best (1899–1978), to be named *insulin* [17–19].

The identification and description of other GEP cells were no less convoluted. Enterochromaffin (EC) cells in the gastric mucosa of rabbits and dogs were first described in 1868 by Peter Heidenhain (1834–1897), a prodigy who received his doctorate at the age of twenty. He noted the cells contained acidophilic granules unlike the surrounding mucosal cells. Two years later, he also identified small, granulated yellow-staining cells on the surface of gastric glands that are now understood to be the histamine-secreting enterochromaffin-like (ECL) cells [20]. In 1891, Adolphe Nicolas (1861–1939) reported the wide distribution of EC cells throughout the GI tract in lizards, laying the framework for the subsequent description of the diffuse neuroendocrine system [21, 22].

In 1897, the Russian Nikolai Kulchitsky (1856–1925) noted similar granular cells with acidophilic properties in the crypts of Lieberkuhn in the intestinal mucosa of cats and dogs. Kulchitsky was uncertain of the function of these cells due to limited understanding of chemical messengers at the turn of the nineteenth century, but he postulated that the "acidophilic granules within the epithelial cells are a result of the digestive activity of the intestinal tract," citing his observation that these granules appear only during times of digestion and are absent during periods of starvation [23]. It is believed that at the time of his publication, Kulchitsky was aware of Heidenhain's discovery 29 years before, but he did not comment on this parallel

observation that potentially may have expedited the realization that the observations were describing the same cellular entity [21].

Unfortunately, at the time of these discoveries, eponyms were the method of choice for labeling new discoveries. This custom proved to confuse the scientific community about the proposed functions of the "clear cells" being independently described variously in the literature as "cells of Nicolas-Kulchitsky," "yellow cells of Schmidt," "enterochromaffin cells of Ciaccio," "argentaffin or silver-reducing cells of Masson," and "chromoargentaffin cells of Cordier" [24]. Finally, in 1906, M.C. Ciaccio (1877–1956) was the first to suggest the term enterochromaffin (EC) cells to replace the various eponymous terms. This name, he explained, simply reflects both the special staining properties of these unique cells and their anatomical location [21, 25].

2.4 Carcinoid Tumors

Around the same time that the GEP neuroendocrine system was being described, two different German pathologists were describing a series of unusual tumors of the small intestine. In 1867, Theodor Langhans (1839–1915) wrote about a small, firm, mushroom-shaped submucosal tumor that was found on autopsy in the ileum of a 50-year-old woman who died of tuberculosis. He described this tumor as unusual in appearance, with sharp borders and no signs of peritumor invasion [10, 26].

Langhans' publication was solely descriptive, and it wasn't until 21 years later that another description of these unusual tumors appeared in the literature. Otto Lubarsch (1860–1933) published a case report of another series of patients with ileal tumors on autopsy. Lubarsch again noted the abnormal growth of these tumors and indicated that their histologic features were not consistent with previously described carcinomas of the small bowel. He also noted that profuse diarrhea was a prominent symptom of one of these patients, a feature that is consistent with what we now call carcinoid syndrome [27].

In 1890, a third pathologist described the first documented case of metastatic disease in the case of a 50-year-old woman with menorrhagia, abdominal pain, diarrhea, and wheezing. In life, her symptoms were attributed to uterine fibroids, but on autopsy, William Ransom (1860–1909) found several ileal nodules with histologic appearance similar to those described by Langhans and Lubarsch as well as corresponding hepatic tumors. He agreed with the German pathologists that the histologic appearance of these tumors was in fact peculiar and atypical of previously described small bowel carcinomas and went a step further by suggesting that they may have malignant potential [28].

It was 40 years after Langhans described the first of these strange tumors of the small bowel that Siegfried Oberndorfer (1876–1944) gave them a name, "karzinoide" – or "carcinoma-like." Oberndorfer, like Langhans, Lubarsch, and Ransom, was a pathologist who had independently identified a series of unusual tumors in the small intestines of autopsy specimens. In his seminal paper in 1907, he described six cases of individuals who had died from a variety of causes (including

tuberculosis and typhoid fever) that had multiple small tumors of the ileum, spaced far apart, with nests of cells and fibrous connective tissues spreading in the peritumor area. He concluded that these must be multiple primary malignant tumors of the same organ and that they were not true carcinoma but rather grew slowly, had sharp borders, and did not metastasize. Given their supposed lack of malignant potential, Oberndorfer categorized them as distinct from "true" carcinomas, hence the name "carcinoid" [29].

Unfortunately, Oberndorfer's initial description did not accurately represent the malignant potential of these tumors, and it would be twenty-two more years before he would revise his initial assessment to reflect the true nature of carcinoid tumors. In 1929, he published a description of thirty-six more cases of carcinoid tumors of the small intestine and appendix, describing their ability to metastasize and describing their characteristics much the way that we understand them today [30].

A. Gosset (1872–1944) and P. Masson (1880–1959) made the connection between carcinoid tumors and EC cells in 1914. Using silver impregnation techniques, they demonstrated the argentaffin-staining properties of appendiceal carcinoid tumors and proposed an EC cell origin [31]. Subsequent studies by H. Kull confirmed that carcinoid tumors exhibited argentaffinity and argyrophilia in response to silver salts and pointed out that similar granular cells exist throughout the gut mucosa. However, he incorrectly postulated that these cells were of mesodermal origin and secondarily migrated into the gut epithelium [32].

Finally, in 1938, Friedrich Feyrter (1895–1973) of Poland proposed that EC cells represent a diffuse endocrine system based on the observation that these argentaffin-positive and argyrophilic "clear cells" existed throughout the gut and pancreas. He postulated, correctly, that this diffuse cellular entity was in fact the source of the mysterious carcinoid tumor [33, 34].

2.5 Cushing, Bauer, and Hypercortisolism

The two endocrine organs now known to be responsible for the clinical presentation of hypercortisolism – the adrenals and the pituitary – have been known to the medical community for hundreds of years. Bartolomeo Eustachius (1520–1574) first described the adrenal glands in 1563. He called them "glandulae renibus incumbentes." For many years following their anatomical description in *Opuscula anatomica*, they were believed to be hollow inside, containing a dark, bilious fluid that was passed from the adrenals to the kidneys and into the urine [35]. This misconception was likely due to the postmortem liquefaction of the medulla observed on autopsy [36]. In the nineteenth century, the idea of a central cavity was entertained, and in 1827, J.R. Coxe published that this central cavity had a function only in the fetus and suggested that they be renamed "diverticula urinae" to reflect this function [37].

Amazingly, the pituitary gland has been known even longer – since antiquity. For almost 1500 years, the gland was believed to secrete phlegm into the nasal pharynx, a view put forth by Galen of Pergamon (129–201 A.D.), and it was not until the seventeenth century that C.V. Schneider – a distinguished anatomist of

Wittenberg – demonstrated that this was anatomically impossible [38]. Even as recently as the twentieth century, the function of the pituitary was largely unknown, which is rather striking given that several descriptions of acromegalic individuals with pituitary tumors had been published by this time [39–42].

There have been reports of adrenal tumors associated with hypertension, truncal obesity, hirsutism, and pyoderma since 1756 [43, 44]. In 1912, Harvey Cushing (1869–1939) reported on a 23-year-old woman, Minnie G., with weight gain, muscle weakness, back pain, irregular menstruations, hypertrichosis, hyperpigmentation, mucosal bleeding, hypertension, papilledema with diminished visual acuity, and a large, round face who had been admitted to Johns Hopkins Hospital in 1910. Cushing believed her hypercorticism to have a polyglandular etiology, involving the pituitary, pineal, thyroid, and adrenal glands as well as the testes and ovaries, although he noted that a primary pituitary disorder was "questionable" [45, 46]. As the patient's vision continued to deteriorate, a decision was made to do a craniotomy to reduce Minnie's intracranial pressure. Although Cushing was reportedly considering a subsequent operation on the adrenals, this was deemed unnecessary after Minnie began to show marked clinical improvement postoperatively. In 1932, Cushing reported that Minnie remained alive and in "reasonable good health," and it is believed that she lived to be 71 [47]. Minnie's unusual course, spanning a period of approximately 50 years, may suggest that she was suffering from what we now understand to be pigmented, adrenocortical dysplasia [48].

In 1919, an autopsy performed for a fatal case of hypercortisolism revealed both adrenal hyperplasia and a pituitary adenoma. In the discussion of the case report, V. Reichman reflected on the possibility of simultaneous hyperfunctioning of both the adrenals and the pituitary, but failed to draw conclusions about a causal relationship between the two [49].

Then, 20 years after his first study, Cushing published his modified views on "pituitary basophilism" based on 12 cases of clinical hypercortisolism. In such a review, it was noted that the disease was not all that uncommon, that severe infections were a common feature, and that the prognosis tended to be poor. He postulated that the main factor in the disease process was a pituitary (basophil) adenoma, as opposed to a primary adrenal lesion as he had previously assumed [50]. In a later paper, he again concluded, "probably the pituitary lesion (the basophil adenoma) is the cause of the syndrome" [51].

Cushing was well aware of the adrenocortical hypertrophy that was commonly found in syndromes of hypercortisolism and knew that hypersecretion of an adrenal substance may have been of significance [52]. He theorized that gonadotropin might be the factor responsible, and pursued this possibility in animal studies, but was unable to recreate the appropriate symptoms [53]. At no point did Cushing consider a connection between the hypertension associated with these patients and adrenal cortex hypersecretion, which is peculiar considering that this relation was well known in patients with symptoms similar to those described by Cushing [54]. In 1932, shortly after Cushing pointed to the pituitary adenoma as the causative agent in this syndrome of hypercortisolism, P.M.F. Bishop and H.G. Close named the disease, "Cushing's syndrome" [55].

For years, the roll of the pituitary versus the adrenals was debated in the pathophysiology of Cushing's syndrome. Breakthroughs in the understanding of this syndrome were made in 1933 when adrenocorticotroph hormone (ACTH) was reported for the first time and in 1935 when ACTH was found to be elevated in the serum of patients with hypercortisolism [56, 57]. In 1950, a clear concept of Cushing's syndrome emerged when Julius Bauer (1887–1977) published a review article on "so-called Cushing's syndrome." He stated that the disease could be caused by either the pituitary or an adrenal tumor and proposed that the eponym "Cushing's disease" be reserved for those cases of hypercortisolism caused by the pituitary adenomas [58]. Bauer even made mention of the possibility of an ectopic Cushing's syndrome, but it was not until the 1960s that a complete description of ACTH-producing tumors of "non-endocrine" tissue was identified and described [59–62].

Interestingly, in 1924, a Russian physician, Nikolai Itsenko (1889–1954), published in a little-known Russian public health journal a description of a case of hypercortisolism. As this article made no reference to Cushing's article from 1912, it appears entirely plausible that both Cushing and Itsenko independently described this syndrome. In Russia, the eponym Itsenko-Cushing's syndrome is still in use to describe the syndrome of hypercortisolism [63].

2.6 Banting, Best, and Whipple: Hyperglycemia and Hyperinsulinemia

The discovery of insulin is arguably the most substantial contribution to endocrinology and medicine in the past hundred years. Prior to its discovery, diabetes was a lethal disease of childhood with a prognosis worse than cancer. During the nineteenth century, the best physicians could offer patients was a strict low glucose diet which at best extended life by a handful of months, and at worst killed by starvation. When Frederick Banting (1891–1941), a little-known surgeon in London, Ontario, suggested in early 1921 to Professor John Macleod (1876–1935), a distinguished researcher in the treatment of diabetes at the University of Toronto, that he might be able to isolate the internal secretions of the pancreas, Macleod was skeptical. Nonetheless, he was persuaded to provide Banting with a small laboratory with minimal equipment, ten dogs, and a medical student, Charles Best (1899–1978) [64]. Banting and Best ligated the pancreatic ducts of dogs, inducing atrophy of the acinar cells and therefore isolation of the islet cells. Pancreatic extracts were made from this isolated material and administered to a diabetic dog. Using this serum, in the summer of 1921, Banting and Best were able to dramatically prolong the lives of several diabetic dogs, convincing Macleod that they were onto something great. He allocated to the pair a larger lab, more equipment, and a biochemist, Bertram Collip (1892–1965), to help purify this internal secretion, now called *insulin*, for human trials [64].

Banting and Best began testing the pancreatic extract, now being obtained from cattle and purified by Collip, on themselves. Besides feeling week and dizzy following the injection, they had no major side effects from the injections and learned they

could counteract the effects of an insulin overdose by eating high-glucose foods. In January 1922, the first person with diabetes, a 14-year-old boy in Toronto, was chosen to receive insulin. Previously near death, he responded dramatically, regaining strength, weight, and appetite. News of this success spread rapidly and caught the attention of pharmaceutical company Eli Lilly, who began large-scale production of the drug. By 1923, the firm was producing enough insulin to supply the entire North American continent [64–66].

In 1924, shortly after Banting and Best discovered insulin, the American Seale Harris (1870–1957) postulated the existence of spontaneous "hyperinsulinism" – as opposed to the "hypoinsulinism" of diabetes [67, 68]. Four scientists – R. Wilder, F. Allan, M.H. Power, and H.E. Robertson – first published verification of the hypothesis in 1927 in the *Journal of the American Medical Association*. This case report described a patient with intractable hypoglycemia who, on surgical exploration, showed evidence of carcinoma of the pancreatic islet cells with metastases to the liver. The hepatic metastases were shown to express insulin [69]. Two years later, the first case of islet adenoma was diagnosed preoperatively, identified on operation, and excised with a favorable result [68, 70].

A review published in 1935 of 157 cases of hyperinsulinism and islet cell tumor by Allen O. Whipple (1881–1963) and Virginia K. Frantz (1896–1967) proposed that although it was possible to cure refractory hypoglycemia due to insulinoma by surgical excision, the majority of patients with symptoms of hypoglycemia had no need for surgery. However, diagnostic tests of the time were rudimentary. Beyond an indirect measure of blood glucose levels through an assay for reducing substances in serum, there was no way to directly measure blood insulin levels or obtain abdominal imaging that might visualize a pancreatic tumor. As such, Whipple – a well-known surgeon who had pioneered pancreatic surgery – proposed in an article titled "The surgical therapy of hyperinsulinism" that no pancreatic surgery be performed for the treatment of hypoglycemia unless (1) symptoms were known or likely to be caused by hypoglycemia, (2) a low plasma glucose was measured at the time of symptoms, and (3) symptoms were relieved when glucose levels are raised to normal [71, 72]. These criteria, known as "Whipple's triad," although less commonly used today given the availability of serum assays and imaging technologies, remain an important means of separating "true hypoglycemia" from other conditions.

2.7 Zollinger, Ellison, and Gastrinoma

Insulinomas were the only functional islet cell tumors recognized at the time that S.Z. Sailer in 1946 described the case of a patient with a large pancreatic islet cell tumor and no clinical or biochemical evidence of hyperinsulinemia. Although a duodenal ulcer was present in this patient, Sailer failed to associate the ulcer with the pancreatic tumor [73] and it would be nine more years before an association between these two findings was recognized [74].

Robert Zollinger (1903–1992) and Edwin Ellison (1918–1970) first made this connection in a presentation of two cases in front of the American Surgical Association in Philadelphia in April 1955. Both cases were young women with intractable abdominal pain, diarrhea, jejunal ulceration, and gastric hypersecretion who had failed multiple surgical procedures in the preceding years, including vagotomies, antrectomies, and subtotal gastrectomies, and they stated, "it would seem a heretofore unrecognized islet cell tumor of the pancreas with an ulcerogenic potential must be considered in certain atypical cases of peptic ulcer disease (PUD)" [75]. This syndrome – now recognized as the triad of primary peptic ulceration in unusual locations, gastric hypersecretion, and the presence of a nonspecific islet cell tumor of the pancreas – would be named Zollinger-Ellison syndrome (ZES) in 1956 [76]. Interestingly, both of the patients presented by Zollinger and Ellison had family histories of multiple pancreatic tumors and were most likely suffering from the yet-to-be-discovered multiple endocrine neoplasia [74].

Following Zollinger and Ellison's seminal paper, clinical awareness of ZES increased dramatically. At that time, preoperative diagnosis was based on clinical suspicion in those patients who presented with recurrent ulceration despite multiple surgical attempts to control gastric hypersecretion. Gastric analysis and plain X-ray of the abdomen in search of gastric hypertrophy or jejunal ileus minimally enhanced the accuracy of preoperative diagnosis [77].

In the 1960s, major advancements were made in the preoperative diagnosis and characterization of the islet cell tumors commonly seen in patients with ZES. In 1960, R.A. Gregory (1913–1990) and his colleagues identified a substance in the pancreatic tumor of a patient with ZES that might be responsible for ulceration. After injecting the extract of a ZES-associated pancreatic tumor into fasting dogs, they observed a robust and prolonged secretory response that resembled the response produced by gastrin (which had been isolated and identified as the major acid-producing hormone in 1942 by S. Komarov, who ironically studied under the Father of Nervism, Pavlov) [78]. This response could not be reproduced with the administration of histamine or insulin [79, 80]. In 1964, the same group showed definitively that the ZES tumors secreted gastrin, and these tumors became known as gastrinomas [79, 80].

In 1968, McGuigan and Trudeau developed a radioimmunoassay technique to measure serum gastrin levels. This technique was tested on four patients with ZES and compared to twenty-four hospitalized patients without any identified GI pathology. They discovered that even in the ZES patient with the lowest serum gastrin level, it was over eight times higher than the median value of the control subjects [81]. This simple development provided clinicians with a cheap and reliable way to diagnose ZES preoperatively and to monitor their disease progression postoperatively [82].

Until the 1970s, the treatment of choice for gastrinomas was the removal of potentially ulcerogenic tissue (total gastrectomy). As early as 1964, nonsurgical options were being explored, as exemplified by Victor Richards' (1919–2003) quote: "the demonstration by Gregory that these tumors produce either gastrin or a gastrin-like substance has led many people to work experimentally on the concept of producing

an antihormone. I do not know of any clinical experience, however, with the use of antigastrin in the management of these cases, but it is an idea that is worth thinking about for the future" [83]. However, it would not be until a decade later that the development of H_2 blockers and proton pump inhibitors would enable a near-total elimination of ulceration in gastrinoma patients [84–86], allowing the focus to shift from preventing ulcer diathesis towards resection of the primary tumors.

2.8 Multiple Endocrine Neoplasia

Although most NETs are sporadic, multiple inherited syndromes associated with NETs exist, including multiple endocrine neoplasia (MEN) types 1 and 2, von Hippel-Lindau disease, neurofibromatosis 1, and tuberous sclerosis. Among pancreatic NETs, an estimated 10–15 % are part of an inherited disorder, whereas the hereditary aspect of carcinoid tumors is believed to be far less common [87, 88].

In 1903, Austrian pathologist Jakob Erdheim (1874–1937) was the first to describe a patient with tumors in two different endocrine organs. At the autopsy of a 43-year-old acromegalic man, he found a large pituitary adenoma and three enlarged parathyroid glands [89, 90]. Between 1912 and 1952, at least thirteen different cases of patients with various combinations of endocrine adenomas or hyperplasia of the pituitary, parathyroids, and pancreatic islets were described, all lacking a suggestion of underlying etiology. Notably, in 1927, Cushing and L.M. Davidoff described an acromegalic patient with four concurrent endocrine organ adenomas in the parathyroid, pancreas, bilateral adrenals, and pituitary, in addition to a lipoma of the thigh. They asserted that the pancreatic lesion was most certainly an islet cell tumor [91]. In 1929, P.C. Lloyd described a similar case and, in his interpretation of the findings, found that "it seems more than likely that [the anatomic changes in the three endocrine tissues] are related, but the evidence is insufficient to draw such a conclusion" [92].

Fifty years after the initial description of concurrent endocrine neoplasia, three Mayo Clinic physicians – L. Underdahl (1907–1997), an endocrinologist; L. Woolner (1913–2014), a surgical pathologist; and B.M. Black (1910–1997), a surgeon – published an article in which they describe in detail eight complicated cases featuring tumors of the pituitary, parathyroid, and pancreatic islets, in addition to fourteen cases with two of the three tumors [93]. Although five of the eight patients had at least one family member with symptoms or biochemical or pathological findings similar to the patients presented in the report, the suggestion of the familial and genetic nature of the syndrome escaped the authors. Paul Wermer (1899–1978), an American internist at Columbia Presbyterian Hospital in New York, commented 20 years later: "It was only in 1953 when an excellent clinical and pathological description of the adenomatosis was published by Mayo Clinic that one began to recognize the syndrome as a very peculiar disorder which merited the interest of clinicians and pathologists. In reading this article, one is impressed to find cases in which the hereditary origin is evident and it seems remarkable to us today that this completely escaped the attention of the authors.

Genetics was not yet popular with clinicians in 1953" [94]. In 1954, Wermer had concluded that the tumor combination described by Underdahl and his colleagues at the Mayo Clinic the year before was a genetic disorder of autosomal dominant inheritance. This genetic syndrome, which originally bore Wermer's name, was later named MEN type 1 [90].

In 1959, John H Sipple (1930–), then a third-year medical student, was the first to suggest an association between pheochromocytoma and carcinoma of the thyroid gland. Sipple encountered a 33-year-old previously normotensive man with unexplained episodic hypertension complicated by several intracranial hemorrhages. On autopsy, Sipple "was astounded by the findings of bilateral pheochromocytoma, bilateral carcinoma of the thyroid gland, and parathyroid adenoma." Perplexed that his patient could have bilateral tumors of multiple endocrine organs, he searched *Index Medicus* for documented cases of pheochromocytoma. Of the 537 cases identified, five were reported to have thyroid carcinoma on postmortem exam. He noted that, "although the overall incidence of malignancy of other organs is not increased in pheochromocytoma, the incidence of carcinoma of the thyroid is increased far beyond expectations based on chance occurrence." Sipple calculated the incidence of thyroid carcinoma in patients with pheochromocytoma to be fourteen times that of the general population [95]. This association between pheochromocytoma and carcinoma of the thyroid became known as "Sipple syndrome," which describes what we now consider to be the key features of both MEN types 2A and 2B [90, 96].

As has been the case for the entire GEP neuroendocrine system, the nomenclature for the inherited NET syndromes was unsatisfactory and led to much confusion in the literature over a number of years. Different authors variously used "multiple endocrine neoplasia," "multiple endocrine adenomatosis," "Wermer syndrome," "Sipple syndrome," "pluriglandular syndrome," and "pluriglandular adenomatosis" for years. It was not until 1989 that a consensus was reached to use MEN type 1, MEN type 2A, and MEN type 2B to describe the syndromes of pituitary, parathyroid, and pancreatic islet adenomas; medullary thyroid cancer, pheochromocytoma, and parathyroid adenomas; and mucosal neuromas, mesodermal dysplasia, medullary thyroid cancer, and pheochromocytoma, respectively [97].

In the last few decades, there has been significant progress in the understanding, detection, and treatment of the MENs and other inheritable NET syndromes, largely due to improved understanding of genetics and molecular genetic screening techniques. The history of identification of the specific genes responsible for these inherited syndromes and their respective new detection and treatment modalities is outside of the scope of this chapter but will be discussed briefly in later sections that focus on each of these disorders.

Conclusion

The history of NETs is filled with confusing pathology, hormones, and diagnoses. Even the nomenclature itself ("carcinoids" and "APUDomas") caused great confusion [98]. However, with the establishment of consensus guidelines in

pathology and a greater understanding of the biology of this disease, the diagnosis and treatment of NETs will only continue to improve.

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Epidemiology of Neuroendocrine Tumors

3

Saadettin Kilickap and Kadir Mutlu Hayran

3.1 Incidence

Neuroendocrine tumors (NETs) are a heterogeneous group of tumors secreting various bioamines and peptides arising from neuroendocrine cells in the endocrine and central nervous systems. NETs are responsible from approximately 0.5 % of all cancers [1]. The crude incidence is about 0.2/100,000. The incidence has gradually increased from 1.9 to 5.2/100,000 people per year during the last three decades [2, 3]. The increase at the incidence of NETs is faster in comparison to other tumors of the same organ [4]. It increases with age and peaks between 50 and 70 years. Improvement in the classification system, diagnostic techniques such as increased use of endoscopy and imaging techniques, and histopathologic examination of these tumors are considered to be largely responsible for the increased incidence [2, 5]. Due to slowly growing nature of NETs, its prevalence is increasing along with its incidence. The prevalence has been estimated to be 35/100,000/year. Frequency, incidence, and survival rates of NETs are presented in Table 3.1.

Most of NETs are diagnosed at advanced stages. According to SEER data including 19,669 cases with NETs, 59.9 % of NETs arising in gastrointestinal tract were at the localized stage followed by regional (19.9 %) and distant stages (15.5 %) [6]. Data from Spanish registry showed that gastroenteropancreatic NETs were often diagnosed at advanced stage with 44.2 %, followed by localized and regional stages with 36.4 % and 14.2 %, respectively, and then unknown stage and undetermined cases with 5.3 % [7]. However, NETs originating in pancreas tend to be aggressive and about 60 % of these tumors are malignant at the time of diagnosis.

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Table 3.1 Frequency, incidence, and survival rates of neuroendocrine tumors

Location	Within NETs (%)	Within GEP-NETs	Incidence SEER (/100,000)	Incidence (% of primary site)	5-year OS (%)
GEP-NETs	67		5.25	<2	75–82
Gastric		9–20	0.3	1	45–64
Small intestine		39–42	1.1	37–52	62–71
Appendix		6	0.15	30	90
Colon		9–20	0.35	<1	67
Rectum		26	1.1	<1	90
Pancreas		7–12	0.5–1.0	1–2	27–38
Bronchopulmonary	27		0.46	<2	44–87
Other sites[a]	6		0.38		

NETs neuroendocrine tumors, *GEP-NETs* gastroenteropancreatic neuroendocrine tumors, *SEER* Surveillance, Epidemiology, and End Results Program, *OS* overall survival
[a]Esophageal, endometrium, ovary, breast, etc.

3.1.1 NETs of Gastrointestinal Tract (GT-NETs)

Although NETs have arisen from neuroendocrine cells located throughout the body including the lung, breast, ovary, and endometrium, gastrointestinal system is the most common localization of these tumors. The incidence of gastroenteropancreatic NETs (GEP-NETs) is similar in male and female [8, 9]. The incidence increases with age. Median age is lower in NETs arising from appendix and pancreas compared to those of other organs of gastrointestinal tract. The median age is less than 50 years for appendix and pancreatic NETs, but more than 60 for the others [9, 10]. Most of GEP-NETs are symptomatic and the most common symptoms were abdominal pain and weight loss.

NETs of gastrointestinal system, accounting for approximately two-thirds of all NETs, are most commonly located in the gastric mucosa, the small intestine, the rectum, and the pancreas [11]. They comprise less than 2 % of gastrointestinal system tumors. GT-NETs are the second most common gastrointestinal tract tumors after colorectal cancer and more frequent than the other NETs [12]. Distribution and stages at the time of diagnosis of NETs arising in the gastrointestinal tract are presented in Figs. 3.1 and 3.2. Incidence rates of GEP-NETs in different populations are illustrated in Fig. 3.3.

In the developed countries, small bowel is the most common primary site of GEP-NETs [2, 13, 14]. They account for 39–42 % of all GEP-NETs followed by the rectum and the colon [11, 13, 14]. The ileum is the most common location of the small intestinal NETs [13]. In Western countries, the small intestinal NETs are more common than in Asian population [15]. The small bowel NETs slowly progress and usually present with advanced stages at diagnosis. Rectum is one of the most common localization of NETs arising in gastrointestinal system and accounts for 14 % of all NETs [11]. About 1 % of all rectal tumors are NETs [16]. However,

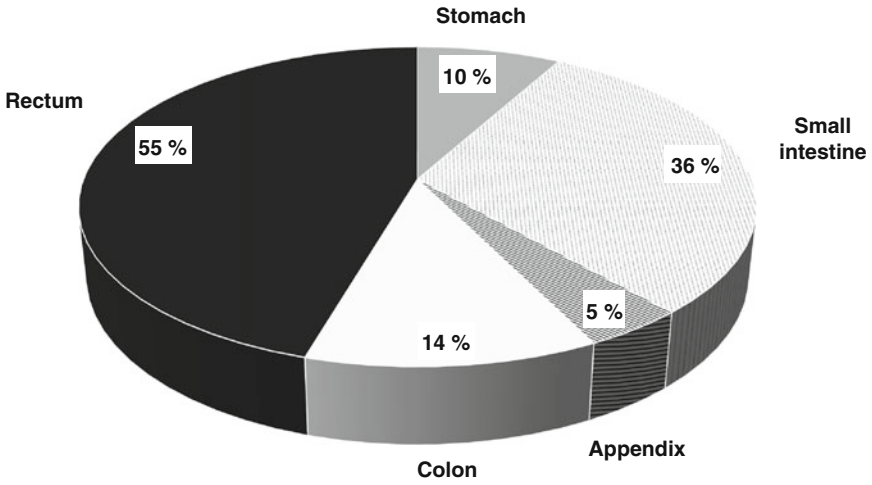

Fig. 3.1 Distribution of NETs arising in the gastrointestinal tract

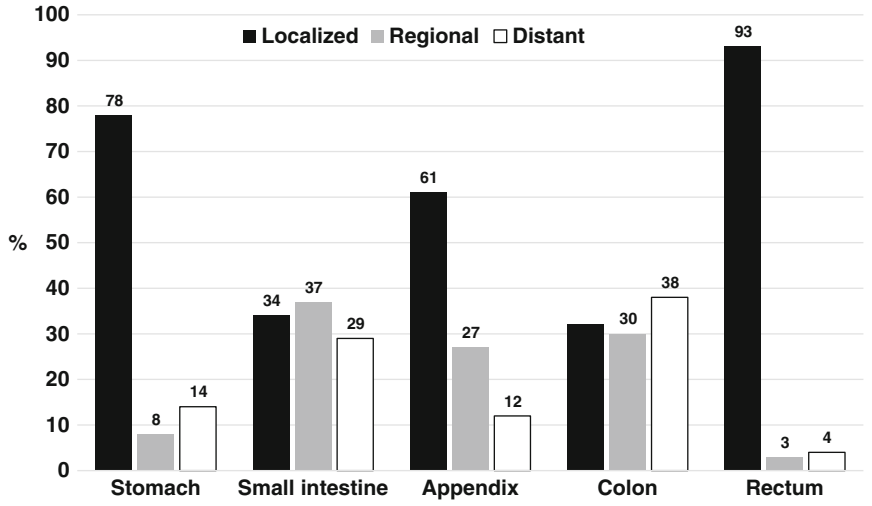

Fig. 3.2 Stage distribution of NETs arising in the gastrointestinal tract

NETs originated from colon represent about 8 % of NETs [11]. Cecum is the most common site for colonic NETs.

NETs originating in gastric mucosa are less common. It accounts for about 9–20 % of GEP-NETs and less than 1 % of all gastric tumors [11, 17, 18]. Recently, the incidence has been increased due to more widespread use of gastrointestinal endoscopy [19]. Gastric NETs are classified into three groups as types I, II, and III. The etiology and survival of these tumors are different. Type I gastric NETs,

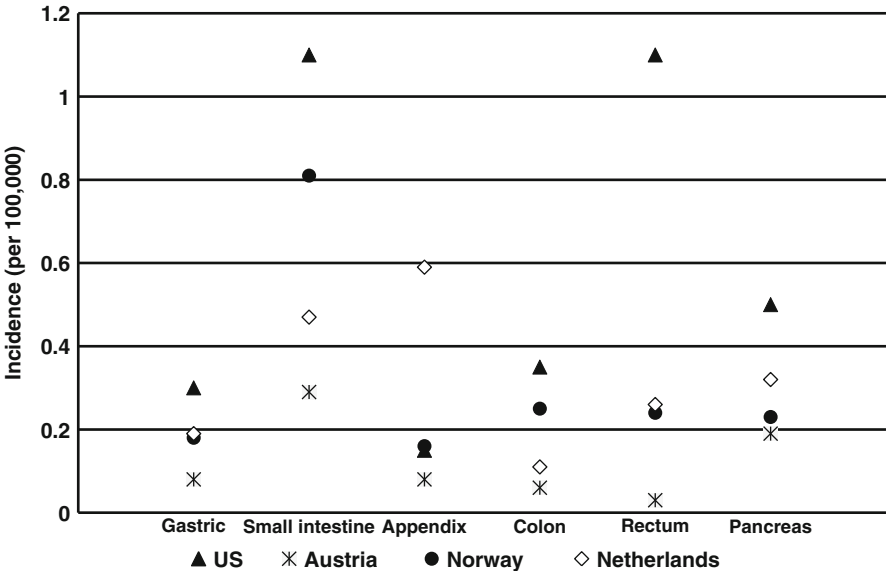

Fig. 3.3 Incidence rates of GEP-NETs in different populations

which are the most common gastric NETs (70–80 %), are associated with autoimmune chronic atrophic gastritis and hypergastrinemia, whereas type II, which accounts for 5–6 % of gastric NETs, develops in patients with multiple endocrine neoplasia (MEN) type 1 [20–23]. Tumor size of type I and II gastric NETs is usually less than 1 cm and the prognosis is good. Type I gastric NETs are usually benign tumors. On the other hand, type III gastric NETs develop in normal gastric mucosa and are responsible for 14–25 % of gastric NETs. Their prognosis is worse than type I and II tumors [20, 22, 24].

In a multicenter registry study, the authors aimed to assess the prevalence and incidence of patients with GEP-NET in Asia-Pacific, Middle East, Turkey, and South Africa [9]. In contrast to the literature reporting the western population figures, pancreas and stomach were the most frequently reported primary sites (42 % and 17 %, respectively).

3.1.2 Pancreatic NETs (PaNETs)

Pancreatic NETs (PaNETs) are responsible for 1–2 % of all pancreatic neoplasms and the overall incidence is 0.5/100,000 people per year [25]. However, the incidence ranges from 0.8 to 10 % in autopsy series [26]. In developed countries, small intestine is the most frequent localization of NETs, but pancreas is the most common in the eastern population [9, 13, 14].

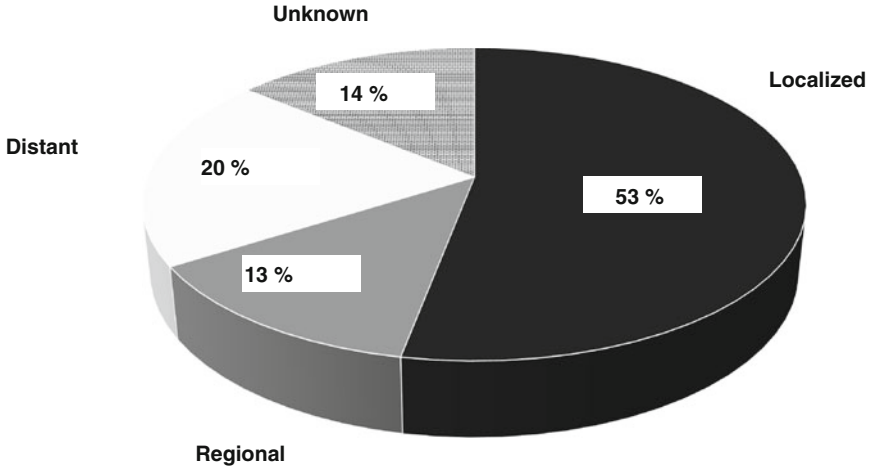

Fig. 3.4 Stage distribution of typical and atypical bronchopulmonary carcinoids

About 10–20 % of PaNETs are associated with MEN type 1 [27]. PaNETs may be functional by secreting various hormones and peptides. The most commonly observed functional tumor is insulinoma with 70–80 % of all PaNETs, but only less than 10 % of insulinoma is malignant [28]. Mostly, the other types of PaNETs such as glucagonoma, VIPoma, and gastrinoma tend to be malignant. PaNETs are usually characterized as slow growing and indolent tumors.

3.1.3 Bronchopulmonary NETs

Bronchopulmonary NETs are classified as three main entities such as carcinoid tumors (typical and atypical carcinoids), large cell NETs, and small cell carcinoma [29]. Well-differentiated low-grade NETs are known as typical carcinoids, and intermediate-grade NETs are atypical carcinoids. Prognosis of typical carcinoids is excellent. Large cell NETs and small cell NETs are considered as poorly differentiated NETs and are known to have a worse prognosis. Stage distribution of typical and atypical bronchopulmonary carcinoids is shown in Fig. 3.4.

The second most common location of NETs after gastrointestinal tract is the bronchopulmonary system and it accounts for about 27 % of all NETs [11]. Less than 2 % of all lung tumors are NETs including typical and atypical carcinoids. Contrary to small cell lung cancer, the lung carcinoids occur in younger and nonsmoker patients [30]. Large cell NETs account for 3 % of all lung cancers [30]. Incidence of bronchopulmonary NETs increases linearly similar to the GEP-NETs. Bronchopulmonary NETs may present with dyspnea, cough, and hemoptysis. In some patients, they are discovered incidentally. Bronchopulmonary carcinoid tumors can be cured with surgical excision.

3.2 Survival

NETs are very heterogeneous tumors. Most of NETs are well or moderately differentiated tumors, with relatively indolent course and slow growth. Thus, overall survival of NETs is different for each tumor. Overall survival in patients who have poorly differentiated tumors and who have distant metastases is shorter than those who have well-differentiated and localized tumors. For example, median survival in patients with distant metastases for nonfunctioning PaNETs was shorter with 23 months than localized and regional disease (124 and 70 months, respectively) [2]. The survival has improved in the last two decades. Prognostic factors influencing survival are known as distant metastasis, poorly differentiated tumor, grade, age, number of liver metastasis, extrahepatic metastasis, and the presence of positive surgical margin [2, 30–35].

While the best survival rates are observed in patients with NETs arising in the rectum and appendix, the prognosis of colonic NETs is worse. The 5-year disease-specific survival rates are about 96 % for rectum and 90 % for appendix [6, 11, 36], while the survival rates are lower in the other GEP-NETs including small intestine (86 %) and colon (67 %). For NETs arising in the small bowel, the 5-year survival varies between 60 and 75 % [11, 37–39]. However, the best survival rates among small intestinal NETs were reported for appendiceal NETs. The prognosis is excellent with the 5-year disease-free survival rates of 89–95 % [6, 38, 40]. In a published study from Denmark, the 5-year survival rate was found to be 77 % in patients with small intestinal NETs [41]. Similarly, the 5-year survival exceeds about 60 % in patients with operable PaNETs [26, 42, 43]. Different survival rates are observed for each country. The 5-year overall survival was reported as 71 % in the US-based SEER database [44]. Higher rates were reported from Italy and Spain with 72–89 %, while in France they were lower with 56 % [7, 45, 46].

For type I gastric NETs, the prognosis is very good and 5-year survival is about 95 % [47]. However, the survival rates for type II and III gastric NETs are 70 % and 35 %, respectively. The survival rates in the USA have been reportedly higher when compared to those of Europe [6, 7, 45, 48]. While the 5-year survival rate based on the SEER database was 75–82 %, the rates were 61 % in Spain, 63 % in Italy, and 45 % in Norway.

Survival rates are lower in PaNETs than other GEP-NETs. The survival is about 27–38 % in advanced stage PaNETs [2, 13, 26, 48, 49]. In patients with functioning PaNETs, the survival is better [50]. Higher 5-year survival rates have been reported from Norway with 43 % and from Spain with 78 % [7, 48]. Incidence of NETs arising in GEP and bronchopulmonary systems has a marked increase in male and female in the last 30 years worldwide.

3.3 Factors Associated with NETs

3.3.1 Age and Gender

Incidence of NETs increases with age. Although the disease can appear at all ages, it peaks between sixth and seventh decades. NETs are rarely seen in pediatric patients. Median age at the time of presentation is 60 years [2, 26, 40, 49, 51, 52].

While incidence rates are similar between both sexes in the USA [11, 53, 54], some NETs such as NETs at rectal location are slightly higher in men than in women [55, 56]. The small intestinal NETs and PaNETs are more common in males [11, 26, 45, 46, 57, 58], but low-grade NETs in the appendix and bronchopulmonary appear to be more often in women compared with men [10].

3.3.2 Race and Ethnicity

According to SEER database, NETs such as carcinoid tumors in the appendix are more prominent in whites. However, certain NETs such as those of the small bowel and PaNETs are more common in African-Americans and Asian population within the USA [2, 11, 26, 48]. Incidence of rectal NETs is about three times higher in Asian than non-Asian populations [11]. These tumors are also more common for both males and females in blacks compared to whites [2, 11, 48, 59].

3.3.3 Genetic and Family History

Although majority of NETs occur sporadically, some may be hereditary and can appear with genetic syndromes such as MEN type 1, MEN type 2, and von Hippel-Lindau disease [2, 18]. The familial von Hippel-Lindau disease-related PaNETs are not as high as MEN type 1 [60, 61]. NETs may also be associated with certain allelic losses. For example, loss of 22q13.1-q13.31 is considered to have associated with development of insulinoma [62].

Data from SEER and Swedish study showed that first-degree relatives of patients with carcinoid tumors are associated with increased risk of also developing a carcinoid tumor [57, 63]. PaNETs may develop in 35–75 % of patients with MEN type 1 [64], and these patients tend to be a younger.

3.3.4 Occupational Risks

The association between occupational exposure and carcinoid tumors has been investigated in epidemiological studies. In a multicenter population-based case-control study, the authors reported that employment in industry of food and beverages (odds ratio, 8.2; 95 % CI, 1.9–34.9) and in the manufacture of motor vehicle bodies (odds ratio, 5.2; 95 % CI, 1.2–22.4), footwear (odds ratio, 3.9; 95 % CI, 1.9–16.1), and metal structure footwear (odds ratio, 3.3; 95 % CI, 1.0–10.4) is associated with the increased risk of small intestine carcinoid [65]. The results of a large prospective study which evaluated risk factors for adenocarcinomas and malignant carcinoid tumors of the small intestine suggested that age (HR for $\geq$65 vs. 50–55 years, 3.31; 95 % CI, 1.51, 7.28), male sex (HR = 1.4; 95 % CI, 1.0–2.1), body mass index (BMI, HR for $\geq$35 kg/m^2 vs. <25 kg/m^2, 1.9; 95 % CI, 1.1, 3.6), and current menopausal hormone therapy use (HR = 1.9, 95 % CI, 1.1, 3.5) were positively associated with malignant carcinoids [66].

3.4 Summary

The incidence and prevalence of NETs have increased about fourfold for the last 30 years because of improvement in the diagnostic techniques. PaNETs are more aggressive tumors compared with NETs originating in the other sites. Also, most of PaNETs are malignant and tend to be in advanced stage at the time of diagnosis. Gastrointestinal tract is the most common location and is responsible for two-thirds of NETs. Small bowel is the most common primary site of GEP-NETs in the developed countries. PaNETs are divided into nonfunctional and functional tumors which secrete hormones and peptides. In association with hormone and peptide secretion, functional endocrine tumors may be symptomatic. Bronchopulmonary system is the second most common location of NETs. These tumors consist of one-third of all NETs. Typical and atypical carcinoids of the lung tend to slowly grow. Most of nonfunctional lung carcinoids have been incidentally diagnosed.

The prognosis of NETs associates with their location, functional status, differentiation, and initial stages. In advanced stage, the prognosis is poor. However, the overall survival of well-differentiated and localized NETs is longer. The best survival rates are observed in patients with NETs arising in the rectum and appendix. The 5-year survival rates of these tumors are excellent with over 90 %.

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Molecular Biology of Neuroendocrine Tumors

4

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Ivana Cataldo, and Matteo Fassan

4.1 Introduction

Neuroendocrine tumors (NETs) are diagnostically challenging tumors, as they comprehend a heterogeneous group of epithelial neoplasms with neuroendocrine differentiation [1, 2].

In the past, medical treatment has been mostly chemotherapy based and has not taken into consideration varying tumor biology. In fact, the molecular study of NETs has been significantly limited for many years, due in large part to their relative scarcity. As a result, the knowledge of their cellular and molecular biology was significantly limited in comparison to that of other more common cancers [3, 4].

Moreover, the clinicotherapeutic management of NET patients considerably suffered by the lack of universally accepted standards for the disease, including both a diagnostic nomenclature and a staging system [1]. This significantly limited the conduction of appropriate clinical trials, and thus, survival rates have remained virtually unchanged for decades.

However, recent findings have significantly expanded our knowledge of NET molecular landscape, which provide better patient stratification and improved targeted therapeutic agents in order to achieve personalized patient care [4, 5].

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4.2 The Lesson from Hereditary Syndromes

The vast majority of NETs are sporadic; however, a small fraction arises in a variety of inherited cancer-predisposition syndromes (Fig. 4.1).

The most common NET-related inherited syndrome is the multiple endocrine neoplasia type I (MEN1; OMIM 131100). MEN1 is an autosomal dominant cancer susceptibility syndrome in which there is a greatly elevated risk of a variety of endocrine tumors, including pituitary tumors, parathyroid tumors, and pancreatic NETs (pNETs). By the clinical point of view, MEN1 patients typically harbor multiple small (<0.5 cm) pancreatic neuroendocrine microadenomas, which are thought to be precursor lesions to pNETs. These data support an early involvement of the *MEN1* tumor suppressor gene in pNET tumorigenesis [6–8].

Menin, the protein encoded by the *MEN1* gene, is a ubiquitously expressed nuclear protein that interacts with a SET1-like histone methyltransferase (HMT) complex containing the MLL (KMT2A) protein which functions to methylate lysine 4 of histone 3 (H3K4), an epigenetic modification associated with actively transcribed genes [9]. In normal pancreatic islet cells, menin appears to negatively regulate islet cell growth by targeting this HMT complex to the gene promoters of two

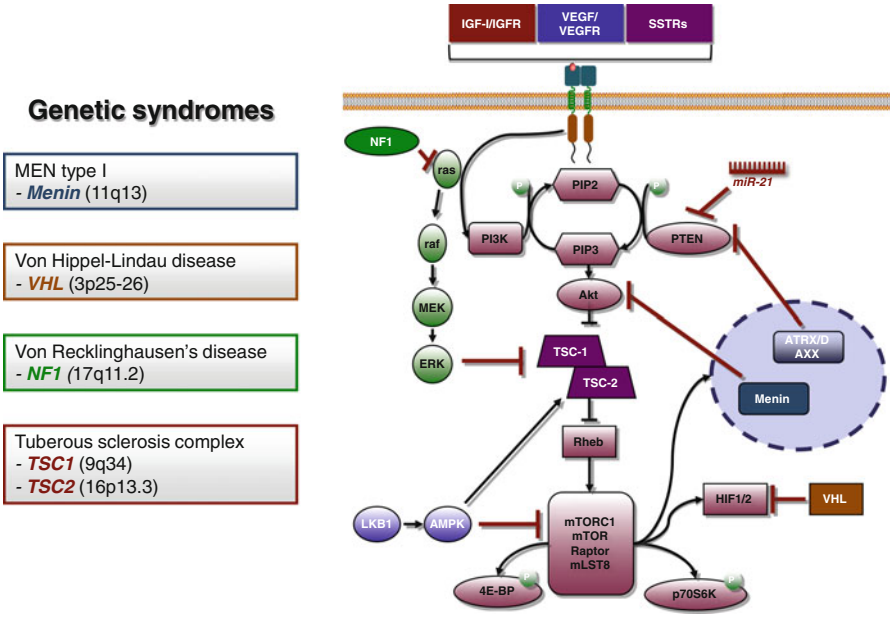

Fig. 4.1 In NETs, the study of inherited syndromes allowed the identification of the common deregulation of genes involved in the mTOR pathway in these tumors. As a master regulator of different cell functions, mTOR activation is subjected to tight and coordinated regulations through diverse positive and feedback regulatory loops. The more recent "next-generation sequencing" effort further identified high prevalence of *ATRX/DAXX* and *PTEN* mutations in pancreatic sporadic tumors. The upregulation of miR-21 also represents a major driver alteration in inherited and sporadic NETs

key negative regulators of the cell cycle: p18 (*CDKN2C*) and p27 (*CDKN1B*), thus fostering their expression. Menin may exert additional effects on the cell cycle via the PI3K/Akt/mTOR signaling pathway by inhibiting the activity of the key serine-threonine kinase Akt1 [10].

In addition to its role on cell proliferation regulation, menin is also involved in the response to DNA damage. In fact, menin activates the transcription of several genes whose protein products are involved in the homologous recombination pathway of DNA repair (e.g., *BRCA1* and *RAD51*) [11]. As a result, depletion of menin leads to increased use of nonhomologous end joining DNA repair activity, a more error-prone repair pathway [12].

Taken together, the above results suggest that loss of menin causes deregulation of normal cell growth control in conjunction with increased genomic instability, an ideal setting for the initiation of malignant transformation. *MEN1* mutations or chromosomal deletions involving the *MEN1* locus at 11q13 locus have been observed in up to 30 % NET sporadic cases [2, 13–21]. To further support these findings, in vivo studies demonstrated that *MEN1* deficiency leads to pancreatic islet cell hyperplasia and neuroendocrine tumors [22]. Weak or negative nuclear expression of menin, as assessed by immunostaining, has been reported in 40 % of sporadic pNET cases, while 27 % of cases had MEN1 gene mutations, most of which were inactivating mutations [13]. In lung carcinoids, the presence of *MEN1* mutation or loss had shorter overall survival and low *MEN1* mRNA levels correlated with distant metastasis and shorter survival [23].

A second autosomal dominant hereditary syndrome is the multiple endocrine neoplasia type 2 (MEN 2). MEN2 is associated with NETs occurring in the thyroid, in the parathyroid, and in the adrenals [24]. The most common tumor among these patients is the medullary thyroid carcinoma (MTC), and overall the 25 % of MTCs are hereditary cases. By the clinical point of view, MEN2 is subclassified in three further inherited syndromes MEN2A, MEN2B, and familial medullary thyroid cancer, which are all caused by mutations of the *RET* oncogene. *RET* encodes a receptor tyrosine kinase that normally binds a family of ligands including glial-derived neurotrophic factor and is thought to provide growth and survival signaling via the RAF-MEK-ERK and PI3K/Akt/mTOR pathways [24]. Thus, activating mutations in *RET* can confer ligand-independent growth and resistance to apoptotic stimuli.

Also the von Hippel-Lindau disease is an autosomal dominant predisposition syndrome featuring an elevated rate of NETs (VHL; OMIM 193300). This syndrome is caused by mutation of the *VHL* tumor suppressor gene located at 3p25 and features nonfunctional pNETs in 5–17 % of patients [25]. Compared to the *MEN1* gene, the *VHL* gene is infrequently mutated in sporadic tumors. However, the *VHL* gene may be inactivated by alternative means, such as promoter hypermethylation or deletion, as has been reported for up to 25 % of pNETs [26]. The *VHL* gene product is involved in the oxygen-regulated degradation of hypoxia-inducible-factor alpha, a transcription factor which regulates gene expression in response to low-oxygen conditions, and tumors harboring *VHL* gene inactivation display elevated expression of HIF1 alpha target genes [26].

Two other inherited autosomal dominant cancer-predisposition syndromes that develop NETs, albeit infrequently, are neurofibromatosis type I (NF1; OMIM 162200) and tuberous sclerosis (TS; OMIM 191100). NF1 (formerly known as von Recklinghausen disease) is caused by a germline mutation of the *NF1* gene at 17q11.2, which encodes the protein neurofibromin. This protein normally functions as a negative regulator of the Ras oncogene and the mTOR signal transduction pathways [27]. Tuberous sclerosis is caused by mutations in one of two different genes: the *TSC1* gene located at 9q34 and the *TSC2* gene located at 16p13.3, which encode for the proteins hamartin and tuberin, respectively. Despite a rare occurrence of NETs in the frame of a TS syndrome [28], the *TSC2* gene has an important role in sporadic pNET pathogenesis. Our group demonstrated that Tsc2 expression is downregulated in 35 % of sporadic pNETs, and, more recently, Jiao and colleagues found the *TSC2* gene to be mutated in 8.8 % of sporadic pNETs [17, 29]. As with neurofibromin, tuberin also functions as a negative regulator of the mTOR pathway, thus lending further support for the potential importance of deregulation of this pathway in NETs.

4.3 Next-Generation Sequencing Era: The Discovery of Novel Cancer Pathways

The advent of massively parallel, high-throughput sequencing, also known as "next-generation sequencing" (NGS), has exceptionally revolutionized the field of cancer genetics over the past decade. This technology allows for the identification of tumor-associated genetic alterations with single-nucleotide level precision, in an unbiased manner, across the entire genome or the entire exome, which consists of the protein-coding regions of the genome [30]. The recognition of driver oncogene mutations in specific tumors has provided a compelling rationale for the introduction of novel targeted therapies [4].

The first NET whole-exome study was published by Jiao and colleagues in 2011 on pNETs [17]. An average mutation rate of 16 mutations per tumor was significantly lower than that previously observed for pancreatic ductal adenocarcinoma (i.e., 66 mutations per tumor) [31]. Furthermore, the genes most frequently mutated in pancreatic adenocarcinoma (*CDKN2A*, *KRAS*, *TP53*, *TGFBR1*, *SMAD3*, and *SMAD4*) were either wild type or only rarely mutated (*TP53*; 5 %) in PanNETs. As anticipated from prior studies, *MEN1* was found to be frequently mutated (44 %). In addition to *MEN1*, genes in the mTOR signaling pathway were also mutated in a significant frequency of cases, with inactivating mutations in *TSC2* (8.8 %) and *PTEN* (7.3 %) observed, plus a single case was found to possess an activating mutation in *PIK3CA*.

Two novel genes were implicated in NET carcinogenesis: the *ATRX* (alpha-thalassemia/mental retardation syndrome, X-linked; OMIM 300032; located on Xq21.1) and *DAXX* (death domain-associated protein; located on 6p21.3) genes. A total of 43 % tumors possessed either an *ATRX* or *DAXX* gene mutation (18 % and 25 %, respectively). Intriguingly, mutations in *ATRX* or *DAXX* were mutually

exclusive, implying that *ATRX* and *DAXX* function in a common pathway important in the pathogenesis of pNETs. In 53 % of cases with either *ATRX* or *DAXX* mutations, *MEN1* was also found to be mutated as well.

Atrx is a large nuclear protein of the SWI/SNF family of chromatin remodeling proteins [32]. Germline mutations in the *ATRX* gene cause the rare syndrome ATRX, which features alpha-thalassemia plus impaired intellectual development [33]. The types of *ATRX* gene mutation differ significantly between those causing the ATRX syndrome and those associated with pNETs. Inherited syndromic *ATRX* mutations are typically hypomorphic missense mutations, whereas sporadic cancer-associated mutations tend to be nonsense mutations or insertions and deletions which lead to a loss of protein expression [17, 33, 34]. This is in keeping with the observation that engineered *ATRX* loss in the mouse is embryonic lethal. Thus, inherited inactivating mutations in humans are likely also to be lethal and, as such, are not observed. Furthermore, individuals with ATRX syndrome do not appear to have an elevated risk of the types of tumors associated with somatic inactivating *ATRX* mutations [17].

Daxx is also a nuclear protein involved in the regulation of chromatin structure and gene transcription. Daxx contains a SUMO-recognition motif, responsible for its association with numerous proteins in their SUMO-modified (sumoylated) state, including a number of transcription factors [35, 36]. Of particular note, Daxx binds the histone variant H3.3 and physically interacts with Atrx to form a histone chaperone-chromatin remodeling complex [37]. This complex acts to deposit H3.3 at a number of locations throughout the genome, including the upstream regions of several active genes, as well as both peri-centromeric and telomeric heterochromatin [37, 38].

Heaphy and colleagues demonstrated that mutations in either *ATRX* or *DAXX* or loss of protein expression were perfectly correlated with the alternative telomerase-independent telomere maintenance mechanism termed alternative lengthening of telomeres (ALT) [34]. At the cellular level, the ALT mechanism is characterized by abnormally enlarged PML nuclear bodies containing large amounts of telomeric DNA repeats, readily detectable in fixed archival tissue specimens via telomere-specific FISH analysis [39]. Intriguingly, whereas ALT is observed in approximately half of all PanNET cases, to date it has only rarely been observed in other NETs: in 5 of 107 cases of gastrointestinal NETs and 0 of 7 lung carcinoids [34, 40].

In order to evaluate the timing of *ATRX* and *DAXX* mutations during pNET tumorigenesis and progression, de Wilde and colleagues assessed Atrx or Daxx nuclear protein expression and ALT status in tissues obtained from a cohort of MEN1 syndrome patients [41]. Loss of either Atrx or Daxx and ALT-positivity were found exclusively in PanNETs and not in any of neuroendocrine microadenomas. In addition, a significant correlation was observed between these abnormalities and higher tumor grade (G2 *versus* G1), and tumor diameter >3 cm. Overall, these findings suggest that *ATRX* and *DAXX* mutations are relatively late events in pNET tumorigenesis.

Of interest, the spectrum of commonly mutated genes in poorly differentiated, high-grade pancreatic neuroendocrine carcinomas (pNECs) differs

substantially from those seen in either pancreatic adenocarcinomas or pNETs [42]. *KRAS* and *CDKN2A*, which are commonly mutated in adenocarcinomas, and *MEN1*, *ATRX*, *DAXX*, and mTOR pathway genes, frequently mutated in pNETs, are mutated infrequently or not at all in pNECs. On the other hand, mutations in *TP53* and *RB1* are very common in this group of tumors. *TP53* is rarely mutated in well-differentiated pNETs, but its pathway is likewise inactivated by the frequent prevalence of extra gene copies of p53-related genes, such as MDM2 (22 %), MDM4 (30 %), and WIP1 (51 %) [43]. In the same way, 80 % of pNETs show an unchanged *RB1* gene but display genetic alterations of other components of the Rb pathway, such as cyclin-dependent protein kinase 4 and 6 and cyclin D [44].

In addition to pNETs, the genomic landscape of small intestinal NETs (SI NET) has recently been investigated [45, 46]. The group led by Matthew Ames [45] performed an integrative analysis of somatic mutations and copy number variations of 48 small intestine NETs. They reported genetic alterations of different members of the PI3K/Akt/mTOR pathway in 30 % of the samples and the loss of SMAD genes in 45 % of samples. Overall, the average per case mutation rate in this study was significantly lower than that of typical carcinomas but comparable to that reported for pNETs [17]. Among these small intestinal NETs, a positive correlation was observed between primary tumors with higher numbers of mutations and the presence of liver metastases. Interestingly, although mutations were identified in several genes previously associated with cancer (e.g., *BRAF*, *EZH2*, *FANCD2*, *FGFR2*, *MEN1*, *VHL*), these mutations were found in only single cases in this series of 48 carcinoid tumors. Notably, many other cancer genes, including those frequently mutated in pNETs, pNECs, and pancreatic adenocarcinomas, were wild type in all of the SI NETs sequenced. Concomitantly, another group identified *p27* (*CDKN1B)* as a putative tumor suppressor in small intestinal NETs [46]. *CDKN1B* encodes a cyclin-dependent kinase inhibitor $p27^{Kip1}$, which is a master regulator of the cell cycle and frequently inactivated in many forms of cancer. Importantly this gene is inactivated in MEN1 patients testing negative for *MEN1* mutations [47]. While these mutations may be present in a subset of carcinoid patients, they do not account for the vast majority of the neoplastic lesions [45, 46].

The whole-exome sequencing of 17 sporadic MTCs showed that approximately 90 % of tumors had mutually exclusive mutations in *RET*, *HRAS*, and *KRAS* genes, suggesting that RET and RAS are the predominant driver pathways in MTC [24]. Relatively few other mutations and no commonly recurrent driver mutations were observed.

A recent gene copy number analysis, genome/exome, and transcriptome sequencing of pulmonary carcinoids demonstrated frequent mutations in chromatin remodeling genes. Covalent histone modifiers and subunits of the SWI/SNF complex were mutated in 40 % and 22 % of the cases, respectively, with *MEN1*, *PSIP1*, and *ARID1A* being recurrently affected [48]. In contrast to small-cell lung cancer and large-cell neuroendocrine lung tumors, *TP53* and *RB1* mutations were rare events.

4.4 Epigenetic Changes in NETs

In recent years, thanks to the advent of molecular techniques allowing genome-wide assessment of epigenetic marks, such as chromatin immunoprecipitation sequencing (ChIP-Seq) and genome-wide DNA methylation analysis, it has become readily apparent that epigenetic abnormalities are widespread in cancer

DNA methylation has been studied rather extensively in a variety of NETs [49]. The long-standing observation that menin is an epigenetic regulator being part of a histone methyltransferase complex further supports that epigenetic, rather than genetic, changes may play a central role in NETs.

Using global analysis of the methylation status of LINE-1 and Alu repeat sequences that are widely distributed throughout the genome, it was found that these sequences were hypomethylated in the vast majority of cases, when compared to adjacent normal tissues and, when present, hypomethylation correlated with clinicopathological parameters such as tumor grade [50, 51].

Another phenomenon involving widespread DNA methylation is CIMP, which stands for CpG island methylator phenotype. First described in colorectal cancer, CIMP-positive tumors display simultaneous abnormal methylation of multiple CpG islands, including several associated with known tumor suppressor genes. In a large series of foregut and midgut NETs, CIMP prevalence was 50 % in gastrinomas and 100 % in VIPomas and glucagonomas [52]. CIMP has also been found in over half of poorly differentiated colorectal neuroendocrine carcinomas, where it was also associated with MSI positivity [53, 54].

Among the others, three genes have been demonstrated to be highly methylated in NETs: *MGMT*, *RASSF1A*, and *CDKN2A*.

MGMT is commonly deficient in pNETs (up to 51 % of cases) and, more importantly, its status can be used to predict response to alkylating agents. On the other hand, MGMT is usually expressed in most G1 GEP-NETs, which likely explains much of the differential sensitivity to temozolomide-based therapies between these two tumor types [55, 56].

The *RASSF1A* tumor suppressor gene, located on 3p21, is rarely mutated in cancer; however, it is frequently silenced via promoter hypermethylation [57]. In pNETs, the *RASSF1A* promoter is reportedly methylated in 75–83% of lesions, where methylation is correlated with reduced *RASSF1A* mRNA expression, increased tumor size, and the presence of metastases [55, 58–60]. However, *RASSF1A* methylation is also observed in a significant fraction of tumor-adjacent normal pancreas tissue, strongly affecting its specificity as potential tumor biomarker [55, 58–60].

The *CDKN2A* gene codes for the p16 tumor suppressor protein, a member of the Rb cell cycle regulatory pathway that, among other cellular processes, regulates entry into S-phase. This pathway is thought to be disrupted in practically all human cancers, and *CDKN2A* has been reported to be methylated in 10–58 % of pNETs and up to 44 % of small intestinal carcinoids [44, 55, 61]. Unlike the case of *RASSF1A*, methylation of the *CDKN2A* displayed good specificity for tumor tissue versus adjacent normal tissue [44, 55, 61].

4.5 microRNA Deregulation in NETs

In contrast to many other tumor types, very little is known about microRNA (miRNA) expression patterns in NETs. However, a significant number of oncogenic and suppressor miRNAs have been identified so far, further supporting the possible use of specific miRNAs signatures to predict clinical outcome in NETs [62–65].

Of interest, unlike most mRNAs, miRNAs are long-living in vivo and very stable in vitro. This structural solidity is fundamental to miRNAs analysis in FFPE samples, in which they can also be investigated at the level of individual cells by applying in situ hybridization [ISH] techniques [66]. Another important point is the evidence for applicability of miRNAs as noninvasive biomarkers, as they are easily and reproducibly detectable in all body fluids [66].

In our seminal report, we investigated global microRNA expression signatures of normal pancreas, Langerhans' islets, pNETs, and pancreatic acinar carcinomas [62]. The overexpression of miR-103 and miR-107, associated with lack of expression of miR-155, significantly discriminated tumor from normal samples. Moreover, a set of 10 miRNAs (miR-125a, miR-99a, miR-99b, miR-125b-1, miR-342, miR-130a, miR-132, miR-129-2, miR-125b-2) allowed the discrimination of PNETs from acinar tumors. This specific miRNA signature is possibly associated with either normal endocrine differentiation or endocrine tumorigenesis.

Among pNETs, miR-204 was primarily expressed in insulinomas (well-differentiated) and correlated with immunohistochemical expression of insulin. On the other hand, miR-21 overexpression was associated with higher Ki67 proliferation index and the presence of liver metastases [62]. The fact that *PTEN* is one of miR-21 targets further supports the increasing evidence of mTOR pathway involvement in NET pathogenesis.

Further confirming miRNAs' stability in body fluids, a nine-miRNA expression pattern (miR-24, miR-30a-3p, miR-18a, miR-92a, miR-342-3p, miR-99b, miR-106b, miR-142-3p, and miR-532-3p) derived from the analysis of selected miRNAs in cyst fluid samples, successfully discriminated cystic forms of pNETs from other – more common – pancreatic cystic lesions [67].

Another group from the Johns Hopkins identified the downregulation of miR-1290 that had the best diagnostic performance in discriminating pNETs from adenocarcinomas [68].

Two relatively recent miRNA expression profile studies find evidence of miRNA deregulation in ileal NET progression [63, 64]. Rubel and colleagues from Mayo Clinic associated miRNA-133a downregulation to progression from primary to metastatic carcinoid tumor, suggesting that it may have an important role in ileal NET development and progression with use for diagnosis and/or prognosis [63]. The same group in collaboration with the Uppsala University investigated miRNA expression in well-differentiated small intestinal NETs. Nine miRNAs were significantly deregulated: five (miR-96, miR-182, miR-183, miR-196, and miR-200) were upregulated during tumor progression, whereas four (miR-31, miR-129-5p, miR-133a, and miR-215) were significantly downregulated in ileal NETs [64].

MiRNAs have been also demonstrated to be significantly deregulated in MTCs, with miR-224 upregulation associated with a better outcome [69]. In lung NETs, members of the miR-29 family seem to have a significant role in the carcinogenetic process [70].

4.6 From Single Gene Alteration to Signaling Pathways Perturbations

In recent years, much attention has focused on identifying key cellular signal transduction pathways that are abnormally activated or deactivated in cancer cells [71]. Such pathways regulate cancer-relevant cellular processes, such as cell growth, cell division, and cell survival. Typically, these pathways involve cascades of cytoplasmic kinases that ultimately impinge on gene transcription. Therapeutically, kinases are attractive drug targets; thus, there is keen interest in elucidating the specific pathways altered in a given tumor in order to identify relevant targets and help predict treatment responses.

Our group sequenced 35 kinase genes implicated in cancer in a series of 36 primary pNETs but found only three mutations – one in *KIT* and two in *ATM* – indicating a low mutation rate for these genes in pNET [72]. Further mutations were identified in pNET-derived cell lines (QGP1, CM, and BON) in the *FGFR3*, *VEGFR1*, and *PIK3CA* genes. Of interest, the membranous immunohistochemical expression of c-Kit was associated with shorter patient survival [72].

The PI3K/Akt/mTOR signaling pathway resulted affected in most NET NGS studies [29, 73–75]. This pathway regulates several cellular processes, including cell growth, proliferation, anabolic metabolism, and apoptosis [76]. The RADIANT-2 and RADIANT-3 trials showed that mTOR inhibitor everolimus had significant antitumor efficacy in the treatment of patients with advanced disease [4]. However, further studies should characterize the molecular bases and find how to overcome the acquisition of therapy resistance.

Somatostatin and its synthetic analogs (e.g., lanreotide, octreotide) act through a family of 5 G-protein couple receptors termed sst1–sst5 to exert a variety of functions, including inhibition of endocrine and exocrine secretions and of tumor cell growth [77]. Somatostatin analogues have been successfully implemented into clinical practice; however, patients may develop resistance to treatment over time [78]. This has been partially explained by the recent finding of novel truncated sst5 receptor variants in humans [79]. One variant, sst5TMD4, which is barely expressed in normal human tissues, shows a marked upregulation in tumors, where it seems to entail pathologically relevant functions. Thus, for example, expression of sst5TMD4 in pituitary adenomas causing acromegaly is related to the reduced ability of octreotide at normalizing hormone secretion in poorly responsive tumors [79].

A pathogenic role for Src family non-receptor tyrosine kinases has been suggested in NETs. In a gene expression profiling study of advanced pNETs, Capurso and colleagues identified the Src-related kinase LCK as one of the genes overexpressed in these cancers [80]. An increased copy number of the *SRC* gene has been

observed in 11/48 (23 %) small intestinal NETs, as well as an increased Src expression in putative GI carcinoid cancer stem cells [45, 81]. Of interest, a potential link between Src and mTOR pathway activation has been identified by immunohistochemical studies, and the concomitant inhibition of the two pathways is more active in impairing cell growth than the use of single agents [82, 83]. Notably, whereas treatment with mTOR inhibitors triggered the activation of a pro-survival feedback dependent on PI3K/Akt signaling, the simultaneous inhibition of both pathways blocked this escape signal.

The VEGF signaling pathway is also deregulated in NETs [84]. NETs are highly vascularized tumors, and a link between the VEGF pathway and pNETs was recognized in the RIP-Tag transgenic mouse model of pNET. Moreover, strong mRNA expression of *VEGFA* and its encoded protein's receptors (*VEGFR1* and *VEGFR2*) were observed in tumors of VHL-related disease [85]. Several VEGF pathway inhibitors including the VEGF inhibitor bevacizumab and the VEGF receptors inhibitors sunitinib, pazopanib, and sorafenib have shown clinical activity [4, 86].

Activation of the epidermal growth factor receptor (EGFR) may play a role in GI carcinoids [87]. In an immunohistochemical study, Shah and colleagues found evidence for activated EGFR in 63 % of 89 GI NET samples as well as activation of Erk and Akt proteins, the downstream targets of activated EGFR [88].

Conclusions

NETs are a challenging group of heterogeneous cancers whose clinical course is often difficult to predict. Currently, there are only a limited number of useful clinicopathological prognostic indicators for these tumors, including tumor grade and the presence of metastases.

In recent years, the data generated in high-throughput studies provided important background for understanding tumor biology in various NET subtype, improving therapeutic decision making and patient stratification. In addition, several novel targetable pathways have been identified, opening the possibility of tailoring treatment to different subtypes of tumors and personalizing medicine.

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Genetic Syndromes Associated with Neuroendocrine Tumours

5

Wouter W. de Herder and Gerlof D. Valk

5.1 Introduction

Neuroendocrine tumors (NETs) are uncommon diseases which can occur either sporadically or in the spectrum of multisystem autosomal dominant inherited genetic disorders (Table 5.1) [1, 2].

The four most common endocrine tumor syndromes are multiple endocrine neoplasia type 1 (MEN-1), multiple endocrine neoplasia type 2 (MEN-2a and MEN-2b), von Hippel-Lindau disease (VHL), and Carney complex (CC). Less commonly, endocrine tumors of the pancreas, parathyroids, and adrenal glands have been observed in neurofibromatosis type 1 (NF-1) and tuberous sclerosis complex (TSC) (Table 5.1) [3–10].

According to the Knudson multiple-hit hypothesis, most endocrine tumor-predisposing disorders are related to inactivation of growth suppressor genes, except MEN-2a, MEN-2b, and familial medullary thyroid carcinoma (FMTC) which occur through dominant activation of the RET tyrosine kinase receptor.

This chapter will only focus on tumor syndromes associated with NET of the digestive tract, pancreas, lung, and thymus.

MEN-1 (MIM 131100) is a multisystem autosomal dominant inherited genetic disorder characterized by hyperplasia and/or multiple adenomas of the parathyroid glands, single or multiple NET of the pancreas and/or duodenum and stomach, adenomas of the anterior pituitary, NET of the thymus and lung and functioning and nonfunctioning hyperplasia, or adenomas of the adrenal cortex.

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Table 5.1 Hereditary tumor syndromes associated with neuroendocrine tumors

Name	Neuroendocrine tumor (NET) (frequency)
MEN-1 syndrome (MEN-1) (MIM 131100) (Wermer's syndrome)	Pituitary adenoma (5–65 %)
	Pancreatic NET (80–100 %)
	Thymic NET (mostly ♂), (<10 %)
	Lung NET (20–25 %)
	Gastric, type 2, NET (ZES related) (5–35 %)
MEN-2a syndrome (MEN-2a) (MIM 171400) (Sipple syndrome)	Medullary thyroid carcinoma
	Pheochromocytoma
MEN-2b syndrome (MEN-2b) (MIM 162300)	Medullary thyroid carcinoma
	Pheochromocytoma
Familiarly medullary thyroid carcinoma (FMTC) (MIM 155240)	Medullary thyroid carcinoma
von Hippel-Lindau (VHL) syndrome (MIM 193300)	*Pancreatic NET* (5–10 %)
	Pheochromocytomas (10–20 %)
Neurofibromatosis I (NF 1, MIM 162200)	*Periampullary NET*
	(*Somatostatinoma*)
	Pheochromocytoma
Tuberous sclerosis (TSC, MIM 191100)	Pituitary adenoma??
	Pancreatic NET?
Carney complex I (CNC1, MIM 160980)	Pituitary adenoma
Carney-Stratakis syndrome (MIM 606864)	Paraganglioma
MEN-4 (MEN-X) syndrome (MIM 610755)	Pituitary adenoma
Familial paraganglioma syndromes (MIM 115310, MIM 168000, MIM 601650, MIM 605373, MIM 614165)	Paraganglioma
	Pheochromocytoma

MIM: OMIM-online catalog of Merdelian inheritance in man catalog number

Less common lesions associated with MEN-1 include cutaneous lesions like angiofibroma, collagenoma, lipoma, and melanoma and peripheral or central nervous system (CNS) tumors such as ependymoma and meningioma [4, 11–14]. The prevalence of the MEN-1 syndrome is approximately 1 in 20,000–40,000 individuals. MEN-1 should be suspected in patients with characteristic endocrine pathology in 2 out of the 3 characteristic affected organs or with a characteristic endocrine disorder in one of these organs plus a first-degree relative affected by the MEN-1 syndrome [11–15].

In 1903, the autopsy of an acromegalic patient with a pituitary adenoma and enlarged parathyroid glands, suggestive of MEN-1, was described by Jakob Erdheim [16]. In 1927, Harvey Williams Cushing and Leo M. Davidoff reported the first patient with the classic MEN-1 tumor triad [17]. In 1953, Laurentius O. Underdahl, Lewis B. Woolner, and B. Marden Black performed the first review of cases with a syndrome of pituitary, parathyroid, and pancreatic NET [18]. Paul Wermer in 1954 was the first who reported that the MEN-1 phenotype was transmitted in an autosomal dominant inheritance pattern giving the syndrome its initial eponym

"Wermer's syndrome" [19]. The MEN-1 clinical phenotype was subsequently fully characterized in the 1960s when radioimmunoassay's for peptides and hormones and standard imaging protocols were developed [20].

The MEN-1 syndrome is the result of an inactivating mutation of the MEN-1 tumor suppressor localized on chromosome 11q13. The 7–10 Kb MEN-1 gene was identified in 1997. It encodes for the 67 kd tumor-suppressor protein menin, consisting of 610 amino acids [21–23]. More than 1,100 unique germline mutations and more than 200 somatic mutations have now been identified in MEN-1 family probands [24]. There is no recognizable genotype/phenotype relation among germline and somatic MEN-1 mutations.

Somatic mutations have also been reported in sporadic forms of endocrine tumors with variable incidence of 20–40 % in pancreatic and lung NET [25, 26]. Clinically, mutations of the MEN-1 and DAXX/ATRX genes seem to be associated with a better prognosis of pancreatic NET [25]. Patients with pancreatic NET with mutations in both the MEN-1 gene and DAXX/ATRX survived at least 10 years, whereas patients without these mutations died within 5 years of diagnosis [25].

Clinical screening of patients remains a prerequisite of genetic analysis [11–15]. Patients with MEN-1 have a shorter life expectancy than the general population. Nowadays this is mainly caused by the malignant potential of the MEN-1-related NET. The 20-year survival of patients affected with MEN-1 is estimated to be 64 % [11–15].

Gastroenteropancreatic (GEP) NET occur in about 30–80 % of MEN-1 patients and are the second most frequent clinical manifestation of MEN-1 [11–15, 27, 28]. Unlike sporadic GEP NET, they are frequently characterized by multiple tumors that are usually diagnosed a decade earlier than sporadic GEP NET. About 2/3 of these tumors are clinically active, i.e., producing an excess of one or more peptides/hormones, which cause the distinct clinical syndromes. The most common functional pancreatic NET are insulinomas (15 %) (11–15; 17). Gastrinomas represent more than half of all functional GEP NET in MEN-1. The great majority of (multiple) gastrinomas (>90 %) in MEN-1 patients is located in the duodenum. In MEN-1 patients, these tumors can manifest with the typical symptoms of the Zollinger-Ellison syndrome before the age of 40 and a generally diagnosed when metastases have occurred [27–29]. Gastrinomas represent one of the major causes of morbidity and mortality in MEN-1 patients and are associated with a poor prognosis [29, 30]. Multiple insulinomas in MEN-1 patients are usually also diagnosed before the age of 40 – many times in association with gastrinomas – which is generally earlier than the diagnosis of sporadic insulinomas [31]. Glucagonomas have also been reported in only a few MEN-1 cases.

Thymic NET almost exclusively occur in male patients with MEN-1. Their prevalence is between 3 and 4 %, and the 10-year survival of patients with these tumors is approximately 25 %. Lung NET occur in 20–25 % of MEN-1 patients. MEN-1 patients with a lung NET, as compared to those with a thymic NET, have a much better 10-year survival (>70 %) [32, 33].

The therapy of NET and NET syndromes in MEN-1 patients is essentially not different from the therapies for their sporadic counterparts. However, there is

discussion as to whether gastrinoma surgery should be generally attempted in MEN-1 patients [29, 34–36]. Gastric NET in MEN-1 patients almost exclusively develop in the presence of the Zollinger-Ellison syndrome [37, 38]. They are generally characterized as type 2 ECL-omas, or gastric carcinoids. These generally well-differentiated lesions usually show a benign clinical course [38].

Periodic screening for tumor manifestations and subsequent treatment of asymptomatic MEN-1 mutation carriers can prevent complications and may lead to a more favorable course of the disease. Therefore, according to the recently published Clinical Practice Guidelines for MEN-1, periodic radiological (and biochemical) screening for pancreatic, thymic, and lung NET is recommended every 1–2 years using thoracic and abdominal CT or MRI [15]. According to these guidelines, MEN-1 germline mutation testing should be offered to index patients with MEN-1 and to their – many times asymptomatic – first-degree relatives from the age of 5 years [13].

von Hippel-Lindau (VHL; MIM 193300) disease is a multisystem autosomal dominant inherited genetic disorder that may manifest with retinal angiomas, Central Nervous System (CNS) hemangioblastomas (involving the cerebellum, spinal cord, or brainstem), clear cell renal carcinoma, uni- or bilateral pheochromocytoma(s), pancreatic lesions (see later), endolymphatic sac tumors of the middle ear, and papillary cystadenomas of the epididymis and broad ligament [8, 39]. The prevalence of the VHL syndrome is approximately 1 in 36,000 newborns [8].

Patients with VHL may be divided into two groups: type 1 and type 2, both leading to a specific phenotype. Patients with type 1 VHL do *not* develop pheochromocytoma(s), whereas those from type 2 disease are at high risk for developing pheochromocytoma(s). Type 2 VHL is further divided into type 2A, 2B, and 2C. Patients with type 2A VHL have a low risk for renal clear cell carcinoma in contrast with type 2B VHL, and patients with type 2C VHL develop pheochromocytomas only [8, 40].

In 1904, the ophthalmologist Eugen von Hippel first described retinal angiomas [41]. Arvid Vilhelm Lindau described the hemangiomas of the CNS in 1927 [42].

In a recent review of patients affected by VHL, 60 % of VHL patients had pancreatic involvement including true cysts, serous cystadenomas, and NET (in 15 %) [27, 28, 43]. Studies also suggest that VHL-related pancreatic NET are mostly nonfunctional [44]. VHL-related pancreatic NET might be distinguished from MEN-1-related NET based on the (1) absence of duodenal tumors, (2) frequent clinically nonfunctional tumors, and (3) frequent occurrence of cystic adenomas around the pancreatic NET. Libutti and colleagues have formulated guidelines for the follow-up of pancreatic NET in patients with VHL [45]. For pancreatic NET ≤1 cm, they recommend follow-up with CT or MRI every 12 months; for NET between 1 and 3 cm, a case-by-case assessment is recommended; and NET >3 cm that are symptomatic or functional or lesions that are increasing in size are considered for resection [45].

The VHL gene is a tumor-suppressor gene located on chromosome 3p25-26. The VHL protein binds to elongin C and elongin B, thereby inhibiting transcription elongation, Cul2, and Rbx1 and degrades the α-subunits of hypoxia-inducible factors (HIF). This mechanism is influenced by oxygen. In absence of the VHL

protein, lack of degradation of HIF results in uncontrolled production of factors, like vascular endothelial growth factor (VEGF), that promote formation of new blood vessels and tumor development [8, 9]. Germline mutations in the VHL gene are now identifiable in virtually all VHL families [8]. VHL gene sequencing has been useful in VHL disease for presymptomatic diagnosis [8]. The exact molecular mechanism of development of NET in VHL is yet unknown.

Neurofibromatosis type 1 (NF-1) (von Recklinghausen disease, MIM 162200) is a multisystem autosomal dominant inherited genetic disorder characterized by neurofibromas, multiple café au lait spots, axillary and inguinal freckling, iris hematomas (Lisch nodules), skeletal abnormalities, CNS gliomas, pheochromocytomas, and paragangliomas and occasionally with periampullary somatostatinomas [46].

The disorder was first characterized by Friedrich Daniel von Recklinghausen in 1882 [47]. The prevalence of NF-1 is about 1 in 3,000 individuals.

Approximately 40 % of the periampullary somatostatinomas are associated with NF-1 [46].

The NF-1 gene is located on chromosome 17q11.2. It encodes for the protein neurofibromin, which inhibits the intracellular phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K-AKT-mTOR) pathway, which is important in apoptosis. Loss of function of the NF-1 gene results in mTOR upregulation and tumor development [10, 48].

Tuberous sclerosis complex (TSC) (OMIM 191100) is a multisystem autosomal dominant inherited genetic disorder, which is characterized by hamartomas in several organs, including the brain, heart, skin, eyes, kidneys, lungs, and liver.

The characteristic tubers (cortical hamartomas) of this disorder were first described by Désiré-Magloire Bourneville in 1880, the disease thereby earning its eponym “Bourneville’s disease” [49].

Pancreatic NET, mostly insulinomas or nonfunctioning tumors, appear to be more frequent in TSC patients than in the general population. However, it is still unclear whether pancreatic NET should be considered as a feature of TSC. Current clinical recommendations for TSC do not include routine investigation for pancreatic NET [27, 28, 50].

The live birth prevalence of the TSC is approximately 10–16 cases per 100,000 individuals.

TSC is caused by inactivating mutations in either of the two genes TSC1 (located on chromosome 9q34 and encoding for the protein hamartin) or TSC2 (located on chromosome 16p13.3 and encoding for the gene product tuberin). Mutations of the TSC1 and TSC2 genes result in an impaired function of the hamartin-tuberin complex, which results in the upregulation of the PI3K-AKT-mTOR pathway [7, 48].

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Pathological Evaluation and Classification of Digestive Neuroendocrine Tumours

6

Anne Couvelard

6.1 The Pathological Diagnosis of Digestive NET

Neuroendocrine neoplasms (NEN) arise from neuroendocrine cells which are distributed in the mucosa of the gastrointestinal (GI) tract and in the pancreas. There are several diagnostic tools to perform the diagnosis of NEN, including imaging, serological tests and endoscopy, but the diagnosis should be formally confirmed by the pathological investigation. The histological feature of NEN, which is generally characteristic in most cases, at least for well-differentiated NEN, must be confirmed by immunohistochemistry and allows to categorise these tumours as NETs (neuroendocrine tumours) or NECs (neuroendocrine carcinomas) according to the last international WHO classification [5]. This is very important to assess the tumour prognosis and to guide patient therapy.

6.1.1 Morphology

Characteristic histopathological features of digestive NEN are held in common. By morphology, there is a clear distinction to be made, in all digestive locations, between the well-differentiated and the poorly differentiated tumours. This distinction is important, since their morphology, prognosis and response to treatments are very different. However, it must be emphasised that a relatively small percentage of tumours is not easy to classify into the well- or poorly differentiated groups, because they share some morphological characteristics of both of them. Well-differentiated tumours (called "neuroendocrine tumours", NET, according to the WHO 2010 classification, as discussed below) are composed of tumour

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cells possessing round nuclei with "salt and pepper" chromatin [5]. Their cytoplasm is eosinophilic and granular. They show regular insular, trabecular or sheet-like patterns, depending on the site of primary, the insular pattern being more frequent in the ileum, with palisading of the peripheral cell layers. Poorly differentiated tumours (called "neuroendocrine carcinomas", NEC, according to the WHO 2010 classification, as discussed below) are classified as small or large-cell carcinomas, according to the histological morphology of their cells [5]. The small cells are, as their name implies, small, round ovoid or spindle-shaped, with very scant cytoplasm; their chromatin is fine and granular without nucleoli. In contrast, large-cell carcinomas are composed of medium-sized or large-sized cells; their nuclei are atypical with evident nucleoli. These morphological characteristics are similar to those used to classify the lung neuroendocrine tumours according to the lung WHO 2004 classifications [50].

6.1.2 Immunohistochemistry

The morphological diagnosis of digestive NEN must be confirmed by immunohistochemistry, as firstly proposed by the European Neuroendocrine Tumor Society (ENETS) and confirmed by the WHO 2010 classification [40–42]. NEN share marker proteins with the neural cell system, such as synaptophysin and neuron-specific enolase. Among the several neuroendocrine markers, chromogranin A and synaptophysin are the most common and those which are required to confirm the diagnosis of NEN according to the ENETS recommendations and to the WHO 2010 classification of digestive tumours [5, 41, 42]. The neural cell adhesion molecule CD56 (NCAM) is also useful, especially in poorly differentiated tumours, because they may weakly, or not at all, express chromogranin A. Hindgut rectal NEN may be negative for chromogranin A and only express chromogranin B. The diagnosis of rectal NET should not be ruled out in the case of negativity of chromogranin A.

6.2 The Pathological Classification of Digestive NET

A new classification of digestive NEN has been formulated in the 2010 revision of the WHO classification of tumours of the digestive system [5]. The terminology and principles were greatly modified in this novel classification as compared to those used previously. Three main grading categories are recognised, irrespective of the site in the digestive system (neuroendocrine tumour Grade 1, neuroendocrine tumour Grade 2 and neuroendocrine carcinoma of small- or of large-cell types) combined with a site-specific TNM staging, which was published in 2009 by the AJCC-UICC following a 2006 TNM proposal by the European Neuroendocrine Tumor Society (ENETS) [41, 47].

6.2.1 The WHO 2010 Classification

6.2.1.1 Introduction

In 2000, the WHO published a classification for the histological typing of digestive neuroendocrine tumours (NETs) [48]. This classification remained unchanged following the WHO 2004 revision and was used until 2010 [9]. A new WHO classification for GEP-NET appeared in 2010 [5]. In the first pages of the WHO classification book, the "nomenclature and classification of neuroendocrine neoplasms of the digestive system" is introduced and described in detail [40]. Its main principle is a clear distinction between histological classification (including grading, the same in any digestive location) and staging (using the AJCC-UICC TNM 7th edition, specific for each location). As for most other tumour types, histological WHO must be associated with TNM staging since WHO and TNM complement each other.

According to the WHO 2010 classification, digestive neuroendocrine neoplasms (the term "neuroendocrine neoplasm or NEN" encompasses well- and poorly differentiated tumours) are classified into three main histological categories (Table 6.1): *neuroendocrine tumours grade 1 or NET G1* (Fig. 6.1a, b), *neuroendocrine tumours grade 2 or NET G2* (Fig. 6.1c, d) and *neuroendocrine carcinomas or NEC*, with two different subtypes, *of large- or small-cell types*; these poorly differentiated carcinomas are of grade G3 (Fig. 6.1e). This parallels well with the pulmonary neuroendocrine WHO classification [50]. Two other categories include *mixed adenoneuroendocrine carcinomas (MANECs) and hyperplastic and preneoplastic lesions*. The WHO 2010 classification deleted the terms "benign" and "malignant" used in the previous classification to describe the well-differentiated tumours assuming neuroendocrine neoplasms (NEN) as a category to be potentially malignant.

6.2.1.2 Basis of the Grading

The histological grading into G1, G2 and G3 is performed on the basis of the assessment of the proliferation fraction according to the ENETS scheme firstly published in 2006 [41], with the same cut-off values (Table 6.2). However, subtle differences appeared in the way to count. Indeed, in the WHO 2010 classification, it is required to count mitosis in 50 HPF (high-power field) (1 HFP = 0.2 mm^2), instead of 40 HPF in the ENETS proposals. It is recommended to count the Ki-67 index using the MIB

Table 6.1 General neuroendocrine neoplasm categories in the WHO 2010 classification

1	Neuroendocrine tumour, NET G1 (carcinoid)
2	Neuroendocrine tumour, NET G2
3	Neuroendocrine carcinoma, NEC (small- or large-cell type)
4	Mixed adenoneuroendocrine carcinoma, MANEC
5	Hyperplastic and preneoplastic lesions

Adapted from Bosman et al. [5]

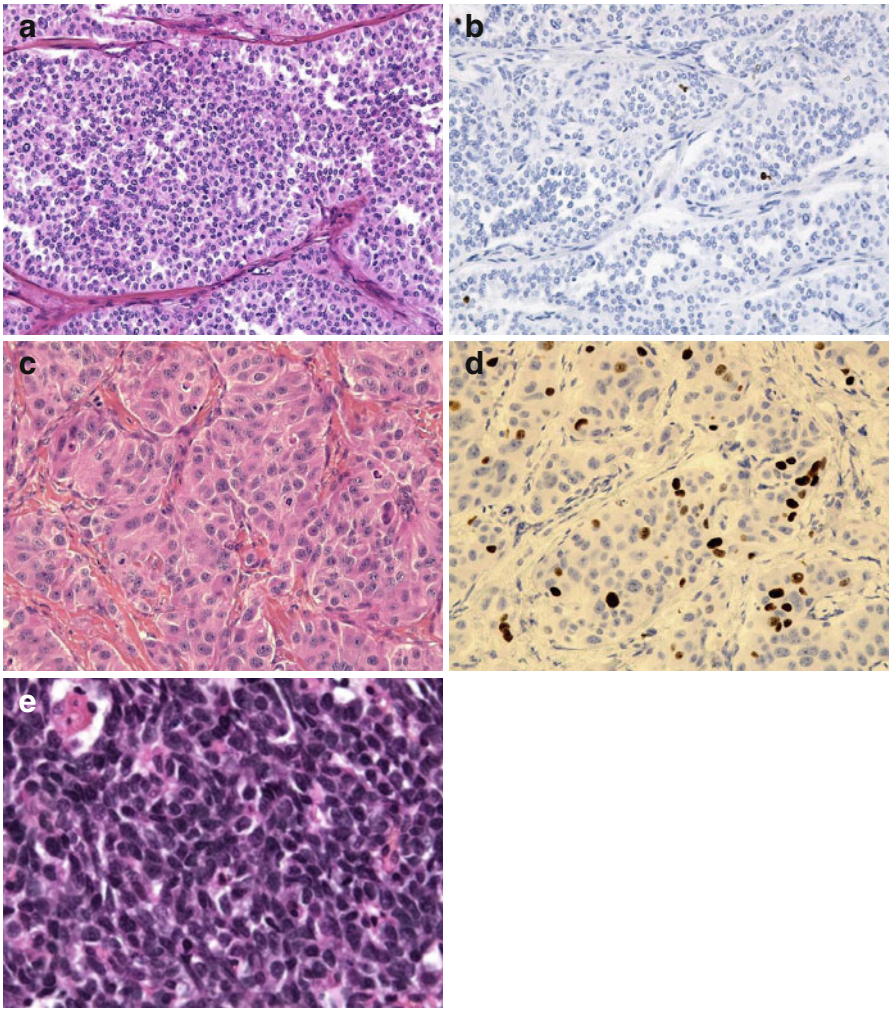

Fig. 6.1 A well-differentiated (**a**) neuroendocrine tumour (NET) of grade G1 (Ki-67 < 1 %, **b**); a well-differentiated (**c**) neuroendocrine tumour (NET) of grade G2 (Ki-67 = 12 %, **d**); a gastric small-cell poorly differentiated (**e**) neuroendocrine carcinoma (NEC)

antibody as a percentage of 500–2,000 cells (whereas it was recommended to count 2,000 cells in the ENETS proposals). Grade 1 tumours have a mitotic count <2 per 2 mm^2 (10 HPF) and/or ≤2 % Ki-67. Grade 2 tumours have a mitotic count between 2 and 20 per 2 mm^2 and/or 3–20 % Ki-67 >20 %. Grade 3 tumours have a mitotic count >20 per 2 mm^2 and/or Ki-67. If grade differs for mitosis and Ki-67 evaluation, it is suggested to consider the higher grade. It is of importance to note that in order to perform a proper evaluation of the mitotic count, the pathological specimen must have a minimal size: indeed, 50 HPF represents 10 mm^2. This is not feasible in a biopsy specimen where evaluation of Ki-67 is consequently required. The

Table 6.2 Grading for digestive neuroendocrine tumours, according to the WHO 2010 classification

Grade	Mitotic count (/2 mm²)[a]	Ki-67 index (%)[b]
G1	<2	≤2
G2	2–20	3–20
G3	>20	>20

Adapted from Rindi et al. [41] and Bosman et al. [5]
[a]10 high-power field [HPF], 40× magnification = 2 mm². It is recommended to count mitoses in at least 50 fields at ×40 magnification in areas of highest mitotic density and to divide the total number of mitoses by 5
[b]MIB1 antibody; % of 500–2,000 tumour cells in areas of highest labelling

prognostic value of this grading was demonstrated for foregut, midgut and hindgut NET [10, 13, 17, 18, 27, 35, 36, 45, 52]. Since the use of the WHO 2010 classification, several publications have pointed differences in the evaluation of tumour proliferation by Ki-67 or mitosis. There is a lack of concordance between grades assigned by both methods, the mitotic count being often lower than the Ki-67 count [19]. The best method to evaluate Ki-67, for example, is the use of manual or digital counting, and the best cut-off to discriminate between G1 and G2 still remains controversial [1, 11, 33, 49]. It has been recently suggested that Ki-67 is a better prognosis marker and predictor of metastases than mitoses [30].

6.2.1.3 Definition of the Five Categories of the WHO Classification

1. Neuroendocrine tumours grade 1: these tumours are well differentiated and possess a low proliferation rate, of grade G1 (see above). The term "carcinoid tumour" can be used in place of NET G1. This term was removed from the WHO 2000 classification, and it is important to recall that it is also used to designate neuroendocrine tumours of ileal origin, secreting serotonin, and often responsible for a carcinoid syndrome. "Carcinoid tumours" have a benign connotation, but it is well known that NET G1 can be malignant and metastatic, as it is observed in the lung (Fig. 6.2).
2. Neuroendocrine tumours grade 2: these tumours are well differentiated and possess an intermediate proliferation rate, of grade G2 (see above). The term "atypical carcinoid" is not recommended in the WHO 2010 classification; it cannot be used for NET G2.
3. Neuroendocrine carcinomas of large or small cells: these tumours are poorly differentiated and malignant, composed of small or large cells expressing the neuroendocrine markers chromogranin A and synaptophysin (staining might be faint or focal). They are of grade G3. The large-cell category was not included in the previous WHO 2000 classification. The small-cell category looks like the pulmonary "small-cell carcinoma" subgroup. All practitioners must be aware of the NEC category. Indeed, in the previous WHO classification, the term "carcinoma" was also used for well-differentiated tumours presenting metastases and/or invading the muscular layer in the digestive tract. It is important to document

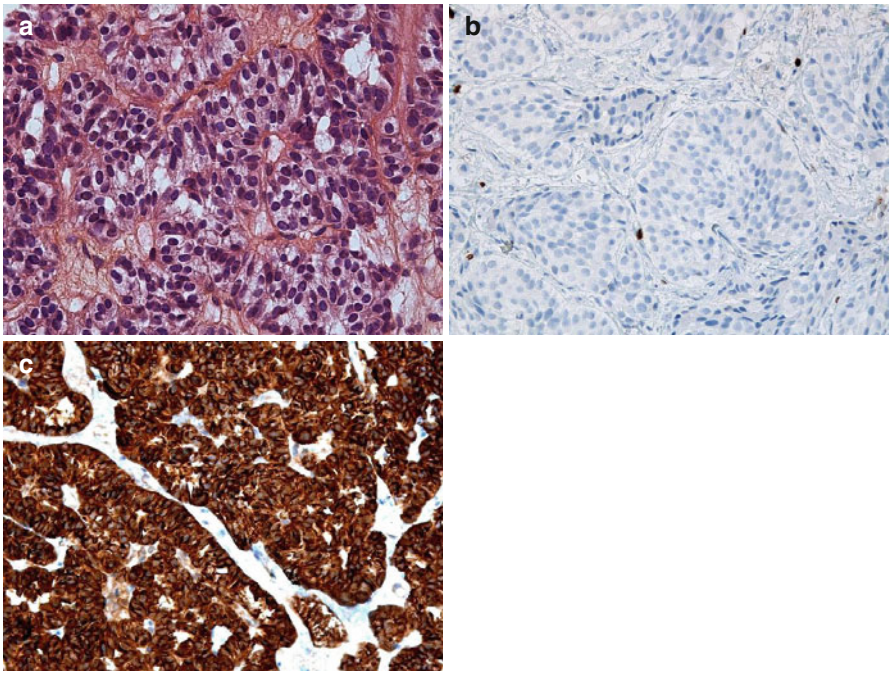

Fig. 6.2 Liver metastasis of a well-differentiated (**a**) pancreatic neuroendocrine tumour (NET) of grade G1 with a very low Ki-67 index <1 % (**b**) and a strong chromogranin A expression (**c**)

the tumour differentiation in the pathology reports in order to make impossible such error. Another important issue is the relationship between the grade G3 and the differentiation in this category. Indeed, the WHO classification suggests that all G3 tumours are poorly differentiated carcinomas. However, it is now known that the G3 group is heterogeneous, containing both well-differentiated NET and poorly differentiated NEC, the former being less aggressive with a lower Ki-67 index and a lower response rate to cisplatin-based chemotherapy [51]. It is not possible to classify "well-differentiated G3" tumours according to the WHO classification.

4. MANECs (mixed adenoneuroendocrine carcinomas) have both a neuroendocrine and an exocrine glandular phenotype. Thirty per cent of each component must be at least identified for this definition. The new term "MANEC" replaces the previous "mixed endocrine-exocrine tumour". Theoretically, the neuroendocrine component may be well or poorly differentiated. The exocrine component may be composed of acinar carcinoma cells. The frequency of MANEC and the type of exocrine or neuroendocrine component depend on the location in the digestive system. For example, in the colon, MANECs are more frequent and they often contain a poorly differentiated neuroendocrine component [28].
5. Hyperplastic and preneoplastic lesions.

6.2.2 The 2009 AJCC-UICC TNM

6.2.2.1 Introduction

In 2009, the 7th edition of the American Joint Cancer Committee-Union Internationale Contre le Cancer (AJCC-UICC) TNM classification was published [47], including for the first time digestive neuroendocrine tumours. It followed the first TNM classification which was proposed in 2006 (for NET of the stomach, duodenum and pancreas) and in 2007 (for NET of the ileum, colon/rectum and appendix) by a working group of the European Neuroendocrine Tumor Society (ENETS) [41, 42]. In the AJCC-UICC classification, high-grade (poorly differentiated) NECs are classified separately, by using the exocrine classification established in respective sites. When considering well-differentiated NETs, the AJCC-UICC TNM is similar to the previous ENETS/TNM proposals for intestinal anatomical sites but differs for other locations (the pancreas, stomach and appendix). It is important to document the pathological features, such as invasion and tumour size, to allow the translation of the staging between the classifications [23].

6.2.2.2 TNM Staging in the Different Digestive Locations

See Table 6.3 for details and comparison between UICC and ENETS T categories.

Table 6.3 T categories in the UICC and ENETS classifications of digestive neuroendocrine tumours, in the pancreas, stomach, small intestine, appendix and colon/rectum

Pancreas-ENETS		Pancreas-UICC[a]
T1	Tumour confined to pancreas, ≤2 cm	Idem
T2	Tumour confined to pancreas, 2–4 cm	Tumour confined to pancreas, >2 cm
T3	Tumour confined to pancreas and >4 cm or invading duodenum or bile duct	Tumour extends beyond pancreas, without involvement of coeliac axis or superior mesenteric artery
T4	Tumour involves coeliac axis or superior mesenteric artery or adjacent organs (stomach, spleen, colon, adrenal)	Tumour involves coeliac axis or superior mesenteric artery
Stomach-ENETS		Stomach-UICC
Tis	In situ/dysplasia (<0.5 mm)	Idem
T1	Tumour invading mucosa or submucosa, ≤1 cm	Idem
T2	Tumour invading muscularis propria or subserosa or >1 cm	Tumour invading muscularis propria or >1 cm
T3	Tumour penetrating serosa	Tumour invading subserosa
T4	Tumour invading adjacent structures	Tumour penetrating serosa or invading adjacent structures
Small intestine-ENETS		Small intestine-UICC
T1	Tumour invading mucosa or submucosa, ≤1 cm	Idem
T2	Tumour invading muscularis propria or >1 cm	Idem

(continued)

Table 6.3 (continued)

Small intestine-ENETS		Small intestine-UICC
T3	Jejunum, ileum: tumour invading subserosa Ampulla, duodenum: tumour invading pancreas or retroperitoneum	Idem
T4	Tumour invading serosa or other organs	Idem
Appendix-ENETS		Appendix-TNM[b]
T1	Tumour ≤1 cm; invading submucosa, muscularis propria	T1a: ≤1 cm T1b: >1–2 cm
T2	Tumour ≤2 cm; invading submucosa, muscularis propria, minimally (≤3 mm) subserosa/mesoappendix	Tumour >2–4 cm or invading the cecum
T3	Tumour > 2 cm or largely (>3 mm) invading subserosa/mesoappendix	Tumour >4 cm or invading the ileum
T4	Tumour invading serosa or other organs	Idem
Colon/rectum-ENETS		Colon/rectum-UICC
T1	Tumour invading mucosa or submucosa, T1a < 1 cm, T1b: ≥1–2 cm	Idem
T2	Tumour invading muscularis propria or >2 cm	Idem
T3	Tumour invading subserosa or mesorectum	Idem
T4	Tumour penetrating serosa or invading adjacent structures	Idem

Adapted from Rindi et al. [41], Rindi et al. [42], Sobin et al. [47], Bosman et al. [5]
According to UICC, the poorly differentiated NECs are classified as exocrine tumours
[a]According to UICC, all pancreatic neuroendocrine neoplasms (including G1, G2 and G3 and well- or poorly differentiated tumours) are classified following the pancreatic exocrine tumour classification
[b]Goblet cell carcinoids are classified according to the exocrine carcinoma classification

Pancreas

In this location, the AJCC-UICC applies the same TNM as the one used for classifying adenocarcinomas, either for well-differentiated or poorly differentiated tumours. In the AJCC-UICC TNM, invasion of the peripancreatic fat applies to pT3 tumours as compared to tumour size >4 cm in the ENETS TNM (Table 6.3). The size cut-off of 4 cm, which is reported to be an important prognostic factor [45], is not included in the AJCC-UICC classification. There is a discrepancy between the ENETS and UICC staging in the pancreas in a large proportion of cases [29].

Stomach

In this location, a Tis stage is defined (in situ tumour, less than 0.5 mm). The UICC and ENETS classifications differ. Tumours invading the subserosa are T2 according to ENETS and T3 according to UICC.

Intestine

The UICC and ENETS classifications are identical in the small intestine. The T2 and T3 stages apply to tumours invading the muscularis propria and the subserosa,

respectively, whereas T4 tumours penetrate the serosa (Table 6.3). In the rectum, the stage and grade according to ENETS/WHO are correlated with survival (Weinstock).

Appendix

According to the UICC classification, the tumour size is a very important criterion to classify NET in this location. According to ENETS, the invasion into the mesoappendix should be evaluated to distinguish T2 and T3 tumours (T3 tumour >2 cm and/or >3 mm extension into the mesoappendix).

Colon/Rectum

In the colon and rectum, the UICC and ENETS classifications are identical. T1 is separated into T1a and T1b, according to size (<1 cm or ≥1–2 cm). This parameter is important for endoscopic resection of rectal tumours.

6.3 Specificities of Pathological Diagnosis According to the Digestive Locations

6.3.1 Pancreas

Pancreatic NETs represent a very heterogeneous group of tumours, depending on functional status, presence of inherited syndromes or tumour differentiation [21, 22]. By definition, tumours are greater than 5 mm (below this size, they are defined as microadenomas). Functional tumours are associated with clinical syndromes caused by inappropriate secretion of insulin, glucagon, somatostatin, gastrin or VIP. Non-functioning tumours are often discovered incidentally, or when they become clinically apparent due to their large size, to invasion of adjacent organs or to the occurrence of metastases. Most PNETs are solitary and well differentiated; when multiple, MEN1 or VHL syndromes should be suspected. Well-differentiated NETs are usually well circumscribed; they may present different histological patterns (such as solid, trabecular, gland-like, oncocytic); they may show invasion of the peripancreatic fatty tissue (then classified as T3 according to UICC TNM, see above). First metastases are usually found in regional lymph nodes and the liver. Poorly differentiated NECs are infrequent in the pancreas, mostly represented by large-cell NEC [3].

Among functional pancreatic NETs, insulinomas are the most frequent. In 4–6 % of cases, they are associated with MEN1 [25]. They are frequently discovered while still small, and most are less than 2 cm, due in part to their earlier detection [26]. A relatively characteristic histological feature is the stroma with deposition of amyloid. Pancreatic gastrinomas are associated with the sporadic form of Zollinger-Ellison syndrome, as compared to duodenal ones which are more frequent, smaller and more often associated with MEN1 syndrome [25]. The histological aspect of gastrinomas has no distinctive features with other functioning or non-functioning pancreatic NETs (Fig. 6.3). Glucagonomas are usually large and solitary tumours more frequent in the tail. They cause a functional syndrome including a skin rash (necrolytic migratory erythema). They represent 8–13 % of functioning tumours.

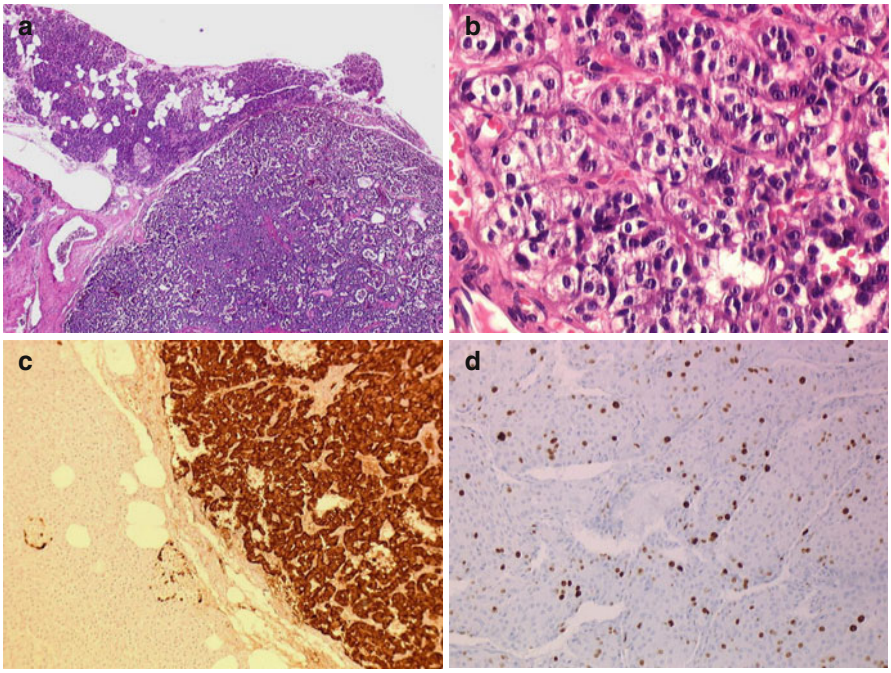

Fig. 6.3 A pancreatic glucagonoma corresponding to a well-differentiated neuroendocrine tumour (NET) G2 at low (**a**) and high (**b**) magnification. Tumour cells are regular and strongly express glucagon (**c**). Ki-67 is calculated at 6 % (**d**)

Inherited diseases, mostly MEN1 and VHL disease, are responsible for multiple PNETs, associated with microadenomas, which are more frequent and numerous in MEN1 (Fig. 6.4).

In VHL disease, pancreatic NETs occur in about 10–17 % of patients, whereas VHL is prevalent in about 0.5 % of pancreatic NETs [4, 7, 12]. The presence of microadenomas is not constant in pancreatic specimen resected for NET (about 70 % of cases) [37]. As compared to sporadic NET, VHL-NETs present a lower malignancy rate [12].

6.3.2 Stomach

Well-differentiated gastric NETs are classified into types 1, 2 and 3 [25, 43]. Type 1 NETs are the most common (70–80 %). They are related to fundic atrophic gastritis and hypergastrinaemia secondary to the deficient production of gastric acid. They occur in the fundus, are multifocal, small (mostly less than 1 cm) and polypoid, usually G1 tumours (Fig. 6.5). They are composed of enterochromaffin-like (ECL) cells and associated with ECL-cell hyperplasia in the adjacent mucosa. The prognosis of type 1 gastric NET is excellent; their small size allows an endoscopic resection in most cases. Type 2 tumours are rare. They are associated with a

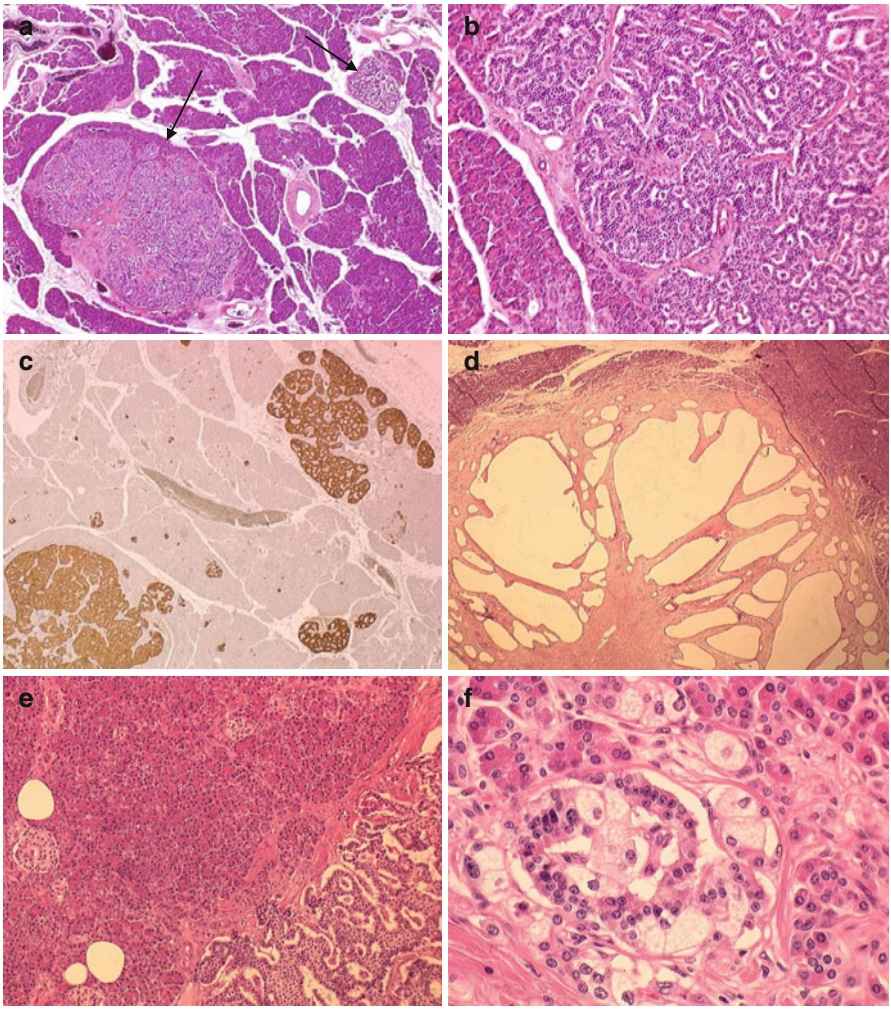

Fig. 6.4 Two pancreatic microadenomas (**a**, *arrows*) in a patient with multiple endocrine neoplasia type 1, well delimited and composed by regular cells (**b**) expressing chromogranin A (**c**). A serous cystadenoma (**d**), a well-differentiated neuroendocrine tumour of grade G1 (**e**) and a small microadenoma containing large neuroendocrine clear cells (**f**) in patient with a von Hippel-Lindau disease

Zollinger-Ellison syndrome. Hypergastrinemia causes fundic ECL-cell hyperplasia and, in the setting of MEN1, small fundic neuroendocrine tumours which are numerous and multifocal. Type 2 tumours are very similar to type 1 tumours but not associated with fundic atrophic gastritis. Moreover, lymph node metastases are more frequent than in type 1 NET [25, 43]. Type 3 NETs occur in any part of the stomach and are not associated with atrophic gastritis, hypergastrinemia, ECL-cell hyperplasia or MEN1. They are well-differentiated, solitary and larger than type 1 or type 2 tumours, with a more aggressive course and more frequent local or distant

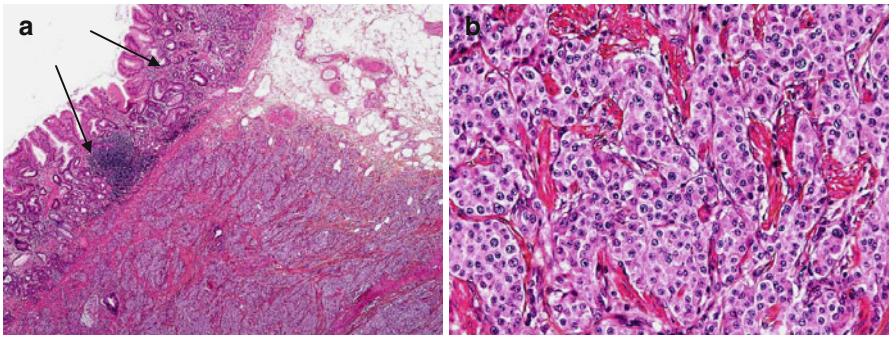

Fig. 6.5 A type 1 gastric NET (**a**, see the overlying inflammatory fundic mucosa, *arrows*), measuring 1.5 cm and infiltrating the submucosa (pT2, according to UICC TNM), well-differentiated (**b**) of grade G1

metastases [44]. Type 4 gastric NEN are poorly differentiate, large, ulcerated tumours of poor prognosis, occurring in any part of the stomach [25]. Poorly differentiated NECs are rare in the stomach.

6.3.3 Small Intestine

6.3.3.1 Ileum

Most of the ileal NETs are serotonin-producing EC-cell tumours. A carcinoid syndrome, due to the effect of serotonin, is present when tumours are of significant size, usually with liver metastases. These NETs are not associated with any of the known inherited syndrome (i.e. MEN1, von Hippel-Lindau disease, neurofibromatosis, etc.). However, familial cases have been described [16, 20]. Ileal NETs occur in the distal part of the ileum and can present as multiple tumours in some cases [53]. Histologically, they present an insular growth pattern with frequent palisading at their periphery and a fibro-sclerotic stroma. They often deeply invade the intestinal wall and give lymph node and liver metastasis, whereas their proliferative index is usually low [18]. The presence of mesenteric tumour deposit is frequent and could be an indicator of poor prognosis and survival [15].

6.3.3.2 Jejunum

A recent study underlines the heterogeneity of jejunal NETs and supports the distinction between "upper" and "lower" jejunal tumours, which, for prognostic purposes, could be grouped with, respectively, duodenal and ileal NETs [8].

6.3.3.3 Duodenum

In the duodenum, well-differentiated NETs are of five main types: (1) gastrinoma or gastrin-producing NET with or without MEN1 syndrome, (2) somatostatinoma or somatostatin-producing NET with or without neurofibromatosis type 1, (3) non-functioning NET, (4) gangliocytic paragangliomas and (5) poorly differentiated NEC.

Non-functioning NET may produce gastrin, somatostatin, serotonin or calcitonin. Gastrin-producing NETs occur mainly in the proximal duodenum, whereas somatostatin-producing NETs occur mainly in the ampulla of Vater [14]. The somatostatinoma syndrome does not usually develop. Histologically, the pseudoglandular pattern with psammoma bodies is characteristic; a neurofibromatosis type 1 syndrome should be suspected. Both gastrin- and somatostatin-producing NETs can be associated with MEN1. In this case, hyperplasia of neuroendocrine cells can be found in the adjacent non-neoplastic mucosa. Most duodenal gastrinomas are confined to the mucosa and submucosa, but lymph node metastases are frequent and often much larger than the duodenal primary, whereas liver metastases are rare [2, 25]. Duodenal gastrinoma can be sporadic or associated with MEN1 (in 20–30 % of cases). They frequently metastasise to the regional lymph nodes, but liver metastases are less frequent than in patients with pancreatic gastrinomas. Gangliocytic paragangliomas possess a triphasic cellular differentiation with neuroendocrine cells, ganglion cells and Schwann-like cells. They mainly occur in the papilla of Vater, and their course is usually benign, but they may spread to a lymph node. Duodenal NECs, which are infrequent, most commonly occur in the papilla of Vater [25].

6.3.4 Colon/Rectum

Rectum NETs are more frequent than colonic NETs, often solitary, sessile and incidentally discovered on colonoscopy. They are increasing in incidence, probably due to increase reporting of small polyps at endoscopy. Average tumour size is <1 cm and G1 tumours account for >80 % of cases [52]. Large tumours may be ulcerated. Rectal NETs are usually negative for chromogranin A and positive for prostatic acid phosphatase (Fig. 6.6). They can be treated by endoscopic resection, depending on their size and on tumour invasion, as determined by endoscopic ultrasound (in general if <2 cm and no invasion of muscularis propria) [39]. The evaluation of the margin and of the grading is important in such specimen [52].

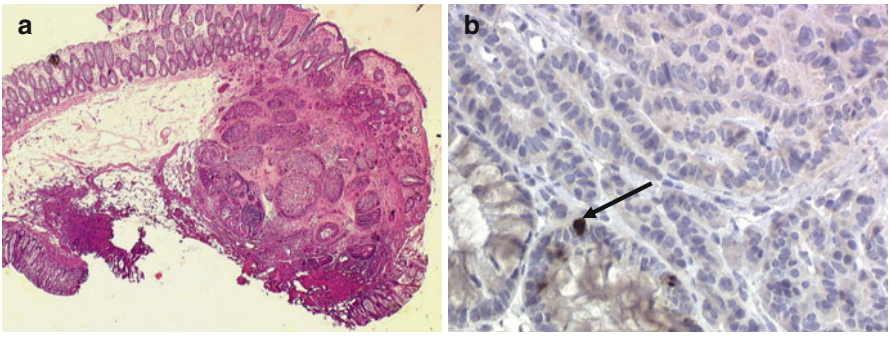

Fig. 6.6 A well-differentiated NET of the rectum, less than 1 cm and infiltrating the submucosa (pT1a), resected by mucosectomy (**a**). Chromogranin A is not expressed by tumour cells; in contrast, it is expressed by normal neuroendocrine cells located in the glands of the mucosa (**b**, *arrow*)

In the colon, NEC are more frequent than in the rectum. Large-cell NEC is the most common type [46]. Mixed tumours (MANEC) are not infrequent, and overlying adenoma or adenocarcinoma is associated with infiltrative poorly differentiated neuroendocrine carcinomas [28].

6.3.5 Appendix

Appendiceal NETs are frequent, often diagnosed after an appendiceal surgical rejection because of symptoms of acute appendicitis. Most are EC-cell NETs, comparable to ileal EC-cell NETs and arise in the tip (>70 % of cases) of the appendix [6]. Tubular carcinoid is a histological variant difficult to diagnose which should not be misdiagnosed as adenocarcinomas. The goblet cell carcinoid is classified, in the 2010 classification, according to the scheme for carcinoma [5]. Those tumours contain both neuroendocrine cells and cells with intracytoplasmic mucus similar to goblet cells, with a predominantly submucosal growth in a concentric manner. Goblet cell carcinoids are more aggressive than conventional NETs. Most conventional NETs of the appendix are less than 2 cm (>80 %) and infiltrate the appendix wall [38]. Poorly differentiated tumours are exceptional. The risk of metastases increases with size and deep invasion of the appendix. Moertel et al. reported metastatic disease in 31 % of patients with tumours > 2 cm [32]. Patients with tumours >2 cm should be treated by right hemicolectomy [34, 38]. The invasion of the mesoappendix is included in the ENETS TNM proposal (T3 if >3 mm; see Table 6.3), but not in the 2009 UICC TNM. Its prognostic impact is still controversial, but McGillivray et al. reviewed 414 appendiceal NETs and found that the mesoappendiceal invasion was related to metastatic disease [31].

Conclusion

Despite a certain degree of morphological and immunohistochemical homogeneity of well-differentiated digestive NETs, these tumours are heterogeneous regarding their presentation (functional status, presence of inherited syndromes), prognosis and staging according to their location in the digestive system. Because of frequent modifications in nomenclature, grading and staging, it is important to identify the minimal data that should be reported in all pathology reports in order to ensure optimal reproducible and uniform data to aid in both clinical management and stratification in therapeutic trials (Table 6.4) [40]. The use of the 2009 international UICC TNM is recommended, but in certain locations criteria of the ENETS classification should be added (e.g. the invasion of mesoappendix). The histological differentiation should clearly appear in order to avoid problems with the "carcinoma" category, different in the WHO 2000 and 2010 classification. It is important to give the exact value of proliferation rate to make comparison possible and to better stratify patient groups, since several data suggest to change the cut-off between G1 and G2. It is now clear that Ki-67 index is not optional and is very important especially in biopsy specimens, which do not allow to properly assess the mitotic counts [24]. It is important to standardise pathological diagnosis, grading and staging of NETs in the clinical management of patients and also to give a uniform basis for research trials.

Table 6.4 Indications for minimal data for the pathological report of neuroendocrine tumours

Diagnostic
Morphology, differentiation (well or poorly differentiated)
Immunohistochemistry (chromogranin A and synaptophysin expression)
Assessment of hormone expression upon specific clinical request
Classification
WHO 2010
Histological grade
Grade G1, G2 or G3
In addition, give the exact value of mitotic and/or Ki-67 index
In biopsy samples, use the Ki-67 index
pTNM
Size, exact site
Distance from resection margin (for resection specimen)
AJCC-UICC TNM 7th edition, 2009 (one may add ENETS/TNM in certain locations)

Adapted from Bosman et al. [5], Rindi et al. [40] and Klöppel et al. [24]

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Circulating Biomarkers in Neuroendocrine Tumours

7

Kjell Öberg

7.1 Background

Neuroendocrine tumours (NETs) constitute a heterogeneous group of neoplasm which originates from neuroendocrine cells of the so-called diffuse endocrine cell system. They may synthesise, store and secrete peptides and amines that can cause distinct clinical symptoms. On the other hand, many are clinically silent until late presentation with mass effects. The clinical presentation of NETs varies according to the site and size of the primary tumour, the presence or absence of metastatic spread, whether the associated features compatible with the hereditary syndrome exist or not, whether the tumour is functional or not, and if so what syndrome is present [1, 2]. Early in the disease process, patients present with vague symptoms, associated with various hormonal symptoms that often are misdiagnosed for many years [3]. In such a situation, early diagnosis depends on syndromic recognition by physicians, and it is achieved by appropriate laboratory testing followed later by imaging studies and tissue diagnosis. A common exception to this rule occurs with midgut carcinoid syndrome-producing tumours that often present symptoms late in the disease course. Non-functioning tumours also commonly present late in the disease course with imageable metastases identified per chance or when studies are ordered for the symptoms attributable to tumour growth rather than hormone production [4].

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NETs derive from neuroendocrine cells (highly specialised cells with both neural and endocrine characteristics) which upon specific stimulation secrete hormones regulating various functions [5]. The various cell types of the neuroendocrine cell system can secrete products such as peptides and biogenic amines that are tumour specific and may serve as markers for the diagnosis and follow-up of treatment. Some tumour markers may have prognostic implications. Others might be predictive for treatment response [5–7]. Neuroendocrine cells can be confined to a specific gland, such as the adrenal medulla (pheochromocytomas) or the thyroid (medullary thyroid carcinoma). These tumours will not be discussed in this review which will concentrate on biomarkers for gastroenteropancreatic (GEP) NETs.

The gastrointestinal tract including pancreas is the largest endocrine organ in the body with 15 different neuroendocrine cell types identified, and each of these has specific hormone products and regulatory functions [5]. Today we recognise more than 30 gut peptide hormone genes, which express more than 100 bioactive peptides. The amine- and peptide-producing cells utilise endocrine, paracrine, neurocrine or autocrine regulatory mechanisms [5]. The cytoplasm of the neuroendocrine cell is occupied by a large number of secretory granules of varying electron densities, size and shape and is the storage site of secretory products (serotonin, chromogranin A and so forth). Upon specific stimulation, granules are translocated to the cell membrane and their content released by exocytosis [5]. Peptide pro-hormones are synthesised in the rough endoplasmic reticulum (RER), together with chromogranin A (CgA) and other granular proteins. Chromogranins may serve as substrates for proteolytic enzymes and thereby modulate this process. The products are then transported to the Golgi apparatus and packaged into secretory granules (large dense-core granules). Amines might be stored in small synaptic vesicles [8–10]. The presence of these secretory products in the serum can be exploited diagnostically as tumour markers for neuroendocrine tumours and they are divided into general markers and specific markers, depending on the cell type involved [3, 5]. This chapter will concentrate on circulating biomarkers divided into diagnostic markers, general and specific markers evaluating response to treatment as well as prognosis and finally new potential markers.

7.1.1 General Biomarkers (Table 7.1)

The chromogranin family and pancreatic polypeptide, neuron-specific enolase (NSE) and alpha HCG constitute general markers for diagnosis and follow-up of patients with NETs.

Table 7.1 General biomarkers

Biomarkers	Specificity
CgA, CgB, pancreastatin	H
PP, NSE, neurokinin, neurotensin	I
HCG-α, HCG-β	L

7.1.1.1 **Chromogranins** (Table 7.2)

The granins constitute a whole family of glycoproteins of which CgA and CgB are the most clinically interesting. These proteins are 27–100 kDa in size and contain 10% acidic (glutamic or aspartic acid) residues, as well as multiple single and dibasic amino acid residues [9–11]. All of the granins are found as major components of the soluble core of dense-core secretory granules in NE cells and are secreted from these cells in a physiologically regulated manner. The granins are major constituents of large dense-core secretory vesicles and are co-secreted with peptide hormones and mines [9–11]. Granins are found in NE cells throughout the body but also located in the neuronal cells in the central and peripheral nerve system [12]. In adrenal chromaffin cells, CgA and CgB are present in about equal amount, but in thyroid cells and entero-chromaffin cells (EC cells) in the stomach contain mostly CgA and very little CgB [9–11]. CgA, a 439-amino acid glycoprotein, has multiple pairs of basic amino acids distributed along its length, these being more abundant in the carboxyl terminal part of the molecule (Fig. 7.1) [9–11]. CgB has a similar chemical structure being glycoprotein but otherwise different from CgA [13, 14]. The precise function of CgA remains unknown but is thought to be involved in packaging and processing of neuropeptide precursor and peptide hormones [10, 11]. It may also play a role in the organisation of the secretory granule matrix. Moreover, CgA has diverse physiologic interaction. CgA or its splice products are inhibitors of catecholamine, insulin and leptin, having a role in carbohydrate and lipid metabolism; moreover, it inhibits parathyroid hormone secretion. It is also known that CgA increases glucagon and amylase release [15]. In addition to its effect on endocrine organs, CgA also regulates reproductive functions and has a role also in the regulation of cardiovascular functions. CgA elevation has been reported in essential hypertension and in chronic heart failure correlating with the grade of hypertension and cardiac dysfunction, respectively. A role of CgA in the regulation of inflammatory response has been described. CgA is found elevated in various inflammatory conditions such as rheumatoid arthritis, systemic lupus erythematosus, chronic obstructive pulmonary disease as well as inflammatory bowel disease. CgA in these conditions positively correlates with inflammatory markers such as C-reactive protein and procalcitonin. It is suggested that CgA participates in a negative feedback that limits the activation of endothelial cells [16, 17].

Tumours of neuroendocrine origin usually present with increased plasma or serum levels of CgA and sometimes also CgB. Plasma CgA levels may be elevated in a variety of NETs including pheochromocytomas, paragangliomas, pNETs, small

Table 7.2 The chromogranin family

Chromogranin A (CgA)
Chromogranin B (CgB)
Secretogranin II (CgC)
Secretogranin III (1B1075)
Secretogranin IV (HISL-19)
Secretogranin V (7B2)
Secretogranin VI (NESP55)

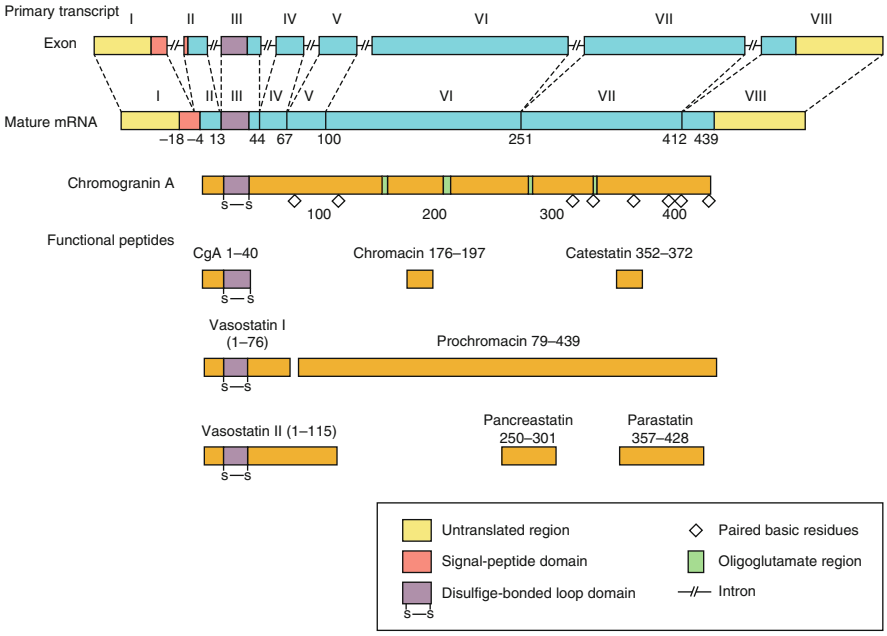

Fig. 7.1 Molecular structure of chromogranin A [10]

intestinal NETs, medullary thyroid carcinomas, parathyroid and pituitary adenomas and also in a proportion of patients with small-cell lung cancer [4, 8, 14, 18–21]. The highest CgA levels have been found in patients with metastatic small intestinal NETs (carcinoid) and also some pNETs. Both tumour burden and the secretory activity reflect circulating CgA levels. The sensitivity and specificity vary between 60 and 100 % and 70 and 100 %, respectively, for different types of NETs, the highest values being for small intestinal NETs [7, 14, 21–24]. CgA has been shown to be an independent prognostic factor for small intestinal NETs, because it correlates not only with the tumour burden, but also with the biological activity in the tumours (Fig. 7.2). Most functioning and non-functioning NETs present increased circulating levels of CgA. It is also noticed that CgA is more frequently elevated in well-differentiated tumours (G1, G2), compared to poorly differentiated tumours, suggesting a loss of CgA expression in poorly differentiated neuroendocrine carcinomas [25] (Fig. 7.3). Effective treatment is often associated with decrease in CgA values, and the CgA correlates with tumour burden and recurrence [4, 6, 26]. However, in patients treated with somatostatin analogues, there is no correlation between circulating CgA levels and tumour mass [21, 27]. The reason for this is that somatostatin analogues are able to block the production and release of CgA in addition to also affecting tumour burden. Recent data are indicating that early response in CgA might indicate an antitumour effect by treatment with targeted agents (everolimus, mTOR inhibitor) [28, 29].

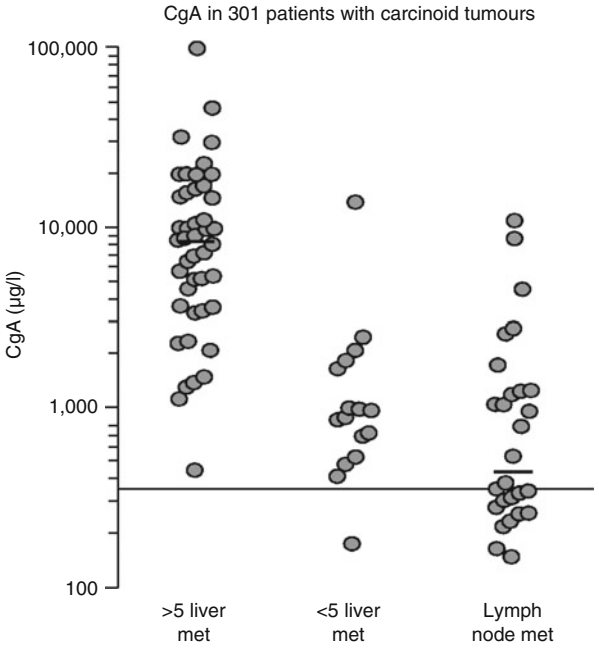

Fig. 7.2 Chromogranin A levels in patients with malignant carcinoid tumours in relation to tumour mass [73]

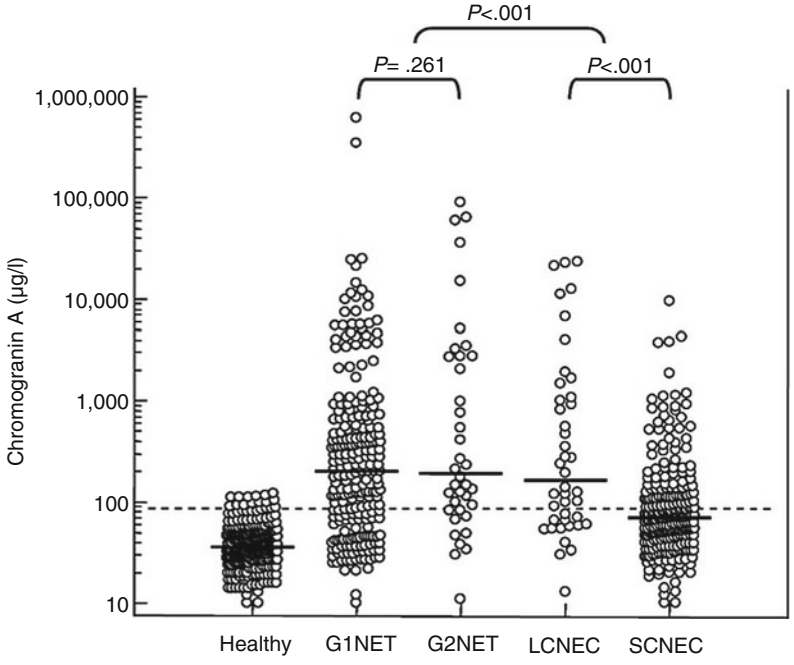

Fig. 7.3 Chromogranin A levels in patients with tumours with different histological grading. Note that G1 and G2 tumours present higher levels than G3 NEC tumours (LCNEC, SCNEC)

Elevated CgA can also occur in normal individuals and in patients with non-NETs although the levels are usually lower than in patients with NETs. Levels of CgA secretion vary on day-to-day bases and healthy subjects as well as in individuals with NETs. The mean day today variation of CgA is approximately 25 %. Food intake may increase CgA levels; therefore, CgA should be measured in fasting patients to ensure standardisation of the results [30, 31]. It is reported that heart disease and long-term exercise as well as extreme physical stress can elevate CgA. Increased serum or plasma levels of CgA have also been demonstrated in patients with other malignancies including colon, lung, breast, liver and prostate cancer [32]. Other conditions with elevation of CgA are impaired renal function [33], Parkinson disease, untreated hypertension and pregnancy [34], steroid treatment or glucocorticoid excess [35] and chronic atrophic gastritis (type A) and finally treatment with proton-pump inhibitors [36]. Other conditions are inflammatory bowel disease, liver disease and hyperparathyroidism [37]. In renal failure CgA increases due to a decreased plasma clearance, and in autoimmune chronic atrophic gastritis, elevated circulating CgA levels are caused by chronic hypergastrinaemia and stimulation of the ECL cell which proliferate and secrete CgA. A major cause of elevated CgA levels in non-NET patients are the widespread use of PPIs and other suppressive medications. A normalisation of CgA levels occurs by withdrawal of PPI in 1–2 weeks [38].

There is no universal standard calibration for serum or plasma CgA assays. The most useful immunoassays for tumour detection have been those that measure the whole CgA molecule. Assays measuring specifically defined parts of the molecule (pancreastatin) usually have lower sensitivity in detecting patients with NETs. Several commercial available immunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs) have been developed for the measurement of circulating CgA concentrations. Moreover, many diagnostic laboratories use in-house assays. It should be noted however that there is a fair amount of variability between assays with sensitivities varying from 67 to 93 % depending on which assay is used. Concerning plasma and serum measurement, a strong positive linear relationship has been reported between plasma and serum CgA values indicating that CgA measurement can be undertaken in both sample types. Measurements of plasma or serum CgA are a valuable tool for both diagnostic and follow-up of patients with NETs [19, 39, 40]. However, there is an unmet need for standardisation of various assays that should be undertaken in a near future.

7.1.1.2 Other Members of the Granin Family (Fig. 7.4)

Chromogranin B (CgB) is the second most abundant member of the chromogranin family. Like CgA it is a strong acid protein containing approximately 25 % acidic amino acid residues. It has 14 dibasic cleavage points but has been less well studied than CgA. Unlike CgA, CgB does not seem to have increased concentration in patients with renal failure, in patients with atrophic gastritis or those receiving acid-suppressing therapy [21]. In NETs where CgA is not found, CgB may be increased. Such patients include those with MEN-1 and those with tumours in the duodenum or rectum. In addition CgB is a major granin of the human adrenal medulla and may

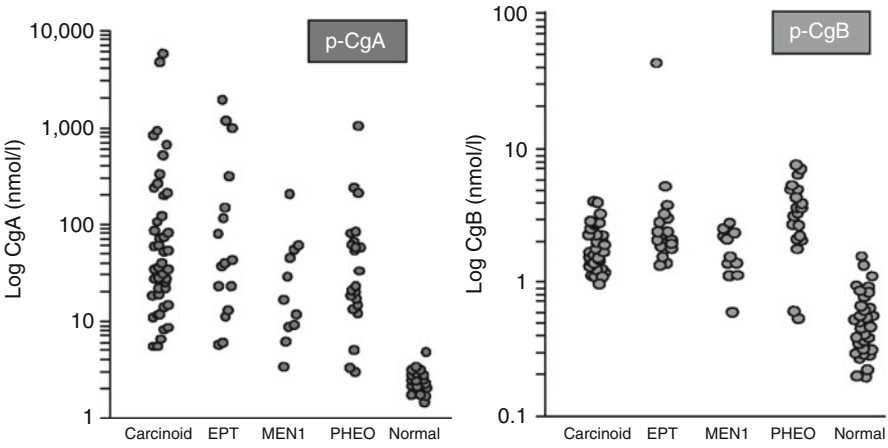

Fig. 7.4 Comparison between circulating levels of chromogranin A and chromogranin B in patients with various NETs. *EPT* endocrine pancreatic tumours, *MEN1* multiple endocrine neoplasia type 1, *PHEO* pheochromocytomas [73]

be a more sensitive marker pheochromocytoma [41, 42]. At the moment there is one commercial assay which measures CgB specifically (Euro Diagnostica).

7.1.2 Pancreastatin

CgA is processed by an endoprotease, a pro-hormone convertase 1 (PC1), into smaller peptides such as pancreastatin (PST), a 49-amino acid peptide, that inhibits insulin secretion, somatostatin release, exocrine pancreatic secretion and gastric acid secretion [43–45]. PST is found in human stomach and colon extracts and in liver metastases of gastrinoma. PST has been found to be significantly increased in patients with NETs metastasised to the liver, and the concentrations are proportional to the number of hepatic metastases [41]. PST is not increased in patients with gastric achlorhydria or hypochlorhydria. It may be a very early biomarker to liver tumour activity even when CgA is normal [42].

Recently it has been suggested that analyses of CgA gene expression can be examined by QRT PCR. It has been done in tumour tissue with higher sensitivity than just analysing protein expression that can also be applied in blood. However, data are still lacking [46].

7.1.3 Pancreatic Polypeptide

Pancreatic polypeptide (PP), a 36-amino acid linear peptide, is another general tumour marker secreted by PP cells, which are located in the gut mucosa and pancreas. The uncinate process of the pancreas is particularly rich in PP cells [1].

Other members of the same family are peptide tyrosine-tyrosine (PYY) and neuropeptide Y (NPY). The release of PP is caused by ingestion of meals, particularly those containing protein [41]. It has been found to be elevated in NETs of the gastrointestinal tract and pancreas, with a sensitivity of about 50–80 % [7]. However, there are a lot of clinical conditions where falsely elevated levels are noted, such as diarrhoea, laxative abuse, high age, inflammatory processes in the gut and chronic renal disease [7, 20]. A combination of CgA and PP has been useful in patients with non-functional pNETs, with a sensitivity of almost 95 %. A specific meal stimulatory test (mixed meal) has been particularly useful in patients with multiple endocrine neoplasia type 1 (MEN-1) and early detects pancreatic tumours [21].

7.1.4 Neuron-Specific Enolase (NSE)

NSE is the neuron-specific isomer of the glycolytic enzyme 2-phospho-D-glycerate hydroxylase or enolase. This isomer is present in the cytoplasm of neurons and neuroendocrine cells and can serve as a circulating marker for NETs. NSE is most frequent elevating in patients with small-cell lung cancer but has also been found to be elevated in 30–50 % patients with intestinal NETs, medullary thyroid carcinoma, pNETs and pheochromocytomas [25, 42]. In patients with poorly differentiated NETs, NSE might be elevated despite normal CgA. NSE is also roughly correlated with tumour size although specificity is lower than that of CgA [25]. The combination of CgA and NSE has a higher sensitivity than either parameter separately [7, 14, 21]. Recently an early response in NSE is related to therapeutic response with an mTOR inhibitor (everolimus) [29].

7.1.5 Human Chorionic Gonadotropin (HCG)

Human chorionic gonadotropin (HCG), a glycoprotein hormone consisting of alpha and beta subunits, can be ectopically produced by neoplasms. Assays for the intact glycoprotein and its alpha and beta subunits have been used as markers to screen for a number of different tumours including NETs. In particular, HCG alpha and beta subunits have been found to be increased in patients with malignant pNETs [47, 48].

7.1.6 Other General Tumour Markers

Pro-gastrin-releasing peptide (Pro-GRP) is a promising tumour marker for small-cell lung cancer. Pro-GRP is the precursor of the neuropeptide gastrin-releasing peptide (GRP). Its production is increased in small-cell lung cancer [49]. It has been described that levels are elevated in patients with NETs, particularly those

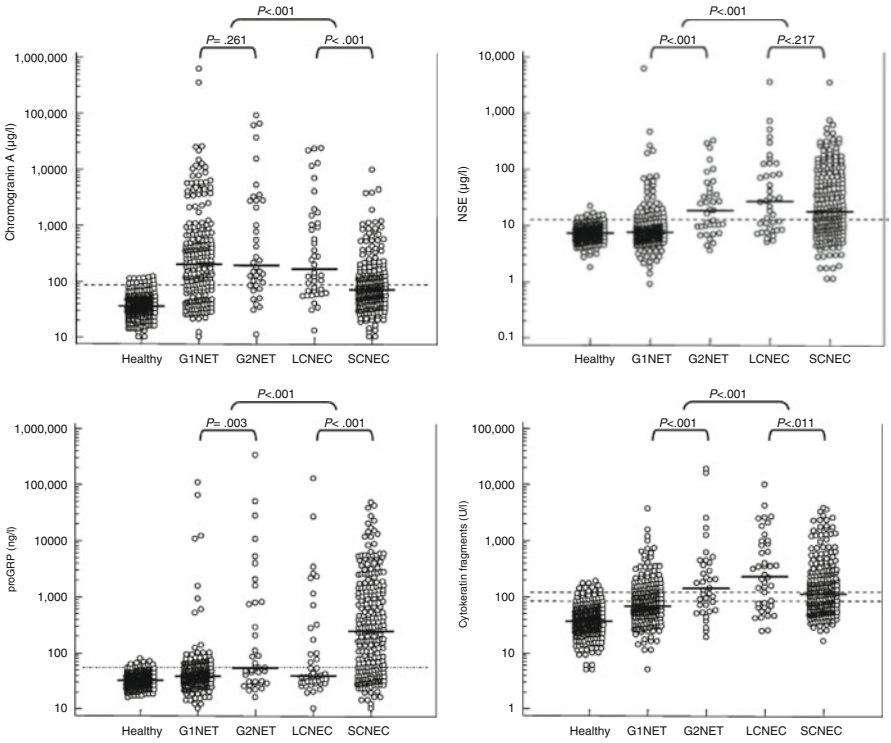

Fig. 7.5 Comparison of different biochemical markers in a material of NETs with different histological grade [25]

with high-grade tumours (G3) [25]. Cytokeratin fragments (CKfr) are sensitive indicators of tumour cell turnover and are especially useful in the management of patients with malignancies of epithelial origin [50]. CKfr is associated with angiogenesis factors which may play a role in NETs [51]. However, limited data are so far presented. The assay currently used is determining CKfr 8, 18 and 19 fragments in serum [25]. In an attempt to look at the choice of general tumour markers in patients with NETs in relation to histological grade, Korse and co-workers have analysed CgA neuron-specific enolase (NSE) pro-GRP and CKfr [25]. The largest area under the ROC curve was for CgA in patients with G1 and G2 NETs. Pro-GRP showed the highest sensitivity (73 %) and 95 % specificity in patients with small-cell NETs (G3). In a multivariate survival analysis, only CKfr was associated with survival ($p < 0.0001$) for patients with well-differentiated NETs (G1 and G2 NETs). In patients with poorly differentiated NEC (G3), both CKfr and NSE were associated with survival ($p < 0.001$ and $p = 0.003$, respectively). The authors recommend in patients with well-differentiated NETs (G1, G2) the use of CgA and CKfr, whilst in patients with NEC G3, pro-GRP and CKfr and NSE are preferred (Fig. 7.5).

7.2 Specific Markers and Related Tumours (Table 7.3)

In addition to general markers, there are biomarkers specific for the different functioning NETs. The various tumours and secretarial products are summarised in Table 7.3.

Carcinoid tumours have traditionally been divided according to the presumed embryological origin of the precursor cell thus divided into the foregut (lung, thymus, stomach and duodenum), midgut (jejunum, ileum, appendix and caecum) and hindgut (distal colon and rectum) carcinoids and together with pNETs collectively considered as gastroenteropancreatic NETs [4, 7, 14, 20]. This old classification has now been abandoned, and a new classification established by WHO is now in clinical practice. Well-differentiated endocrine tumours, WHO G1 tumours, are characterised by a low grade of proliferation, with Ki-67 < 2 %. Well-differentiated endocrine tumours, WHO G2, Ki-67 between 3 and 20 % and poorly differentiated endocrine carcinoma with high grade of proliferation Ki-67 > 20 % (NEC G3) [2, 51–53]. Besides this classification NETs are also divided into functional tumours, which produce a clinical hormone-related syndrome, versus non-functional tumours which might present symptoms related to tumour growth [4, 20].

7.2.1 Bronchial and Thymic NETs

Bronchial NETs are essentially divided into typical, atypical, large-cell and small-cell lung NETs. This paragraph will concentrate on typical and atypical lung NETs. These tumours can produce almost any of the described hormones or amines in the body. The carcinoid syndrome including flushing, diarrhoea, cardiac fibrosis, wheezing and dyspnoea occurs in lung NETs but is usually a atypical variant and not quite similar to the syndrome in the small intestine. The syndrome is rarely related to production of serotonin and tachykinins but because of the lack of enzymes converting hydroxytryptophan (5-HTP) to serotonin patients show a

Table 7.3 Site-specific markers

Site	Biomarkers	Specificity
Thymus	ACTH	I
Lung	ACTH, ADH, serotonin, 5-HIAA	I
	Histamine, GRP, GHRH, VIP, PTHrp	L
Stomach	Histamine, gastrin	I
	Ghrelin	L
Pancreas	Gastrin, insulin, proinsulin, glucagon, somatostatin	H
	C-peptide, neurotensin, VIP, PTHrp, calcitonin	L
Duodenum	Somatostatin, gastrin	H
Ileum	Serotonin, 5-HIAA	H
	NKA, neuropeptide K, SP	I
Colorectum	Peptide YY, somatostatin	I

H high, *I* intermediate, *L* low

prolonged flushing, headache, palpitation and lacrimation as well as broncho-constriction [4, 7]. Lung NETs may also produce Cushing syndrome due to ectopic adrenocorticotrophic hormone (ACTH) production or acromegaly due to ectopic growth hormone-releasing hormone (GHRH) secretion [4, 7, 14]. Besides these specific biomarkers, these tumours also produce CgA as well as prepro-gastrin and NSE. Thymic carcinoids are usually of the non-functioning type, but about 30 % of the patients can present the Cushing syndrome with increased secretion of ACTH or corticotrophin-releasing factor (CRF) [54–56].

7.2.2 Gastro-duodenal NETs

Duodenal NETs (carcinoids) may secret gastrin and cause the typical Zollinger-Ellison syndrome with recurrent ulcers, diarrhoea and abdominal pain [20, 21]. The duodenal gastrinoma may be part of MEN-1 syndrome. Somatostatin-producing NETs are also found in the duodenum. These are usually not presenting any hormone-related symptoms, but histologically they present with psammoma bodies rather than the presence of somatostatinoma syndrome. Some of these tumours are part of the von Hippel-Lindau disease (VHL) [1–3]. Gastric NETs are divided into ECLoma type 1, 2 and 3 and are derived from ECL cells in the corpus fundus region of the stomach. Another type of gastric NETs is G-cell tumours of the antrum. Most antral carcinoids are producing not only gastrin but also ghrelin and sometimes serotonin [57, 58]. The type 1 ECL tumours are associated with chronic atrophic gastritis and present high levels of CgA and sometimes also histamine production and the atypical carcinoid syndrome. Type 2 tumours are associated with MEN-1, Zollinger-Ellison syndrome and hypertrophic gastropathy. Type 3 tumours are sporadic and are not associated with any distinctive gastric pathology. Type 1 and 2 tumours are sharing common hypergastrinaemia, whereas type 3 tumours are independent of any overt hormonal imbalance. All three subtypes of ECL tumours present elevated plasma chromogranin A levels [59–61].

7.2.3 Small Intestinal Neuroendocrine Tumours (Midgut Carcinoids)

Carcinoid syndrome is a typical clinical presentation of metastatic ileal carcinoid occurring in about 18 % of patients and is characterised by flushing, diarrhoea and abdominal pain [3, 4]. Less frequent events are carcinoid heart disease as well as broncho-constriction and pellagra-like skin changes. The syndrome is related to massive release of serotonin which is no longer metabolised in the liver. Other substances involved in the carcinoid syndrome might be tachykinins (neuropeptide K, substance B, NKA), prostaglandins and bradykinins [6, 7, 14]. Patients with carcinoid syndrome present with increased levels in the urine of the breakdown product of serotonin 5-HIAA, with levels between 100 and 3,000 μmol/24 h (ref. <50 μmol/24 h). The overall sensitivity and specificity of u-5-HIAA in the presence of carcinoid syndrome is 70 and 90 %, respectively [4, 7, 14, 62]. Therefore, this marker is the most frequently performed assay in the clinical setting of the

carcinoid syndrome. High-pressure liquid chromatography (HPLC) with electrochemical detection is currently recommended to measure urinary 5-HIAA. In some laboratories automated assays using mass spectrometry are available. It has been recently suggested that plasma 5-HIAA might be a valid alternative to the collection of u-5-HIAA [63]. Some studies have demonstrated higher sensitivity (100 %) for the measurement of platelet poor plasma serotonin compared with u-5-HIAA in patients with small intestinal NETs [14]. However, there are large fluctuations in the plasma levels of serotonin depending upon food ingestion, time of the day, stress and sampling procedures. In addition, certain malabsorptive conditions including Whipple's disease and celiac sprue may be associated with elevated urine 5-HIAA levels. It is also important that the patient gets strict diet instructions, avoiding plums, pineapples, bananas, eggplants, tomatoes, avocados and walnuts during the collection of urine. Certain medications made also interfere with the assay: paracetamol, fluorouracil, methysergide and naproxen. On the contrary levodopa, aspirin, ACTH, methyldopa and phenothiazine may give false-negative results. Somatostatin analogues are known to decrease levels of 5-HIAA. Serotonin plays a key role in the development of peritoneal and cardiac fibrosis with activation of the 5-HT2B receptor and the cascade of connected tissue growth factors. Reduction in plasma serotonin levels correlated with decreased incidence of carcinoid heart disease. Moreover, u-5-HIAA excretion also correlates with the severity of carcinoid heart disease and prognosis in patients with carcinoid syndrome. In the clinical practice, it is useful to have two consecutive 24 h collection of urine for measurements of 5-HIAA and take the mean value, but there is a strong argument to change to measurements of plasma 5-HIAA.

7.2.4 Colorectal NETs

Colorectal NETs generally present in the clinic as non-functional tumours. Patients develop abdominal pain, gastrointestinal bleeding and an enlarged liver with tumour growth. In recent years more hindgut carcinoids have been discovered at an earlier un-symptomatic stage during routine screening for colorectal cancer. These tumours might secret pancreatic polypeptide, somatostatin, PYY and CgA. Single tumours might also secrete serotonin.

7.2.5 Pancreatic NETs

Pancreatic NETs present with a wide variety of well-known clinical syndromes such as Zollinger-Ellison syndrome (gastrinoma), characterised by abdominal pain, diarrhoea and peptic ulceration. The insulinoma syndrome (characterised by hypoglycaemia-induced neuroglycopenic and sympathetic overdrive symptoms), the glucagonoma syndrome (characterised by glucose intolerance and a specific rash called migratory necrolytic erythema and thromboembolism) the Verner-Morrison or VIPoma syndrome (characterised by watery diarrhoea, hypokalemia and

achlorhydria), the somatostatinoma syndrome including diarrhoea, hyperglycaemia and cholelithiasis [4, 20]. In each case identification of the specific elevated plasma level of the syndromic product may be useful. In some situations provocative testing may be useful but are used less and less because of more sensitive immunoassays during the last years [4, 7, 20]. For patients with the Zollinger-Ellison syndromes, secretin testing might be an alternative for patients with insulinomas, 24–48 h fasting, measuring insulin, blood glucose, proinsulin and C-peptide [20]. The standard test for patients with gastrinoma is plasma or serum gastrin and, for patients with insulin-producing tumour, blood glucose combined with insulin, proinsulin and C-peptide. For patients with glucagonoma plasma glucagon and for patients with Verner-Morrison syndrome, VIP should be analysed. The rare cases with somatostatinomas can be picked up by measurement of plasma somatostatin [14, 20]. About 50–60 % of patients present the so-called non-functioning tumours which mean that they have no hormone-related symptoms. Some of them include somatostatin-producing tumours as well as pancreatic polypeptide (pp)-producing tumours and neurotensin-producing tumours. These tumours are picked up by measurements of pp somatostatin and neurotensin, but in particular it is a general tumour marker such as CgA or CgB that will be more helpful. Some tumours might also produce ghrelin. A proportion of pancreatic NETs might also produce ACTH or CRF giving rise to Cushing syndrome. Calcitonin might also be produced by pNETs and sometimes giving diarrhoea but are mostly part of the group of non-functioning NETs. Another rare type of pNET is the PTHrp-producing tumours giving hypercalcaemia and similar symptoms like hyperparathyroidism [7, 14, 20].

7.3 New Potential Biomarkers in NETs

7.3.1 MicroRNAs in NET

MicroRNAs (miRNAs) are a class of natural occurring small non-coding RNA molecules. Mature microRNAs consist of 19–25 nucleotides and are derived from hairpin precursor molecules of 17–100 nucleotides. As 50 % of human microRNAs are localised in fragile chromosomal regions which might exhibit DNA amplification deletions or translocations during tumour development, their expression is frequently deregulated in cancer. Therefore, microRNAs have important roles in deregulation of gene expression in cancer. Today studies on solid cancers (e.g., ovarian, lung, breast and colorectal) report that microRNAs are involved in the regulation of different cellular processes, such as apoptosis, cell proliferation, epithelial to mesenchymal transition and metastases. In blood microRNAs seem to be highly stable because most of them are included in apoptotic bodies, micro-vesicles or exozones and withstand known microRNA degradation factors [64–66].

In a recent paper, Li and co-workers are describing microRNA profiling of well-differentiated small intestinal NETs [67]. They identify nine microRNAs of importance; five were upregulated during tumour progression, whereas four were downregulated (see Fig. 7.6). Future studies will further illuminate the potential

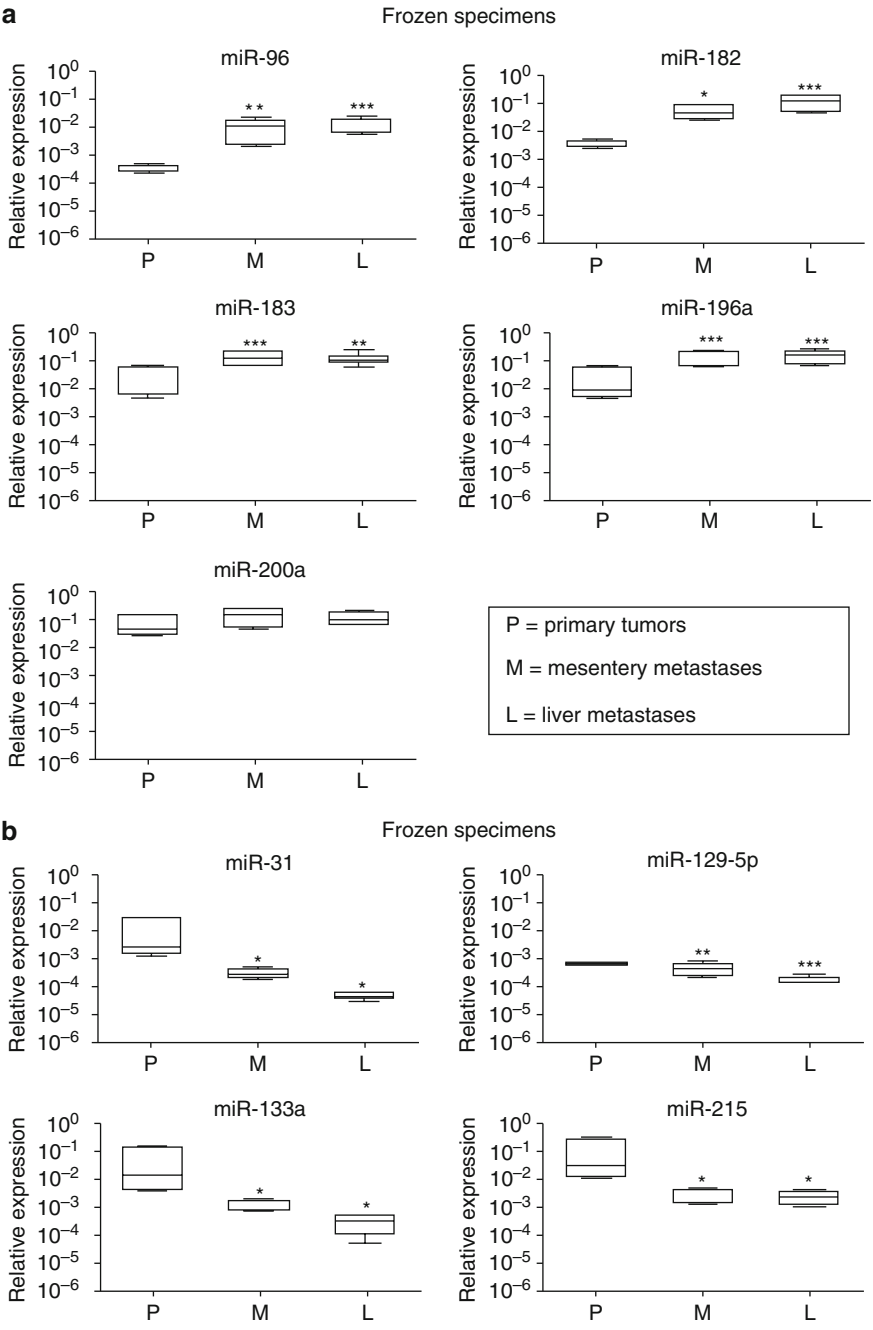

Fig. 7.6 Quantitative real-time PCR analysis validating the expression of nine selected microRNAs in tumour specimens from patients with midgut carcinoid tumours. Panel (**a**) is indicating upregulated microRNA expression in metastatic disease comparing with primary tumours. Panel (**b**) is showing downregulated microRNA expression in metastatic disease compared with primary tumours. Significance was calculated by one-way analysis of variance (ANOVA) followed by Bonferroni test. $*P<0.05$; $**P<0.01$; $***P<0.001$ [67]

value of microRNAs as diagnostic and therapeutic tools. A previous study has indicated that the downregulation of microRNA-133a was related to tumour progression [68]. The recent paper also demonstrated low level of microRNA-133a in lymph node and liver metastases compared to primary tumour and normal EC cells [67].

7.3.2 Circulating Tumour Cells (CTCs)

Recent technological advances have enabled circulating tumour cells (CTCs) in numeration and characterisation by different methods. The CellSearch® platform an automated immuno-magnetic enrichment and staining system has been utilised to detect CTCs with high sensitivity, specificity and reproducibility. Large studies have reported that a number of CTCs in patients with metastatic breast cancer before commencing a new therapy are an independent predictor of progression-free and overall survival with similar results reported in metastatic colorectal and prostate cancer [69, 70]. The CellSearch® platform requires EpCAM epithelia cell adhesion molecule expression to isolate CTCs and enables the CellSearch® platform to enrich CTCs via immuno-magnetic separation with iron particles coupled to EpCAM antibodies [71]. This platform was used in a series of patients with NETs [72]. Forty-three per cent of midgut and 21 % of pancreatic NETs had CTCs detected in the blood. The absence of CTCs was strongly associated with stable disease. There was a moderate correlation between CTC levels and urinary 5-hydroxyindolacetic acid and between CTC levels and burden of liver metastases. Measurements of CTC have to be explored in a larger material to precisely delineate the value in clinical practice.

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Cross-Sectional Imaging of Neuroendocrine Tumours

8

Shema Hameed, Mark Wills, and Andrea Rockall

8.1 Introduction

Neuroendocrine tumours (NETs) may arise from the gastrointestinal tract and pancreas (collectively known as gastroenteropancreatic neuroendocrine tumours, GEP-NETs) and from neuroendocrine cells scattered in other tissues. Non-functioning tumours, in which there is no recognisable clinical syndrome, often present late with symptoms due to mass effect, although small non-functioning lesions may be detected when screening family groups with genetic disorders associated with NETs. Functioning tumours usually present relatively early due to clinical symptoms but may be a challenge to localise as they are often small. The malignant potential of NETs varies.

Surgery is often the only form of curative treatment, and imaging assists in selecting patients for surgery and planning the procedure. Imaging is primarily directed at accurate localisation and staging of the tumour. Preoperative localisation increases the chances of successful surgical resection and reduces the likelihood of complications. Cross-sectional imaging is also valuable for monitoring treatment response and in the detection of recurrent or metastatic disease. The imaging work-up for NETs does vary depending on local availability of particular techniques and expertise. This chapter will describe the cross-sectional imaging techniques and appearances of NETs.

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8.2 Medullary Thyroid Carcinoma

These tumours arise from the parafollicular C-cells of the thyroid gland and secrete calcitonin. They account for 3.5–10 % of all thyroid tumours, with 25 % occurring in association with a genetic syndrome, including familial autosomal dominant medullary thyroid cancer (MTC) and MEN IIA and B syndromes [1]. The commonest presentation is with a solitary thyroid nodule or presumed multi-nodular goitre, unless metastatic disease is present by which time the patient may experience flushing and diarrhoea [2]. Tumours are often firm, encapsulated masses. Lymph node metastases are common at presentation.

8.2.1 Ultrasound (US)

Ultrasound (US) is the modality of choice for initial assessment. Lesions may appear ill-defined on US, with a spiculated border. They are typically round or ovoid, internally solid and markedly hypoechoic in comparison to surrounding thyroid tissue and strap muscles. Micro- or macrocalcifications are found in approximately half of cases [3]. Fine-needle aspiration cytology (FNAC) of suspicious nodules should be performed promptly, ideally at the time of examination to ensure rapid diagnosis. Although some studies have shown calcitonin levels to be more sensitive at diagnosing MTC than FNAC [4], hypercalcitonaemia is also associated with C-cell hyperplasia and thyroiditis [5]; therefore, biochemistry and imaging should be complimentary.

8.2.2 Computerized Tomography (CT)

CT may be performed for staging purposes. MTC appears as a well-circumscribed, heterogeneously enhancing mass. Inherited forms are likely to have multifocal disease, with numerous nodules within the thyroid gland and lymph node metastases with similar enhancement characteristics [6].

8.2.3 Magnetic Resonance Imaging (MRI)

MRI can be used to determine local invasion and detect nodal metastases. Lesions are isointense to hyperintense on T1-weighted (TIW) imaging and hyperintense on T2-weighted (T2W) imaging and show marked enhancement post-contrast administration. Although T2 hyperintensity is highly suggestive of MTC, this is not specific to MTC; diagnosis is therefore only possible with a confirmed hypercalcitonaemia [7].

8.3 Adrenal Tumours

8.3.1 Adrenocortical Carcinoma

These rare but aggressive tumours arise from the adrenal cortex and are more often functioning in children (85 %) than in adults (15–30 %) [8, 9]. Adrenocortical carcinomas (ACC) may secrete a variety of hormones including cortisol, androgens, oestrogens and aldosterone, often in combination [10]. Co-secretion of cortisol with androgens is highly suggestive of malignancy [11, 12]. Functioning tumours thus typically present with Cushing's syndrome, virilisation or a combination of the two, with Conn's syndrome also being a feature in children. However, the majority of tumours in adults are non-functioning and present late with pain and a large adrenal mass, ranging between 2 and 25 cm [10, 13]. Up to 30 % have metastatic disease at presentation [8, 14]. The mass may first be detected by US (Fig. 8.1a), but preoperative staging is by CT with MRI used for problem-solving.

8.3.1.1 CT

CT Imaging features suggestive of an ACC include a suprarenal mass measuring >4 cm with irregular margins, central intratumoural necrosis or haemorrhage, heterogeneous enhancement and fatty regions due to intracytoplasmic lipid, calcification (present in 30 %), invasion into adjacent structures and venous extension into the IVC and renal vein, more commonly found with right-sided lesions (Fig. 8.1) [15, 16]. Metastases to lymph nodes, lungs, liver and bone are frequently present on initial imaging.

In smaller lesions, measuring the density of adrenal lesions on both unenhanced and enhanced CT is a well-established tool that aids in differentiating benign from potentially malignant adrenal lesions. Adrenal adenomas have attenuation values of <10 Hounsfield units (HU) in 98 % of cases [17], in contrast to ACCs that will almost always have a HU of >10 on the unenhanced study. Following intravenous contrast administration, ACCs retain contrast for longer than a benign adenoma would, with an absolute percentage washout of <60 % and relative washout of <40 % at 10 min.

8.3.1.2 MRI

Lesions are typically iso- or very slightly hypointense to normal liver parenchyma on T1W images with high T1 seen in areas of haemorrhage. On T2W images lesions are usually hyperintense to liver with a heterogeneous appearance due to intratumoural cysts and haemorrhage. Lesions usually demonstrate restricted diffusion and avid enhancement throughout (Fig. 8.1d–f), with slow washout on delayed imaging; however, other enhancement patterns have been described, including peripheral mural-based enhancing nodules [18]. Chemical shift imaging (CSI), which is routinely performed to characterise adrenal nodules, typically shows no

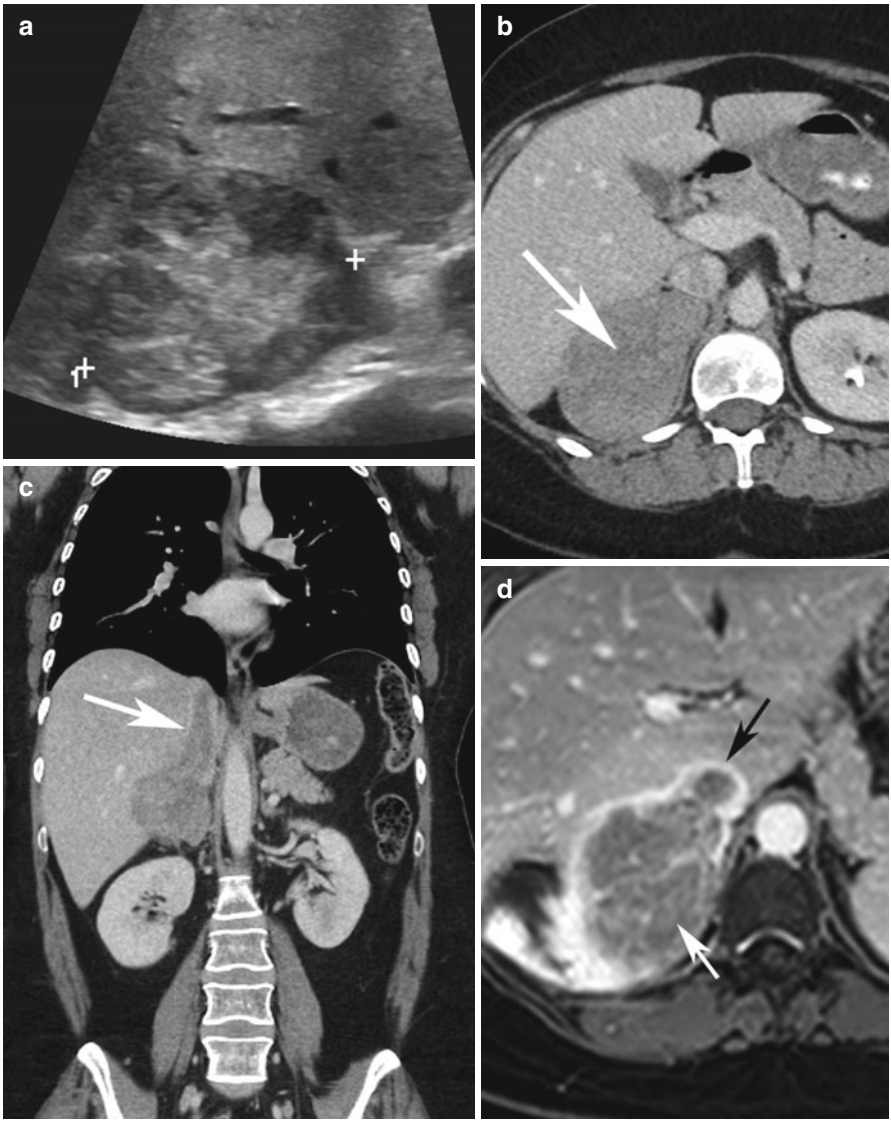


Fig. 8.1 Adrenocortical carcinoma. A 39-year-old lady presented with Cushing's syndrome and right-sided flank pain. (**a**) Ultrasound showed a large solid right suprarenal mass (between calipers) abutting the liver, with heterogeneous internal echoes. (**b**) Contrast-enhanced CT demonstrates a 7-cm-diameter heterogeneous hypodense right suprarenal lesion (*arrow*) abutting the right lobe of the liver. (**c**) Enhancing tumour extending into the IVC (*white arrow*). (**d**) Dynamic contrast-enhanced MRI showed slightly atypical gradual patchy and heterogeneous enhancement, with poor enhancement internally (*white arrow*) and avid rim enhancement (*black arrow*) after 5 minutes. Restricted diffusion is demonstrated by high signal on the b500 DWI (**e**) and low signal on the ADC map, (**f**) typical of malignancy (*arrow*). The lesion was excised via thoracotomy

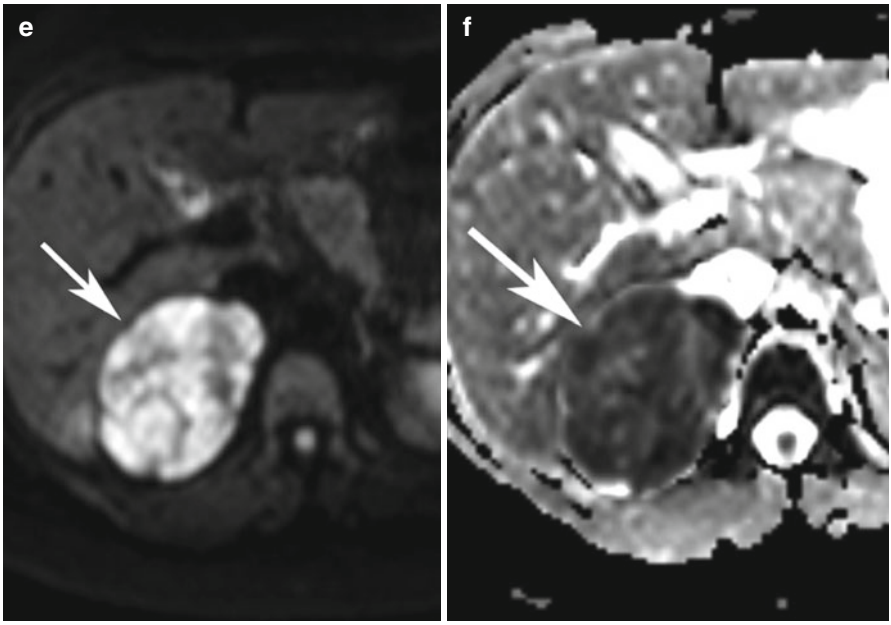


Fig. 8.1 (continued)

loss of signal in ACCs. However, in <30 % of functioning lesions, small irregular regions of signal dropout are demonstrated due to the scattered intracytoplasmic lipid found in these ACCs [18–20]. MR spectroscopy may be a potentially useful method of differentiating between lesions such as ACC, phaeochromocytoma, adrenal metastases and adrenal adenomas, with ACCs having a choline-creatine ratio of >1.20 and choline-lipid ratio of >0.38 and a 4.0–4.3 ppm/creatine ratio of >1.50 in one study [21].

8.3.2 Pheochromocytoma

These rare catecholamine-secreting tumours arise from the chromaffin cells of the adrenal medulla. Extra-adrenal pheochromocytomas can also be found in the paraspinal paraganglia, where they are termed paragangliomas, discussed separately below.

Pheochromocytomas are usually unilateral and benign; however, 10 % are bilateral and 10 % are malignant [22]. Bilateral pheochromocytomas are usually found in MEN II (50–80 %) and von Hippel-Lindau disease (40–80 %) and rarely in sporadic cases. Hypertension is the commonest presenting symptom; however, these lesions are rare and found in less than 1 % of hypertensive patients.

Pheochromocytomas often have relatively non-specific imaging features that overlap with other benign and malignant adrenal tumours on CT and MRI. Although generally large at presentation, there is marked variability in reported sizes, from 1.2 to 15 cm [23]. Lesion size may also affect the imaging characteristics of the tumour, with smaller lesions typically appearing more homogeneous and larger lesions heterogeneous [23], usually due to intratumoural haemorrhage [24].

8.3.2.1 Ultrasound

Ultrasound may locate moderately sized adrenal tumours, but it can be technically challenging and is operator dependent. The majority of lesions are completely solid, round and well-defined with a homogeneous echotexture which may be iso- or hyperechoic to liver and therefore similar to that of adjacent renal parenchyma [25]. However, approximately half of the solid tumours will appear complex, demonstrating a heterogeneous appearance with interspersed hyper- and hypoechoic regions. This correlates with macroscopic findings of intratumoural haemorrhage and necrosis respectively [25]. Cystic tumours appear as primarily anechoic lesions with posterior acoustic enhancement confirming their liquid property, correlating with old evolved haemorrhage and necrotic debris at pathology.

8.3.2.2 CT

An adrenal protocol scan (unenhanced, 60 s portal-venous phase and 15 min delayed post-contrast phase) is usually performed in order to quantify the intracellular lipid content of the lesion and to measure enhancement and washout characteristics (Fig. 8.2). Pheochromocytomas vary widely in appearance on non-contrast CT, with a range of densities from low to soft tissue. Although the majority will have an attenuation value of around 40–50 HU rarely HU may be much lower [17, 26]. Conversely higher-density lesions are found in cases of intratumoural haemorrhage. Approximately 10 % of lesions are reported to contain calcification, with nearly a quarter of symptomatic lesions being calcified. Following contrast administration, these tumours typically show avid enhancement of their solid components and may show homogeneous or heterogeneous enhancement depending on how complex their composition and relative amounts of internal haemorrhage and necrosis. Lesions show an absolute washout of less than 60 % and a relative percentage washout of less than 40 % at 15 min, or an absolute washout of less than 50 % and a relative percentage washout of less than 40 % at 10 min [27, 28]. However, variations can occur, and one study has shown pheochromocytomas to have similar washout patterns to adenomas [29].

8.3.2.3 MRI

Pheochromocytomas have been described as having a classical ‘light-bulb’ bright appearance on T2W, being brighter than fat and comparable with CSF [30]. They are typically isointense to muscle and hypointense to liver on T1W imaging. However, variations in appearance occur due to the often complex haemorrhagic and necrotic

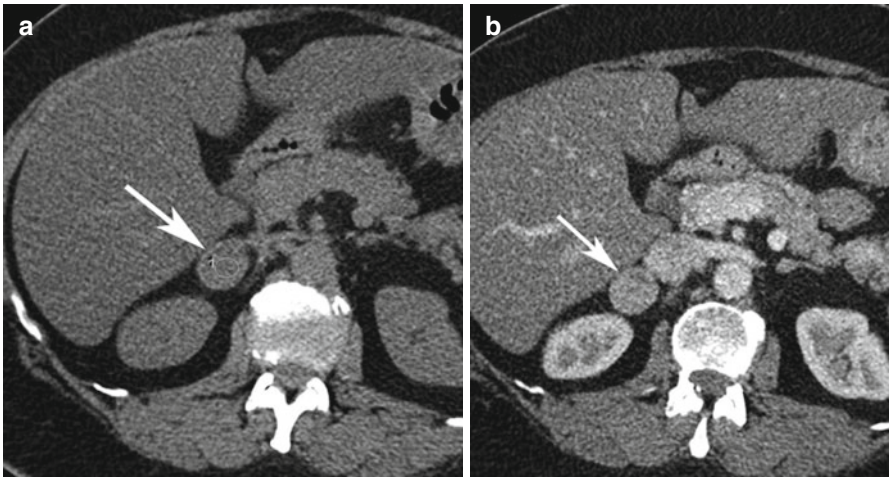


Fig. 8.2 Pheochromocytoma (adrenal protocol CT). A 58-year-old female presented with refractory high blood pressure and palpitations. (**a**) Non-contrast CT demonstrates a 2.7 cm spherical solid lesion arising from the lateral limb of the right adrenal gland with a HU of 25 and no sign of fat or calcification (*arrow*). (**b**) It enhances heterogeneously with a HU of 92 on the first phase and 60 on the delayed phase. This gives the lesion a relative washout of 35 % and absolute washout of 48 %, in keeping with a non-adenomatous lesion. Histology proved this to be a phaeochromocytoma following surgical resection

components. Haemorrhage of varying ages can have different signal characteristic on T1W images, with methaemoglobin in subacute haemorrhage resulting in high T1 and low T2 signal intensities. The rare cases that contain intracellular lipid will show loss of signal intensity on CSI (dual echo in and out of phase T1-weighted MRI) [31]. Pheochromocytomas typically demonstrate avid contrast enhancement post gadolinium administration, with a heterogeneous appearance when there are haemorrhagic, necrotic or cystic components [32].

8.3.3 Paraganglioma

These tumours (also known as extra-adrenal pheochromocytoma) arise from neuroectodermally derived paraganglionic cells anywhere within the body. Sympathetic paragangliomas typically secrete catecholamines, and common sites include the posterior mediastinum and abdominal paraaortic region including Zuckerkandl's body [2]. Clinical presentation is typically with headache, palpitations and sweating, with potentially fatal cardiovascular effects including sudden myocardial infarction, malignant hypertension and cerebral haemorrhage [33]. Parasympathetic paragangliomas tend to be nonsecretory tumours, presenting insidiously with an enlarging palpable mass or pain related to the site of tumour. These lesions include aortic body tumours found in the aorticopulmonary window, as well as those found within the head and neck regions, including carotid body, jugular foramen and

middle ear [33]. Most paragangliomas are solitary and sporadic; however, familial paraganglioma are recognised in 10 % of cases, including patients with succinate dehydrogenase B (SDHB) mutation where tumours can be multiple and have a higher rate of malignancy [34].

8.3.3.1 Head and Neck Paragangliomas

The carotid body is the commonest site, followed by jugular foramen, middle ear and along the distribution of the vagus nerve. Less common sites include the orbit, nasal cavity, nasopharynx, thyroid gland, pineal gland and cheek [35]. The carotid body, within the medial aspect of each carotid bifurcation, is the largest compact collection of paraganglia in the head and neck [33]. Carotid body tumours appear as a well-defined soft tissue mass within the carotid space of the infrahyoid neck, splaying the internal and external carotid arteries [36]. On CT, these lesions demonstrate avid homogeneous enhancement due to their marked hypervascularity (Fig. 8.3a–d) [33]. Occasionally, particularly in larger tumours, haemorrhage and necrosis will result in heterogeneous non-enhancing regions. On MRI they are typically of intermediate signal intensity on T1W and high on T2W imaging. A classical feature is the so-called 'salt-and-pepper' appearance, characterised by multiple punctate and serpiginous signal voids due to fast-flowing internal vessels, interspersed with high-signal regions due to slow flow or haemorrhage [37].

8.3.3.2 Thoracic Paragangliomas

These constitute 1–2 % of all paragangliomas and occur predominantly in mediastinal compartments, with less common sites being the trachea, lung, heart and oesophagus. Aorticopulmonary paragangliomas, which are located within the anterior mediastinum, tend to present late as incidental lesions on chest radiographs, whereas the sympathetic posterior mediastinal lesions present earlier with symptoms related to catecholamine secretion [38]. On CT, lesions appear as well-enhancing typically homogeneous masses; however, in cases where there has been haemorrhage or cystic degeneration, patchy areas of low attenuation within the lesion may be seen [33]. Angiography demonstrates marked hypervascularity with multiple feeding vessels; preoperative embolisation may be considered in cases of bulky or surgically challenging tumours [39]. On MRI, lesions are homogeneous or heterogeneous intermediate signal intensity similar to liver parenchyma on T1W imaging, and are hyperintense compared to liver parenchyma on T2W images (Fig. 8.3e) [40].

8.3.3.3 Abdominal Paragangliomas

Retroperitoneal paragangliomas are most commonly seen in the tissues surrounding the aorta and inferior mesenteric artery. CT demonstrates a soft tissue mass with either homogeneous enhancement or central areas of low attenuation. Focal areas of high-density or punctate calcification due to haemorrhage may be seen [41]. On MRI lesions appear isointense or hypointense compared to liver on T1W imaging and are markedly hyperintense on T2W imaging [42].

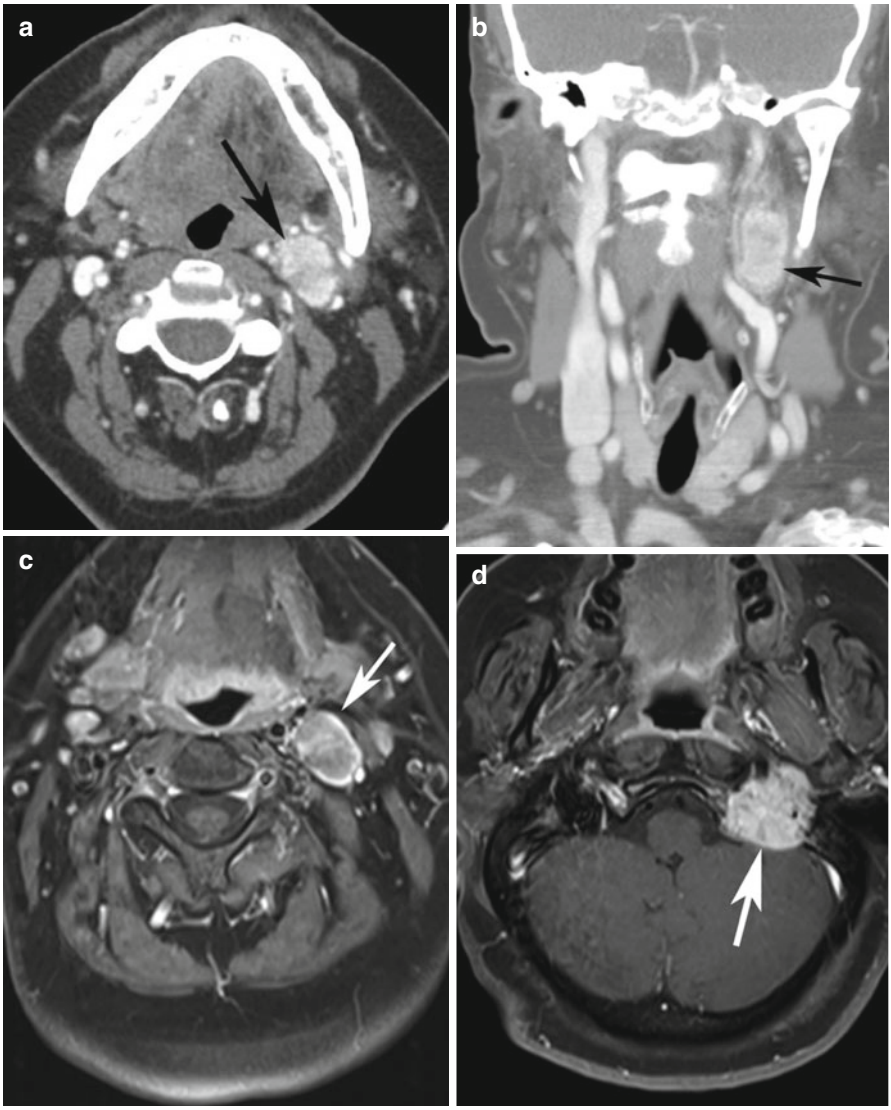
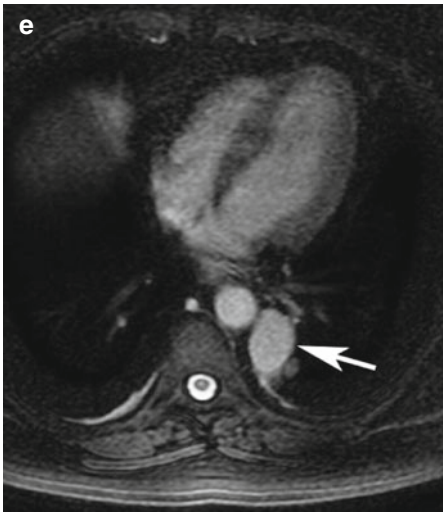


Fig. 8.3 SDHB mutation with multiple paragangliomas. A 57-year-old female presented with a pulsatile neck mass and persistently raised metanephrines. (**a**) Axial (*arrow*) and (**b**) coronal (*arrow*) reformatted arterial-phase CT demonstrates an avid arterially enhancing lesion at the left carotid bifurcation in keeping with a carotid body paraganglioma. (**c**) Post-contrast T1 fat-suppressed image showing the avidly enhancing paraganglioma at the carotid bifurcation, which slightly splays the bifurcation and extends posterior to the common and external carotid arteries. (**d**) Post-contrast fat-suppressed T1-weighted image shows a large avidly enhancing lesion within the left jugular foramen which has eroded and destroyed adjacent bone. Histology following surgical removal confirmed a glomus jugulotympanicum. (**e**) T2-weighted imaging through the thorax revealed a 3 cm hyperintense left paraspinal lesion (*arrow*). The lesion was not PET avid and was proven to be a paraganglioma on histology

Fig. 8.3 (continued)



8.4 Pancreatic Neuroendocrine Tumours (pNET)

Pancreatic endocrine tumours (pNETs) arise from the islet cells of Langerhans and include functioning tumours (insulinoma, gastrinoma, VIPoma, glucagonoma, somatostatinoma) and non-functioning tumours (including pancreatic polypeptidoma (PPoma) and tumours with no associated clinical syndrome).

Insulinomas are the commonest pNET. Diagnosis is based on clinical presentation and biochemical findings (Whipple's triad). Lesions are typically benign (90 %), solitary and almost always intrapancreatic (>90 %), being equally distributed between the head, body and tail of pancreas [43, 44]. Due to their early presentation, they are often small at diagnosis, with 90 % measuring <2 cm and 40 % <1 cm in diameter [45–47].

Gastrinomas are the second commonest pNET. Clinical presentation is usually with the Zollinger-Ellison syndrome (ZES), due to gastrin-driven gastric acid secretion; the diagnosis is usually confirmed biochemically. The majority of cases are malignant; approximately 60 % of patients have hepatic metastases at presentation, and if associated with MEN1 there is an even higher incidence of malignancy [43, 48]. Regional nodal metastases are frequent, and bone metastases have been reported in approximately 30 % of cases [49]. The primary lesion is located within the 'gastrinoma triangle' in more than 90 % of cases (between the junction of the neck and body of the pancreas medially, the second and third parts of the duodenum inferiorly and the junction of the cystic and common bile ducts superiorly) [50]. Gastrinomas may be very small in size and are often extrapancreatic [51].

Other functioning pNETs are very rare (VIPomas, glucagonomas and somatostatinomas), frequently associated with MEN 1 and frequently malignant (between 50 and 80 %). They usually present late with a large mass and metastases. *VIPomas* may be extrapancreatic (in 20 %), predominantly in the retroperitoneal sympathetic

chain and adrenal medulla [43, 50, 52]. *Somatostatinomas* are intrapancreatic (usually in head of pancreas) in over half of cases, the remainder being located in the duodenum and jejunum, usually in association with neurofibromatosis [54].

Non-functioning pNETS including PPoma and other tumours with no clinical syndrome tend to be sporadic and slow growing, presenting late as large tumours causing mass effect [45]. They usually lie in the pancreatic head, and thus presentation may be with biliary obstruction; 60–90 % are malignant [43, 52, 53]. These tumours may also be seen in von Hippel-Lindau syndrome.

8.4.1 Localisation of Pancreatic NETs on Imaging

A wide variety of imaging methods may be used for localising small functioning primary tumours, reflecting the difficulties encountered in detection. Local expertise also dictates the type of imaging used.

8.4.2 Ultrasound

8.4.2.1 Transabdominal Ultrasound

Transabdominal ultrasound (US) has a high specificity but low sensitivity for localising small pNETs in the range of 20–86 % [44, 55, 56]. As with most imaging techniques, sensitivity increases with the size of the lesion [56, 57].

Sonographic appearances are usually of a well-defined round mass, which is homogeneously hypoechoic in comparison to the relatively hyperechoic normal pancreatic tissue and may exhibit a hyperechoic halo. Hypervascularity is typical on Doppler imaging. Tumours that lie along the surface of the pancreas or in the duodenum are less conspicuous on ultrasound.

Contrast-enhanced ultrasound (CEUS) has shown a high specificity of 90–100 % in differentiating pancreatic ductal adenocarcinoma from other solid neoplastic masses, including pNETs which are typically hypervascular [58, 59]. CEUS has shown promising results in detecting small pNETs, with a sensitivity of 94 % and specificity of 96 % [60].

8.4.2.2 Endoscopic Ultrasound

Endoscopic ultrasound (EUS) has been reported to have a high sensitivity (90 %, range 77–100 %) for the detection of small functioning pNETs [61–64].

EUS with a high-frequency probe (7.5–10 mHz) results in superior image resolution due to the close proximity of the transducer to the pancreas. Contrast enhancement may also be used. The probe is positioned within the stomach for visualisation of the pancreatic body and tail and in the duodenum for the head and duodenum [63, 65]. The advantages include localisation of small tumours, particularly in the pancreatic head which may be difficult to palpate at surgery; multiple tumours, in cases of MEN 1; tumours arising in the duodenal wall; and accurate depiction of the relations between tumour and vascular and biliary

structures. FNAC can also be undertaken which has a close correlation to the final histology [66, 67].

EUS can identify regional nodal enlargement, but it cannot fully assess the liver. It is technically challenging and highly specialised, therefore not widely available. Detection of lesions in the tail or extrapancreatic area can be challenging. It also may not be suitable for all patients, for example, where there is duodenal scarring in ZES.

8.4.2.3 Intraoperative Ultrasound

Intraoperative ultrasound (IOUS) has similar advantages to EUS and may improve the intraoperative sensitivity to 92–97 % for identifying small lesions in the head and multiple lesions and is a useful adjunct to palpation [55, 68]. It has been shown to change operative management by identifying multiple gastrinomas or by demonstrating the malignant nature of a lesion in up to 10 % of ZES cases [69]. IOUS has the advantage over EUS of being able to assess the liver, although it is not as sensitive as surgical palpation in detecting extrapancreatic lesions. Disadvantages of the technique include increasing the time and complexity of operation, as complete mobilisation of the pancreas is required, specialist experience for performing and interpreting the scan and poor sensitivity in detecting extrapancreatic/duodenal lesions.

8.4.2.4 CT

Multidetector CT is the most widely used diagnostic tool for the localisation and staging of pNETs.

CT Technique

The patient should be fasted to ensure that the stomach and duodenum are empty. The stomach is then distended with water just prior to scanning, and an IV antiperistaltic agent is administered. An initial pre-contrast scan is performed at the level of the pancreas followed by biphasic post-contrast scanning. Depending on the contrast medium used, around 150 ml should be administered at a rate of 3–5 ml/s. Arterial-phase scanning should be performed either by bolus tracking or after a delay of 25–30 s, followed by portal-venous scanning after 60–70 s with a section thickness ≤5 mm, and the entire liver should be included in both phases. The images are then reconstructed to 1–2 mm in slice thickness, and coronal or sagittal reformats should be reviewed. Images should also be viewed on narrow window settings in order to augment the difference between the enhancing tumour and pancreatic tissue [70, 71].

CT Appearance

Functioning pNETs are usually small and subtle at presentation, appearing isodense to normal pancreatic tissue on pre-contrast images. The majority of pNETs are hypervascular and will only be seen after IV contrast medium. Some tumours are seen more easily on arterial phase and others on portal phase images (Fig. 8.4) [72–75]. Rarely, insulinomas may be hypovascular or cystic and will therefore appear hypodense to the surrounding pancreas (Fig. 8.5) [76]. Cystic pNETs are usually benign and non-functioning; they cannot be reliably differentiated from

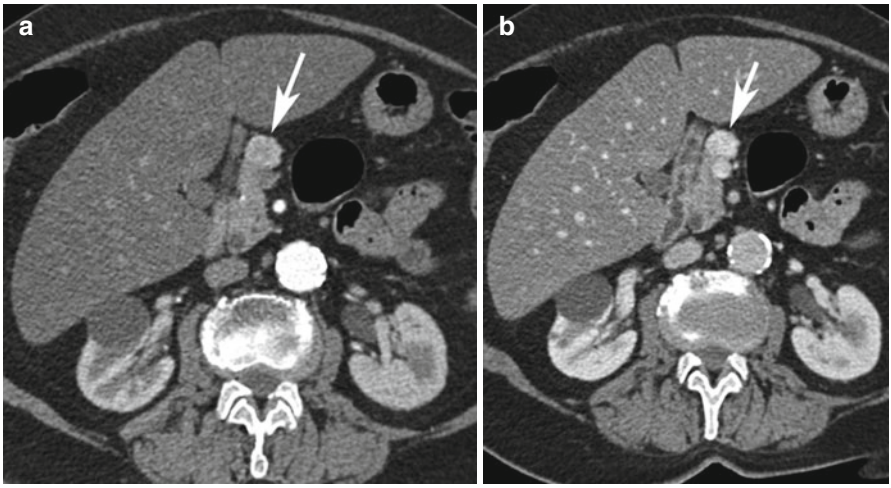


Fig. 8.4 Insulinoma in neck of the pancreas. An 81-year-old female presented with hyperinsulinaemia. (**a**) Arterial-phase CT shows an 18 mm arterially enhancing lesion in the neck of the pancreas (*arrow*); (**b**) portal-venous-phase CT shows that the lesion remained avidly enhancing (*arrow*)

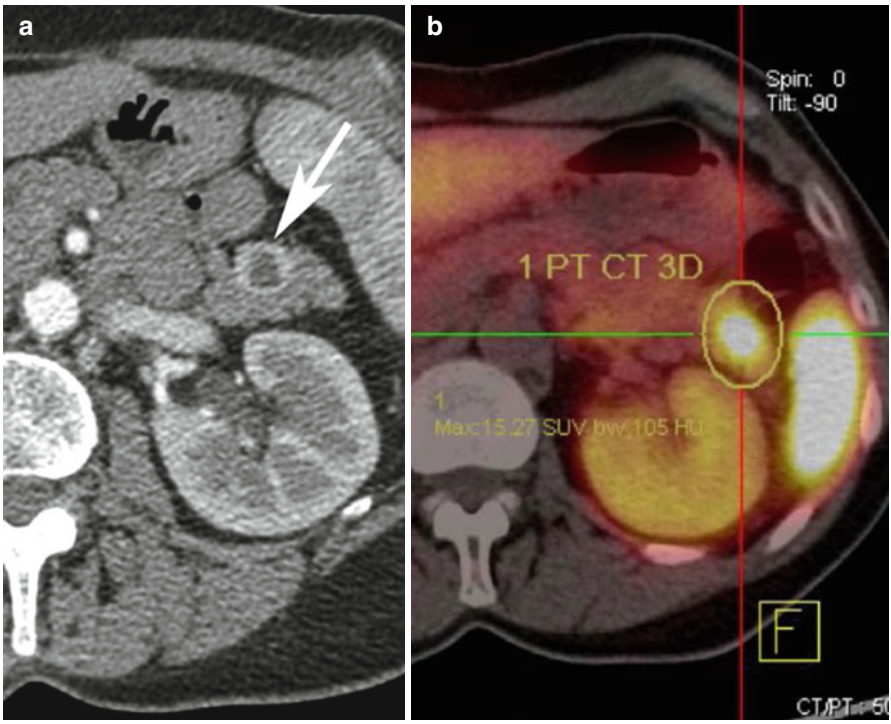


Fig. 8.5 Cystic pancreatic NET. A 61-year-old female. (**a**) Incidental 16 mm lesion in the tail of the pancreas which demonstrated increased vascularity peripherally, suggestive of a cystic NET (*arrow*). (**b**) Gallium PET demonstrated avidity in keeping with a NET

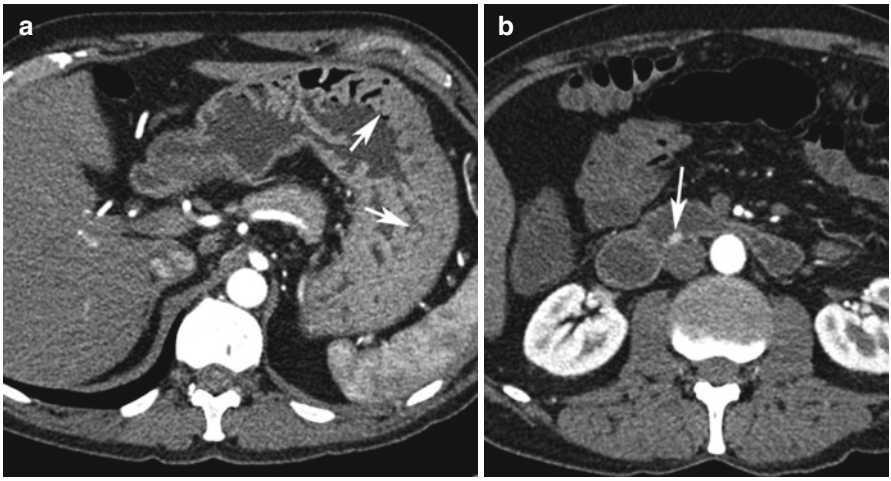


Fig. 8.6 Duodenal gastrinoma. A 49-year-old gentleman presented with Zollinger-Ellison syndrome. (**a**) Arterial-phase CT shows generalised gastric rugal hypertrophy consistent with hypergastrinaemia. (**b**) A small hypervascular lesion on the posterior wall of the third part of the duodenum was identified consistent with a gastrinoma, shown here on axial CT (*white arrow*)

other cystic pancreatic neoplasms on imaging alone [76, 77]. In patients with a suspected gastrinoma, particular attention should be given to the 'gastrinoma triangle' and duodenal wall, where lesions are commonly found (Fig. 8.6).

Large tumours are more likely to be non-functioning and malignant, with signs of necrosis (central non-enhancing low density), calcifications and overt infiltration of the surrounding retroperitoneal structures such as vessels [45, 70].

Diagnostic Performance of CT

Multidetector CT with multiplanar thin reformats has improved the sensitivity for detection of the primary tumour, particularly insulinomas, with one study reporting a sensitivity of 94 % on MDCT alone and 100 % when combined with EUS [82]. Detection is directly related to tumour size, with significant difficulty in detecting tumours <1 cm, detection of 30 % of tumours between 1 cm and 3 cm and 95 % of tumours >3 cm [45, 50, 78]. Tumours within the head and body have a higher detection rate than those in the tail [78, 79]. Small duodenal tumours <1 cm are often missed on CT, and CT sensitivity for the detection of extrahepatic and extrapancreatic gastrinomas, which are often small at presentation, is only 30–50 % [51, 78].

8.4.2.5 Magnetic Resonance Imaging

Advances in MR technology have improved the diagnostic performance in lesion detection [75, 80, 81]. A sensitivity of 94 % for pancreatic lesions, but less for extrapancreatic lesions, has been reported [80, 82]. Although angiography has been reported to be more sensitive for identifying the primary tumour, MR has a higher sensitivity than angiography and CT for regional metastatic disease [83]. Tumour

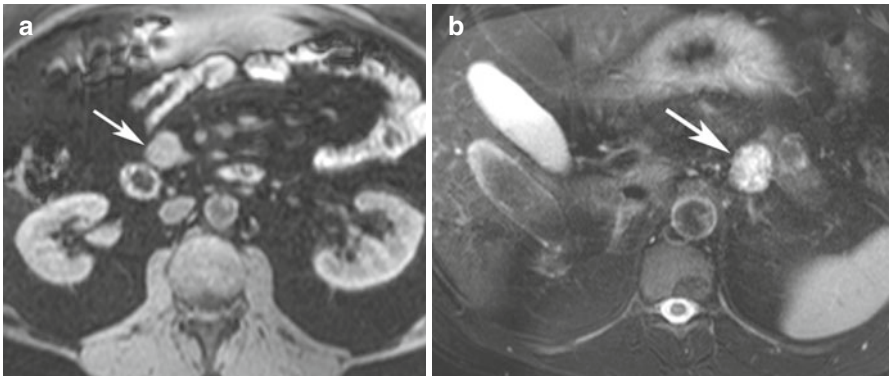


Fig. 8.7 Multiple pancreatic NETs in patient with MEN1. MRI evaluation. (**a**) T1W fat saturated demonstrates a lesion in the uncinate process; (**b**) T2W fat-saturated images shows a high-signal-intensity lesion arising from the tail of the pancreas

detection increases with tumour size, and small multiple tumours in patients with MEN 1 remain difficult to detect.

Optimal technique is needed for high sensitivity as degradation of images due to artefact leads to difficulty in detection. The sequences should include axial T1W, T2W, T1 fat-suppressed spin echo and gradient echo, dynamic contrast enhancement and diffusion-weighted imaging (DWI).

Lesions are usually of low signal intensity on T1W and high on T2W sequences in relation to normal pancreatic tissue, often appearing most conspicuous on the fat-suppressed T1W images (Fig. 8.7) [80, 82]. Rarely, tumours contain a high collagen or fibrous tissue content, with low T2 signal intensity [75]. Following IV gadolinium, characteristic marked homogeneous enhancement is demonstrated. Cystic lesions may demonstrate rim enhancement [80]. Liver-specific contrast agents have been reported to improve detection of liver metastases [84, 85].

DWI is an MRI technique that measures water diffusivity in biological tissues, generating the quantifiable apparent diffusion coefficient (ADC). Small pancreatic NETs may be sensitively detected due to high contrast resolution, and the ability to distinguish benign from malignant lesions is under investigation [86].

8.4.2.6 Angiography

Diagnostic angiography is used in selected cases in specialist centres and is combined with venous sampling (see below). Detailed assessment of the vasculature includes selective catheterisation of all the branches of the coeliac and superior mesenteric arteries. Primary tumours and liver metastases are seen with high sensitivity as a well-defined blush in the capillary and early venous phase. Diagnostic difficulties can arise if a tumour is very small, multiple, or hypovascular or when a lesion lies adjacent to a loop of bowel or spleen and the blush is not separately visible. False positives arise from the blush of a splenunculus or normal pancreas or bowel.

8.4.2.7 Transhepatic Portal-Venous Sampling

Transhepatic portal-venous sampling (TSPVS) involves percutaneous catheterisation of a portal-venous branch. Blood is sampled from the splenic vein, superior and inferior mesenteric veins, pancreatic veins and portal trunk, with the principle that one of these veins will be draining the tumour and therefore contain high concentrations of the secreted hormone. This method is only useful for functioning tumours, although false-negative results occur if hormone secretion is intermittent.

8.4.2.8 Arterial Stimulation and Venous Sampling

Arterial stimulation venous sampling (ASVS) combines simultaneous hepatic venous sampling with selective arterial injection of a pancreatic secretagogue (usually calcium gluconate), a technique that is less invasive than THPVS. Following injection of the secretagogue, hepatic venous sampling is performed every 30 s for 2 min. A two- to threefold increase in the level of hormone indicates the regional presence of tumour. This technique is most useful in cases where a functional tumour remains occult on other imaging modalities. Reported sensitivities are as high 93 %, and the stimulation technique improves the sensitivity of angiography alone [50].

8.5 Gastrointestinal Neuroendocrine Tumours

Gastrointestinal NETs (previously known as carcinoids) are traditionally classified according to their site of origin. The secretory products and therefore the clinical manifestations and immunohistochemical staining patterns are similar for tumours arising from different anatomical sites. 'Foregut carcinoids' include NETs arising from the thymus, bronchus, gastric or duodenal mucosa and pancreas; 'midgut carcinoids' arise in the jejunum, ileum and proximal colon; while 'hindgut' NETs arise in the distal colon and rectum [87]. The most commonly reported sites are the bronchus (32.5 %), jejuno-ileum (20 %), appendix (8 %) and rectum (10 %). They are usually highly vascular tumours, resulting in their characteristic brightly enhancing appearances on imaging.

8.5.1 Bronchial Neuroendocrine Tumours

Bronchopulmonary NETs arise from Kulchitsky cells, neuroepithelial bodies or pluripotential bronchial epithelial stem cells in the bronchial epithelium [88, 89]. Tumours are graded by the World Health Organization according to their malignant potential, from low grade typical carcinoid to small-cell carcinoma [90].

Imaging features vary depending on whether the tumour is located in the airways of the central/middle third of the lung (80 % of cases) or in the peripheral airways [91]. Plain radiography may demonstrate a well-demarcated round or ovoid mass, which is often notched [92]. Centrally located tumours will appear as a central mediastinal or hilar mass, usually measuring 2–5 cm in diameter. These lesions may result in airways obstruction, with recurrent infection and/or lobar collapse [92].

However, as they are often small, CT scanning is more sensitive for detection. Lesions within the bronchial lumen typically brightly enhance (which can mimic a vessel), usually with both an intra- and extraluminal component being visible. Distal collapse or air trapping may be seen due to ball-valve obstruction of the bronchial lumen. Peripheral bronchial NETs are seen in 20 % of cases, appearing as solitary pulmonary nodules, typically round or ovoid with a smooth or lobulated border, commonly with punctate or diffuse calcification. Cavitation and hilar adenopathy and mediastinal invasion may be seen in the more aggressive histological subtypes [92]. In patients with occult ectopic ACTH secretion, a bronchial NET is the commonest source; however, these can be difficult to identify. When a pulmonary lesion is suspected but not seen on CT, MR may aid localisation. Bronchial carcinoids have high signal intensity on T2W and short tau inversion recovery (STIR) images, allowing differentiation between a small mass and the pulmonary vasculature [93].

8.5.2 Thymic Neuroendocrine Tumours

NETs of the thymus are extremely rare, typically presenting with an anterior mediastinal mass. Thought to arise from thymic cells of neural crest origin (Kulchitsky cells), these tumours have similarities to bronchial NETs with a range of tumour differentiation and behaviour. Approximately 50 % of tumours are functioning, with about 40 % presenting with Cushing's syndrome, in which case the mass is usually smaller than those patients presenting with non-functioning tumours [94]. Patients with non-functioning tumours (Fig. 8.8) usually present with symptoms related either to mass effect and/or local invasion. Lesions may be heterogeneous on CT (Fig. 8.9) and may show calcification. Assessment of the SVC for obstruction is vital [95, 96]. Invasive disease as evidenced by extension into the pleura, pericardium, great vessels or regional lymph nodes is also frequently seen in this group of patients [97]. Bilateral adrenal hyperplasia may be present in those with functioning ACTH-secreting tumours [95]. Metastatic disease in lungs, liver and bone, which may be sclerotic, may also be present at the time of diagnosis [96–99].

8.5.3 Gastric Neuroendocrine Tumours

Gastric NETs account for 0.3 % of gastric neoplasms but 11–41 % of all GI NETs [100]. Gastric, duodenal and colorectal neuroendocrine tumours are usually diagnosed by endoscopy with EUS to determine depth of invasion and regional nodal status and for fine-needle aspiration [62]. CT is predominantly done to detect regional and distant metastatic disease. Administration of an antiperistaltic agent and a negative oral contrast agent (water) may also improve detection of the gastric NETs. CT should include an arterial phase acquisition at 25–30 s following the injection of contrast agent at a rate of 4–5 ml/s, followed by a delayed portal-venous phase at 60 s.

Type I gastric NETs, associated with atrophic gastritis, are poorly seen on CT and MR, being multicentric and typically <1 cm in size. The disease is almost

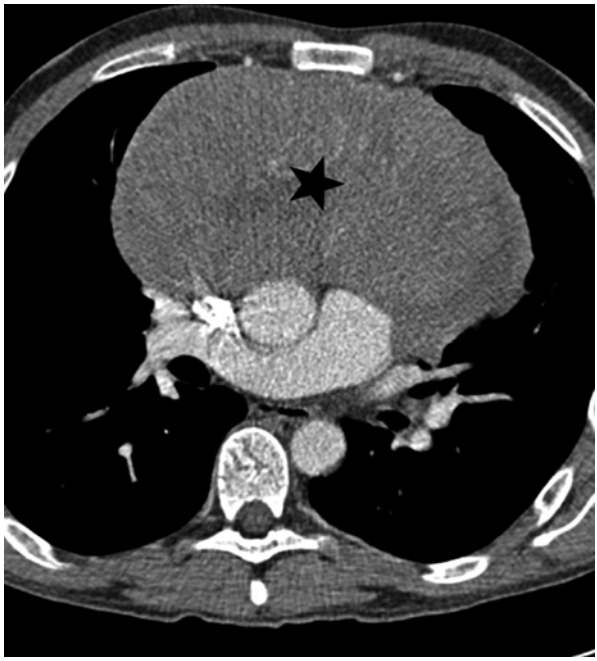


Fig. 8.8 Neuroendocrine tumour of the thymus. A 37-year-old man presented with recurrent chest pain for several years and a previous episode of pericarditis. Chest radiograph showed a large lobulated mediastinal mass. The patient proceeded to a CT scan, which demonstrates a 16 cm heterogeneously enhancing anterior mediastinal mass (*star*), displacing the aorta and pulmonary trunk posteriorly. Functional imaging in the form of gallium-68 DOTATAE showed the lesion to be somatostatin receptor positive. The lesion was unsuitable for resection, and the patient underwent lutetium-177 therapy

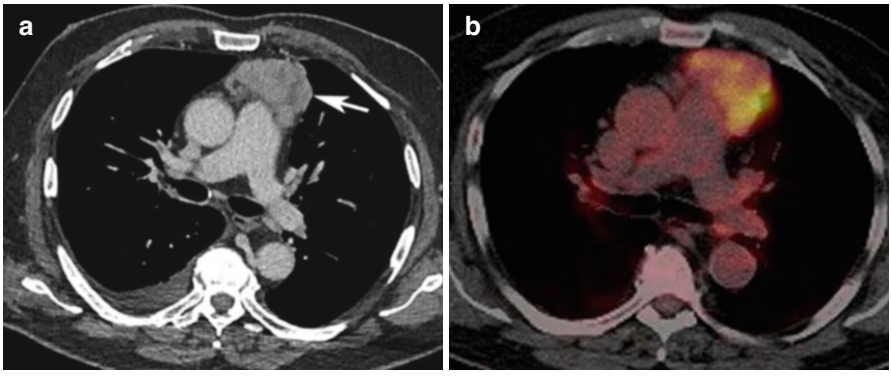


Fig. 8.9 Thymic NET. (**a**) A 77-year-old male presented with chest sepsis. CT chest revealed an incidental 5 cm lobulated, heterogeneously enhancing anterior mediastinal mass with hypodense regions suggestive of necrosis and tiny calcific foci. (**b**) F-18 FDG PET shows metabolic activity within the lesion. Histology showed neuroendocrine carcinoma, with an atypical marker profile

always benign, with metastases present in only 2 % of cases. Type II gastric NETs are associated with MEN-1. There are typically multiple lesions within the gastric wall, which is thickened due to the association with the ZES. There is an increased tendency to metastasise to regional lymph nodes, although the prognosis is generally good [101]. Type I and type II gastric NETs may appear similar to other gastric polyps as numerous enhancing submucosal lesions [102]. Type III sporadic gastric

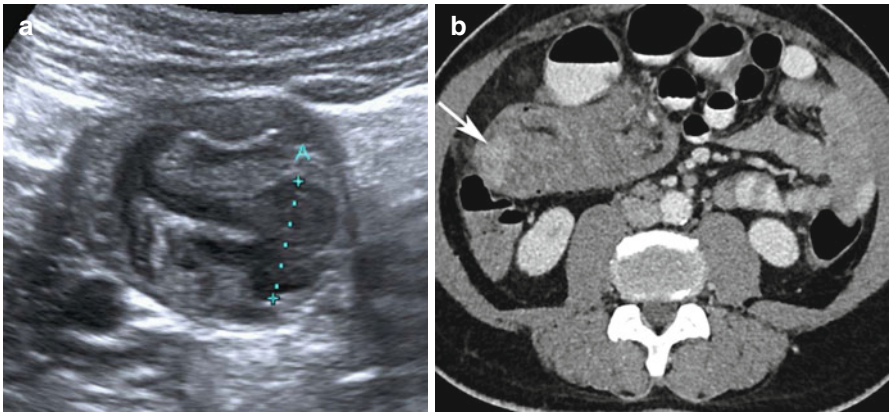


Fig. 8.10 Intussusception due to small bowel NET. (**a**) Ultrasound demonstrates an intussusception, and the lead point is a small bowel NET, shown here between the calipers. (**b**) CT demonstrates the enhancement of the primary lesion

NETs are usually solitary, large lesions with an irregular contour and diffuse margins that may ulcerate. Local invasion with extension into the surrounding gastric fat and metastases are common at presentation.

8.5.4 'Midgut' Neuroendocrine Tumours

Midgut NETs arise beyond the ligament of Treitz to the level of the mid-transverse colon. They are the commonest primary malignant tumour of the small bowel, as well as being the commonest location of all GI NETs [103]. Patients present with abdominal pain, usually with associated colic and diarrhoea, or with symptoms of obstruction that may be due to the primary tumour or due to intussusception (Fig. 8.10). Tumour-associated desmoplastic mesenteric fibrosis due to the local production of fibrogenic agents results in tethering and kinking of the small bowel mesentery that can also result in obstruction. Metastases are often present at diagnosis, most frequently to the liver, bone and lung [104–106]. The incidence of metastases is dependent on tumour size [104, 106], with tumours <1 cm having metastases in 15–25 % of cases, 1–2 cm in size 58–80 % of cases and >2 cm in over 70 % of cases.

Appendiceal NETs are often an incidental finding in surgically removed appendices. Imaging findings are often of an acutely inflamed appendix or an avidly enhancing appendiceal mass.

8.5.4.1 CT

Primary Tumour

Detection of the primary tumour on conventional CT is extremely challenging, as these are usually small lesions not easily detected against the bowel from which

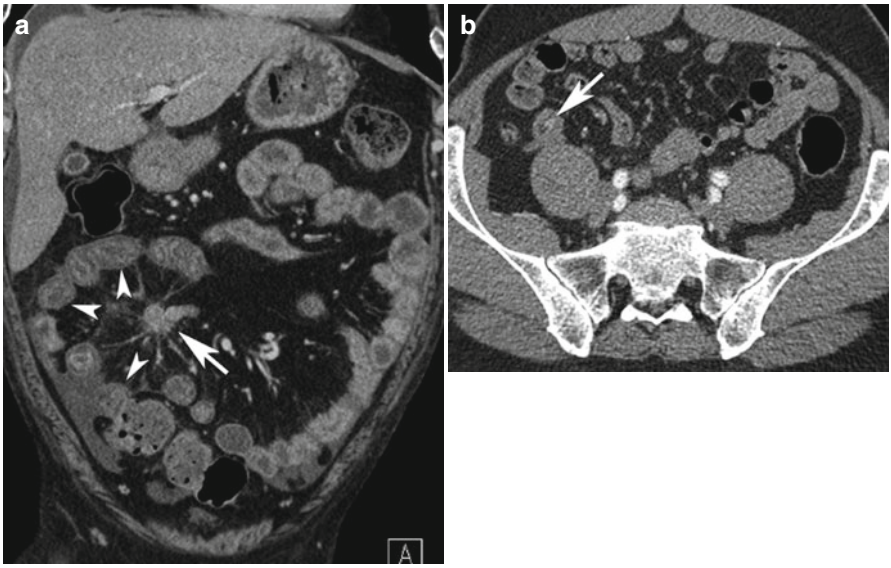


Fig. 8.11 Mesenteric mass secondary to small bowel NET. (**a**) A 56-year-old man presented with a 2-month history of weight loss and rectal bleeding. Contrast-enhanced CT, coronal reformat, shows a 3-cm-diameter soft tissue mass within the small bowel mesentery which contains tiny foci of calcification. The lesion (*arrow*) is causing a desmoplastic reaction, with in-drawing of surrounding bowel loops (*arrowheads*) which demonstrates mural thickening and hyperenhancement. There are a few small adjacent lymph nodes. (**b**) A focal nodular enhancement was thought to represent the primary tumour

they arise; in one large series, only one of 52 primary lesions was identified, where an ileal tumour was causing an intussusception into the caecum (as in Fig. 8.10) [107, 108]. CT enteroclysis is a superior and more reliable imaging technique for diagnosing small bowel lesions [109], with excellent potential for diagnosing small bowel NET. The small bowel is distended with 2.5 l of negative contrast agent through a nasojejunal tube (or orally ingested for CT enterography). An antispasmodic drug is given to reduce peristalsis, and an arterial and portal-venous phase CT is performed, allowing visualisation of the entire small bowel, including intra- and extraluminal lesions.

NETs are seen as a solitary focal nodular mass, a smooth submucosal mass within the bowel wall or an intraluminal polyp (Fig. 8.11). If detected early, tumours may only manifest as subtle wall thickening with most lesions demonstrating avid contrast enhancement. Larger lesions may cause distortion and fixation of the bowel loop and surrounding mesentery, with a pathognomonic appearance on CT of mesenteric strands radiating from the nodule due to a desmoplastic reaction (Fig. 8.11) [110]. CT enteroclysis has a reported sensitivity of up to 100 % and specificity of 96.2 %, with a negative predictive value of 100 % and positive predictive value of 94.7 % [111]. NETs as small as 5 mm in diameter may be detected due to the excellent distension of bowel lumen. Multiplanar reformats optimise visualisation of the bowel wall allowing high diagnostic accuracy (up to 85 %) for transmural and

extramural lesions [112]. The disadvantages of CT enteroclysis include patient discomfort and the radiation dose, as the NJ tube requires insertion under fluoroscopic guidance and there is a minimum of two phases of CT image acquisition. Wireless capsule enteroscopy has been shown to be highly effective at detection of bowel lesions; however, it is limited to intraluminal evaluation [113].

8.5.5 Mesenteric Masses

Mesenteric masses occur in 50–75 % of small bowel NET and appear as soft tissue density lesions, usually identified on CT imaging (Fig. 8.11). These masses typically demonstrate a 'spoke-wheel' appearance, with radiating strands of soft tissue, reflecting the fibrosis secondary to hormonally active substances such as serotonin [107, 114]. Calcification is often present (40–70 %), either as small stippled calcifications or as more bulky, conglomerate foci of calcification [107]. The superior mesenteric artery and vein may also be encased by the contracted mesentery, resulting in vascular compromise and possible bowel ischaemia [61]. On histology, the calcification is localised within areas of mature fibrous scarring within the mass [115].

Diffuse peritoneal metastatic disease occurs in a smaller percentage (20–30 %) of patients with GI NET [107]. On CT, this appears as foci of irregular nodularity throughout the mesentery and on the peritoneal surfaces, often with ascites and abdominal distension, and may result in bowel obstruction.

8.5.5.1 MRI

MRI of the small bowel is now established in inflammatory small bowel disease, but there is less published evidence in noninflammatory conditions. The examination may be poorly tolerated by some patients, and so the use of MRI is determined by local expertise [118]. Patients must be able to breath-hold, as fast sequences are crucial for good image quality, in addition to adequate bowel distension. Sequences include breath-hold T2W (single-shot fast spin echo, single-shot turbo spin echo and a balanced gradient echo sequence such as true fast imaging with steady-state precession) and T1W pre- and post-gadolinium fat-suppressed sequences. Axial and coronal images are obtained.

MR enterography and MR enteroclysis are two techniques that allow distension of the small bowel lumen, which is essential for accurate detection of NETs. Enterography involves the patient drinking a large volume (approximately 2 l) of fluid, an easy, quick and well-tolerated method. Enteroclysis is the distension of small bowel via a nasojejunal tube as described above [116]. Most studies have concluded that enteroclysis results in more adequate and uniform luminal distension of the entire small bowel [117–119]. However, there is limited data directly comparing the diagnostic performance of these two techniques in the detection of small bowel neoplasms. Both have the advantage over conventional barium enteroclysis of the ability to evaluate indirectly the state of the bowel wall, detecting submucosal and extramural primary lesions or extension of small bowel disease, as well as resolving the limitations caused by overlapping bowel loops [119, 120].

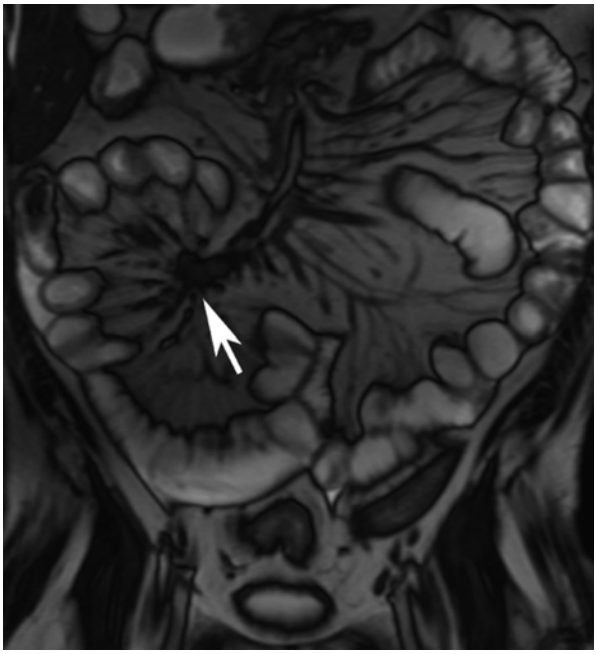


Fig. 8.12 Mesenteric mass secondary to small bowel NET. T2W coronal MRI shows a soft tissue mesenteric mass which is of low T2 signal intensity (*arrow*). The locally tethered adjacent small bowel loops may represent the site of primary disease, but the primary lesion could not be seen

Dynamic sequences in MR enterography also allow evaluation of small bowel peristalsis and distensibility, and imaging may be performed following further oral contrast administration to reassess possible regions of narrowing or suspected intraluminal masses.

As with CT, small bowel NETs typically appear as a focal nodular region of bowel wall thickening or a smooth submucosal mass [121]. Focal wall thickening of more than 3 mm in a well-distended small bowel loop should be considered abnormal [116]. On the unenhanced T1W images, lesions are isointense to muscle and appear iso- or mildly hyperintense to muscle on T2W images, occasionally exhibiting radiating spiculated strands. Multifocal enhancing polypoid lesions are segmentally distributed, and numerous tiny enhancing nodules have also been described [122].

The diagnostic accuracy of small bowel MR enteroclysis at detecting all small bowel neoplasms has been reported to be as high as 0.95 [123], with a stated sensitivity of 91–94 % and a specificity of 97 %.

Mesenteric masses are low signal on both T1- and T2-weighted imaging and show no or negligible enhancement (Fig. 8.12) [121, 124].

8.5.6 Hindgut NETs

Hindgut NETs include tumours arising from the mid-transverse colon extending distally to the rectum, which is the commonest hindgut NET accounting for 27 % of

all GI NETs, a number that has increased over the last decade [103]. One percent of all anorectal neoplasms are NET [125]. The tumours are usually slow growing. Metastases are more common in patients with atypical histology, and as would be expected, the incidence increases with the size of the tumour [126, 127]. Lesions are usually diagnosed at colonoscopy and are typically small and confined to the rectum [128, 129]. Imaging may be used to identify cases suitable for local resection, including stage T1 (tumour is confined to the mucosa and submucosa) and T2 (invasion of muscularis propria) disease, with no evidence of extension to the serosa. Endoscopic US may be useful in demonstrating invasion of the full rectal wall (T3) and invasion into adjacent structures (T4) [129], with the primary lesion appearing as a homogeneously hypoechoic submucosal mass [130]. MR may have a role in local staging. Both CT and MR can be used to stage local lymph node disease and distant metastatic disease in preoperative planning, with up to 30 % of patients having metastatic disease at presentation [126, 127].

8.6 Appearances of Metastatic Disease in NET

Nodal metastases are the most frequent metastatic site at histology, but liver metastases are the most frequently identified metastases on imaging [131]. Mesenteric disease is described in the section on small bowel NETs. The lungs and bones are other common sites of metastatic disease.

8.6.1 Liver Metastases from GEP-NET

8.6.1.1 Ultrasound

The ultrasound technique used to assess for liver metastases is the transabdominal (TA) approach, which allows visualisation of all or most of the liver, for most patients. Endoscopic ultrasound (EUS) does not allow full visualisation of the liver. Whereas metastases from adenocarcinomas (e.g. colorectal, lung) tend to appear hypoechoic on ultrasound, NETs are typically seen on ultrasound as hyperechoic foci within the liver parenchyma, which have irregular and poorly defined margins [52, 57]. In some cases these appearances may mimic a liver haemangioma, and additional imaging will be required to further characterise the lesion. In the context of hepatic steatosis (where the liver has a generalised increase in its echotexture), the presence of a hyperechoic liver metastasis may be masked. Less commonly, liver metastases from GEP-NETs may appear as hypoechoic foci, with or without a hyperechoic halo.

CEUS is a technique used to characterise focal liver lesions on ultrasound following intravenous injection of a microbubble contrast agent. NET liver metastases classically demonstrate avid enhancement in the arterial phase followed by washout to appear as a non-enhancing defect in the late phase [132]. This contrasts to adenocarcinoma metastases, which do not typically demonstrate avid arterial enhancement.

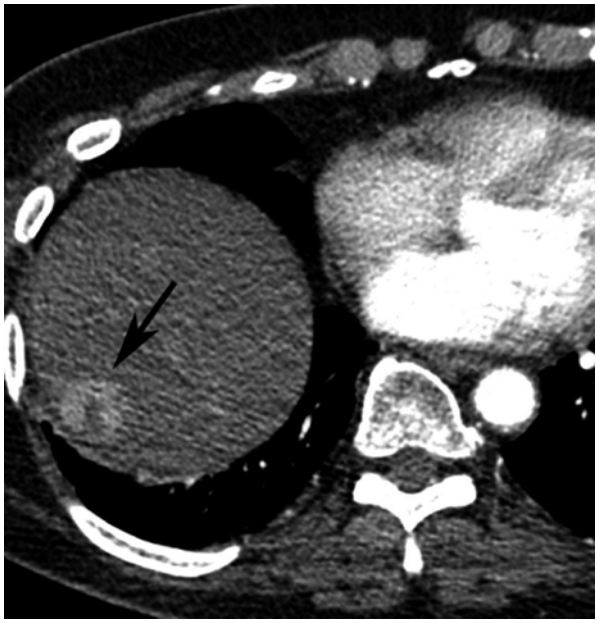


Fig. 8.13 Liver metastasis secondary to pancreatic NET. An avidly arterially enhancing 19 mm liver lesion was found on surveillance CT 7 years later following resection of a pancreatic NET, in keeping with a metastatic deposit

Although traditionally used as a tool for lesion characterisation, CEUS is comparable to contrast-enhanced CT for response assessment of NET liver metastases following radiopeptide therapy [133].

8.6.1.2 CT

NET hepatic metastases are often masked by appearing isointense to normal enhancing liver parenchyma on portal-venous phase (PVP) CT imaging and are therefore best imaged using 'triple-phase' CT scanning, i.e. pre-contrast, hepatic arterial phase (HAP) and PVP (Fig. 8.13).

On the pre-contrast imaging, liver metastases are not well delineated and may not be identifiable. They may appear of lower attenuation than normal liver parenchyma or occasionally contain foci of calcification. Larger lesions may become necrotic and appear of fluid density.

NET hepatic metastases typically demonstrate avid enhancement during the HAP imaging and therefore appear hyperintense with respect to liver parenchyma. During the PVP, the metastases may become isointense to liver parenchyma (reducing their conspicuity) or may washout to appear as a hypointense lesion [134]. In contrast, adenocarcinoma metastases do not typically enhance in the arterial phase and usually manifest simply as hypoenhancing lesions in the PVP.

8.6.1.3 MRI

On MR imaging, the majority of NET liver metastases are of low signal intensity on T1W and intermediate/high signal intensity on T2W imaging. These hypervascular

lesions typically demonstrate avid enhancement in the HAP, and a proportion of these lesions will be solely identifiable in this phase [114].

DWI has been demonstrated to be more sensitive than T2W imaging for the detection of NET metastases, although it does not reliably differentiate between metastases of different origins (e.g. NET from adenocarcinoma) [135].

8.6.2 Lymph Node Metastases

Features suggestive of malignant involvement include a rounded rather than oval appearance, loss of the normal fatty, hypervascular hilum and an increase in the short-axis diameter above that considered to be the upper limit of normal [136]. For most lymph nodes this upper limit is taken as 10 mm, although this should be used with caution as smaller lymph nodes may still be malignant, and larger lymph nodes may be 'reactive' and thus not contain any malignant cells. Involved lymph nodes in NET may also contain foci of calcification, although this finding is not specific for malignancy. Retroperitoneal and mesenteric lymph node enlargement is commonly seen in patients with midgut NET (20–30 %) and may result in retroperitoneal fibrosis, potentially leading to ureteric obstruction [107].

8.6.3 Lung Metastases

The diagnosis of lung metastases arising from NETs is made on CT, manifest by rounded pulmonary nodules of non-uniform sizes in a bronchovascular distribution which enlarge with progression of disease. Following local metastasectomy for pulmonary metastases, follow-up CT studies of the thorax evaluate for local disease recurrence.

8.6.4 Bone Metastases

NET bone metastases are frequently sclerotic, often appearing as small punctate deposits. Less commonly, bone metastases appear as mixed osteolytic/osteosclerotic lesions, and a minority (10 %) appear as purely osteolytic. Treated bony metastases may become more sclerotic and thus be misdiagnosed as disease progression [137]

The presence of metastatic bone disease is associated with a poor prognosis, particularly in the presence of metastatic disease elsewhere [49]. Whole-body MR imaging has been found to have a sensitivity of 86 % in the detection of bone metastases in patients with GEP-NET cancer, similar to that of somatostatin receptor scintigraphy (81 %) [138]. Nuclear medicine studies for the detection of bony metastatic disease are discussed in other chapters.

On MRI, bone metastases typically appear as lesions of reduced signal on T1W imaging, with variable signal intensity on T2W imaging dependent upon the extent

of associated oedema. Bone metastases from NETs may enhance following gadolinium administration [139].

8.6.5 Response to Treatment

Response of metastatic NETs to medical treatment is widely assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria and is defined by changes in diameter of target metastatic lesions, usually on CT or MRI. However, more research is needed into novel ways to evaluate response, particularly in light of the increasing use of targeted chemotherapy agents, which may offer improved patient outcome in the absence of a RECIST response [140].

Surgical resection is the only curative technique in GEP-NETs. However, palliation of symptoms may be achieved by radio-frequency ablation or embolisation or chemoembolisation of liver metastases.

Radio-frequency ablation (RFA) is a technique employed to induce thermal coagulation necrosis within hepatic metastases. Following ablation, the lesion will typically appear as a focus of necrosis on CT (i.e. an area of lower attenuation than the surrounding liver) with smooth margins. Immediately following treatment the area of necrosis is expected to be slightly larger than the pretreatment tumour. There may be peripheral enhancement, but the central necrotic part of the tumour would not be expected to enhance. On subsequent imaging the defect should remain stable or reduce in size, indicating stable disease or a response to treatment. An increase in size or enhancement within the lesion itself suggests disease recurrence or incomplete therapy [141].

Transcatheter chemoembolisation (TACE) is an interventional radiology technique that selectively embolises and delivers chemotherapy to the branches of the hepatic artery supplying the hypervascular liver lesions. A CT performed post-treatment will demonstrate very high-density material within the liver metastasis due to the Lipiodol administered during chemoembolisation. Response to treatment may be assessed by a change in the size of the lesion on subsequent CT imaging, with an increase in tumour size indicating disease progression. Additionally response to TACE may be assessed on contrast-enhanced MRI and DWI, demonstrated by a reduction in the percentage enhancement of the lesions and a reduction in the extent of restricted diffusion [142]. However, these techniques remain under investigation.

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Nuclear Medicine Imaging in Neuroendocrine Tumors

9

Andreas Kjaer

9.1 Introduction

With the paradigm of personalized medicine in cancer, it has increasingly become necessary to diagnose and characterize tumors at the molecular level. Whereas this is possible through invasive methods, e.g., analyses of biopsies, it can also be achieved in a noninvasive manner using nuclear medicine imaging methods [1]. These image-based methods for phenotyping tumors have several advantages over invasive methods. First, they are not prone to sampling error as are analyses based on tissue samples that often turn out not to be representative of the entire disease. Accordingly, WHO grading is based on measurement of proliferation, e.g. Ki67 in biopsies [2]. However, there is no guarantee that such biopsies are taken from the areas with highest proliferation, which is likely to determine the destiny of the patient. Based on experience with nuclear medicine imaging, indeed large regional variation in uptake of functional tracers is seen. Moreover, exact phenotyping of therapeutic targets is important before initiating treatment. Accordingly, somatostatin receptor scintigraphy prior to peptide receptor radionuclide therapy (PRRT) reveals if the whole or only part of the tumor burden in a neuroendocrine tumor (NET) patient will be targeted and thereby predicts the likelihood of success. In many ways, PRRT and somatostatin receptor imaging using the same ligands have been the proof of concept of theranostics within cancer.

For reasons mentioned above, somatostatin receptor scintigraphy based on gamma camera technique has been used extensively and on a routine basis for decades in NET. However, recent developments within nuclear medicine are increasingly moving the field toward the use of positron emission tomography (PET) due

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to the higher sensitivity, better spatial resolution, and quantitative nature. Also the use of non-somatostatin receptor PET imaging may become increasingly important in the future, especially for therapy monitoring with early detection of nonresponders that may then be changed in therapy.

9.2 Somatostatin Receptors as Target for Imaging in NET

Most gastroenteropancreatic (GEP) and bronchopulmonary NETs, which are the focus of this chapter, overexpress somatostatin receptors and especially the subtype 2 receptor [3, 4]. Therefore, somatostatin receptor imaging is effective in diagnosing, staging, and restaging of NET. However, in vitro measurements have shown that the proportion of NETs that express somatostatin receptors varies with high proportion of approximately 90 % of carcinoids but only approximately 70 % of insulinomas [5]. However, since high-expressing NETs, e.g., carcinoids, are much more abundant than low-expressing NET types, e.g., insulinomas, the overall expression rate for NETs is close to 90 %. Overexpression of somatostatin receptors is also used as a target for therapy using long-acting somatostatin analogues as well as PRRT with beta-emitting somatostatin receptor ligands, e.g., ^{177}Lu-DOTATATE and ^{90}Y-DOTATOC [6]. Due to this, somatostatin receptor imaging is also used for selecting patients suited for therapy and to monitor therapy response.

9.3 Gamma Camera Imaging of Somatostatin Receptors

Historically the first gamma camera imaging of somatostatin receptors in NET was performed more than 25 years ago using ^{123}I-labeled octreotide. However, soon thereafter ^{111}In-labeled octreotide using DTPA as a chelator was introduced and later commercialized as OctreoScan. The two initial studies using ^{111}In-DTPA-octreotide reported overall sensitivity on a patient-based level for carcinoids of 88 and 89 % and of 61 % in insulinomas [5, 7]. In line with this, a larger retrospective study of 104 patients from Vienna found sensitivity for detection of primary or recurrent carcinoid tumors to be 91 % [8]. However, a meta-analysis of 720 patients calculated a lower sensitivity of ^{111}In-DTPA-octreotide of 78 % (CI, 76–82 %) to detect abdominal carcinoids [9]. An explanation could be that many of the studies were not performed state of the art, using low dose of tracer and suboptimal imaging protocols. Specifically these early studies in general did not use SPECT or image fusion with computed tomography (CT). Addition of CT may improve the clinical value of the investigation [10]. Currently, the state-of-the-art imaging protocol is described in procedure guidelines of the European Association for Nuclear Medicine (EANM) [11] and recommends imaging at 4 and 24 h or 24 and 48 h. The latter is especially useful if intestinal focus is found after 24 h, and it is unclear whether this is due to intestinal excretion or a NET focus. In general, lesions seen at 4 h are also seen at 24 h so we do not perform 4 h imaging on a routine basis. Laxatives are

generally recommended whereas there is no support of the necessity of pausing somatostatin analog treatment [11]. Also today SPECT should be performed at one time point, 24 h at our institution, and if available the investigation should ideally be performed as a SPECT/CT. Recently, we performed a larger prospective study of 96 consecutive NET patients using state-of-the-art SPECT/low-dose CT and planar images at 24 h as well as planar images after 48 h [12]. The overall sensitivity in this study for detection of primary tumor or metastases was 89 %. Sensitivity did not vary with origin of tumors, and sensitivities for ileal, pancreaticoduodenal, lung, and unknown origin were all between 86 and 91 %. Also we performed an analysis of sensitivity based on Ki67 grading. As expected, tumors with Ki67 > 15 % were only ^{111}In-DTPA-octreotide positive in 69 % of the cases, whereas tumors with Ki67 at or below 15 % were detected in 90 % of the cases indicating the more well-differentiated nature of the latter with somatostatin receptor expression.

A few larger studies have used other gamma camera tracers targeting somatostatin receptors, e.g., ^{99m}Tc-EDDA/HYNIC-Tyr^3-octreotide [13, 14]. In general, these tracers did not perform better than ^{111}In-DTPA-octreotide, which is more widely available.

Taken together data support the key role of somatostatin receptor imaging with ^{111}In-DTPA-octreotide in staging of NETs. This tracer has served well for decades and still does in many areas of the world. In the USA it is currently the method of choice. However, somatostatin receptor imaging is now quickly moving into use of PET tracers.

9.4 PET or SPECT Imaging?

The field of nuclear medicine is increasingly moving into use of PET, and many tracers that served well for many years in gamma camera-based imaging and SPECT are substituted with new PET tracers visualizing the same characteristics. There are major advantages using PET-based imaging compared to SPECT-based imaging.

Firstly, the sensitivity of PET is at least 100-fold higher than that of SPECT allowing for better count statistics with less noise in images obtained at lower tracer doses. This in general translates into less radiation burden.

Secondly, the spatial resolution is typically two-fold better and around 4–5 mm for state-of-the-art PET scanners vs. 8–10 mm for SPECT scanners. However, it should be noted that spatial resolution does not refer to the minimal detectable lesion but whether foci can be discriminated from each other. Accordingly, a small, e.g., 2 mm, focus with high tracer accumulation can be detected but will be represented as a larger focus (blurred). However, due to the averaging of activity in the image, increasing amounts of activity are necessary for detection when spatial resolution drops.

Thirdly, due to the physics in PET, an accurate attenuation correction can be performed which leads to the ability to quantify tracer uptake in absolute terms. In contrast, uptake in SPECT has to be expressed in relative terms, e.g., relative to liver

as used for somatostatin receptor imaging using the Krenning scale. With PET it is possible to follow absolute uptake over time, and typically the reproducibility allows for detection of changes of 10 % or more between two scans. This can be and is used for therapy response monitoring.

On the negative side PET scanners are still more expensive than SPECT scanners. However, if effectively utilized the scanner cost and depreciation is not the major cost driver for nuclear medicine investigations – major costs are personnel and radioactive tracers. Accordingly, PET scans often are less expensive than comparable SPECT imaging.

Another challenge in PET imaging is the necessity of a cyclotron and radiochemistry laboratories. However, recently generator-based PET isotopes have become available, most notably ^{68}Ga, thereby making production of PET isotopes possible without an expensive on-site cyclotron. Also, the use of PET isotopes with long half-lives, e.g., ^{64}Cu with 13 h half-life, has made it possible to centralize production of tracers for distribution to PET centers. In part, this is also possible, if logistics is in place, with ^{18}F-labeled compounds, since ^{18}F has a half-life of 110 min. Therefore, at present nuclear medicine departments may implement PET technology by installing PET scanners without the need of creating a whole PET center with cyclotrons and advanced radiochemistry facilities. This has lowered the entry barrier to perform PET scans considerably.

9.5 PET-Based Imaging of Somatostatin Receptors

9.5.1 ^{68}Ga-Labeled PET Tracers

Considering the obvious advantages of using PET for somatostatin receptor imaging and the convenience of ^{68}Ga labeling using a generator, several new PET tracers targeted toward the somatostatin receptor have recently been introduced. The majority is based on the same peptides as used for SPECT imaging, i.e., octreotide and octreotate using DOTA as the chelator. The affinity for the different subtypes of somatostatin receptors is dependent on radionuclide, chelator, and peptide. Discussion on which PET tracer is best includes the affinity for the somatostatin receptor subtype 2 as well as affinity for other somatostatin receptors, especially subtype 5. However, we have shown on a gene expression level that somatostatin 2 receptors are expressed to a much higher level on NETs than any other of the receptors [3], so we suggest that affinities toward the other 4 receptor subtypes are of limited interest. The three most commonly used ^{68}Ga-labeled somatostatin receptor PET tracers are ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC, and ^{68}Ga-DOTANOC. In Table 9.1 in vitro affinities of the different PET tracers toward the five somatostatin receptor subtypes as reported in the literature are summarized [15, 16]. ^{68}Ga-DOTATATE has a ten-fold higher affinity for the somatostatin 2 receptor and a very low affinity toward the somatostatin 5 receptor compared to the other two tracers. ^{68}Ga-DOTANOC has the highest affinity toward the somatostatin 5 receptor, which we believe is of minor importance in NETs. A difference between the three

Table 9.1 In vitro binding affinities (IC50 in nM ± SEM) of chelated somatostatin analogues

Name	SST_1	SST_2	SST_3	SST_4	SST_5
Ga-DOTATATE	>10,000	0.2 ± 0.04	>10,000	300 ± 140	377 ± 18
Ga-DOTATOC	>10,000	2.5 ± 0.5	613 ± 140	>1,000	73 ± 21
Ga-DOTANOC	>10,000	1.9 ± 0.4	40 ± 5.8	260 ± 74	7.2 ± 1.6

Data from Refs. [15, 16]
SST somatostatin receptor, *Ga-DOTATATE* Ga-DOTA-tyr^3-octreotate, *Ga-DOTATOC* Ga-DOTA-tyr^3-octreotide, *Ga-DOTANOC* Ga-DOTA-l-Nal^3-octreotide

tracers in normal tissue distribution could lead to differences in tumor-to-background ratios and thereby easiness to detect lesions. However, for most organs the tumor-to-background ratios are comparable [4].

Performance of the three ^{68}Ga-based PET tracers has recently been reviewed [17]. For ^{68}Ga-DOTATATE, sensitivity on a patient basis was reported to be 72–96 % in 6 smaller studies including between 18 and 38 patients (total of 144 patients) [18–22]. The study with the median sensitivity reported 93 % (CI, 70–99 %; 20 patients) [21]. For ^{68}Ga-DOTATOC, sensitivity on a patient basis was found to be 92–100 % in 6 small- or medium-sized studies including between 8 and 84 patients (total of 211 patients) [23–28]. The largest study with 84 patients reported a sensitivity of 97 % (CI, 90–100 %) [26]. Finally, for ^{68}Ga-DOTANOC, patient-based sensitivity was reported to be 68–100 % from 9 studies including 11–1,239 patients (total of 1,677 patients) [21, 22, 29–34]. The study with 1,239 patients reported a sensitivity of 92 % (CI, 90–94 %) [34].

Taking into consideration that the number of patients as well as the composition of the groups evaluated with the different ^{68}Ga-based tracers varied, it seems fair to conclude that no major differences have been documented especially since the populations studied varied between the investigations. So far, only three studies have compared these tracers pair-wise on a head-to-head basis [21, 22, 35]. In all these studies, comparisons were undertaken lesion based rather than patients based since only minor differences were expected and since the superiority of one tracer could be ability to detect more lesions. Two studies compared ^{68}Ga-DOTATATE with ^{68}Ga-DOTANOC [21, 22], and one compared ^{68}Ga-DOTATATE with ^{68}Ga-DOTATOC [35]. Although minor differences were reported, many were nonsignificant on a patient basis, and considering clinical relevance in our view, there were no major differences favoring use of one of these three tracers. Local availability, experience, and wish to theranostic pairing, e.g., use of ^{68}Ga-DOTATATE before ^{177}Lu-DOTATATE is to be used for PRRT, may decide which tracer to use. Absolute uptake of tracer between the tracers did vary although not systematically. Therefore, if comparing absolute values as SUV longitudinally, e.g., in therapy response monitoring, the same tracer has to be used.

Recently, another ^{68}Ga-labeled PET somatostatin receptor PET tracer, ^{68}Ga-DOTA-lantreotide (^{68}Ga-DOTALAN), was introduced mainly as a companion diagnostic for ^{90}Y-labeled lanreotide. Two studies compared ^{68}Ga-DOTALAN on a head-to-head basis with ^{68}Ga-DOTATOC [36] and ^{68}Ga-DOTATATE [37],

respectively. Both studies found substantially less lesions visualized with ^{68}Ga-DOTALAN, questioning the usefulness of this tracer as a stand-alone imaging agent but not necessarily as a companion diagnostics.

9.5.2 ^{64}Cu-DOTATATE

Recently we introduced the somatostatin receptor PET tracer ^{64}Cu-DOTATATE for human use [38]. Theoretical advantages compared to ^{68}Ga-labeled tracers include the longer half-life of ^{64}Cu (13 h) compared to ^{68}Ga (68 min) allowing for delayed imaging for up to 24 h. Indeed, we showed that imaging after 3 h rather than 1 h with ^{64}Cu-DOTATATE seems optimal since most kidney activity is then cleared. Imaging with ^{68}Ga-tracers at 3 h would not be possible due to the short half-life. Also, the positron range, which is the distance a positron travels from emission until it is annihilated and sends out two photons, differs between the radionuclides. Since the PET scanner detects the coincident photons and not where the emission took place, which is really what should be visualized, the image is off focus (blurred). For ^{64}Cu the average positron range is only 1 mm whereas it is 4 mm for ^{68}Ga. This translates into better resolution and potentially better detection of small foci using ^{64}Cu as radionuclide. Finally, the long half-life and a shelf life of >24 h of ^{64}Cu-DOTATATE allow us to produce the tracer and distribute it if wanted. Finally, logistically ^{68}Ga needs a radiochemist to be on standby, as doses need to be produced in close relation to when needed. In contrast, ^{64}Cu-DOTATATE is produced as one batch for use throughout the day and the next morning.

In our first-in-human study of 14 patients where we performed a head-to-head comparison with ^{111}In-DTPA-octreotide [38], in 6 of these 14 patients, additional lesions were found using ^{64}Cu-DOTATATE compared to ^{111}In-DTPA-octreotide, and in 5 patients these were in organ systems not previously known as metastatic sites. After 18 months of follow-up, all additional lesions seen on PET could be confirmed as true positive. Based on the first 100 prospectively enrolled patients, we found a sensitivity of 91 % for ^{64}Cu-DOTATATE. In 35 cases PET identified pathological foci in organs not identified with ^{111}In-DTPA-octreotide. Of these, 31 were later confirmed to be true-positive lesions [39]. Taken together ^{64}Cu-DOTATATE seems promising for clinical use. Currently, we are undertaking head-to-head comparison of ^{64}Cu-DOTATATE with ^{68}Ga-DOTATOC. Data are not yet available, but based on image quality better resolution as reflected by degree of detail is found using ^{64}Cu-DOTATATE (Fig. 9.1).

9.6 Catecholaminergic and Serotonergic Pathways for Imaging of NETs

Neuroendocrine tumors have the ability for uptake of amine precursors by the *L*-type large neutral amino acid transport system (LAT) transporter and through further metabolism including decarboxylation to form catecholamines and serotonin.

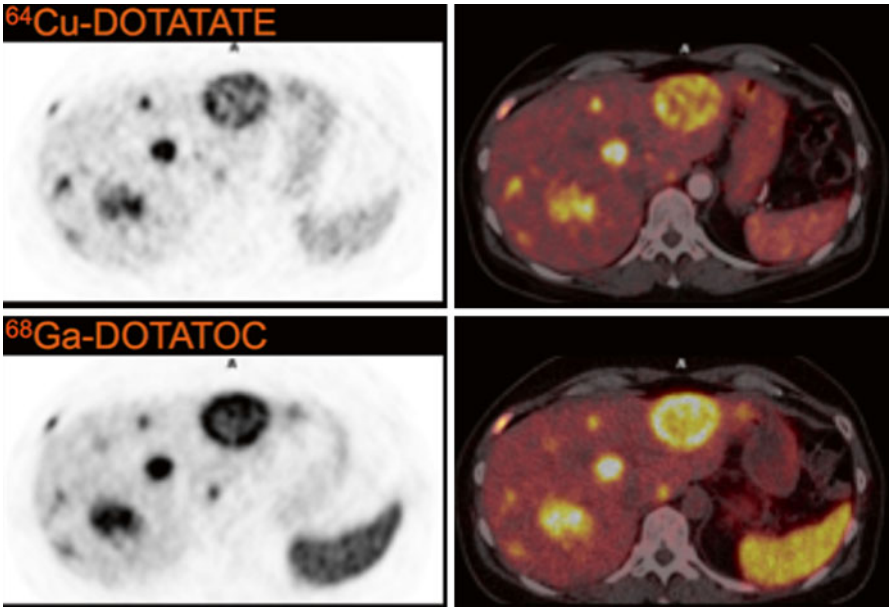

Fig. 9.1 Imaging of same NET liver lesions with ^{64}Cu-DOTATATE and ^{68}Ga-DOTATOC. Please note the greater detail in ^{64}Cu-DOTATATE images, probably due to difference in positron range of ^{64}Cu and ^{68}Ga as well as the lower uptake in spleen (Reproduced with permission from Ref. [17])

9.6.1 ^{18}F-DOPA

The PET tracer and dopamine precursor ^{18}F-L-dihydroxyphenylalanine (^{18}F-DOPA) is taken up by neuroendocrine tumor cells by LAT, transported into the cytoplasm, and metabolized (decarboxylated) to dopamine, which is then transported into secretory vesicles by the vesicular monoamine transporter (VMAT). In the secretory vesicles dopamine is further metabolized to noradrenaline and adrenaline.

Early studies showed promising results for ^{18}F-DOPA in GEP and bronchopulmonary NETs with sensitivities on a patient basis ranging from 65 to 96 % based on four studies with a total of 116 patients (meta-analysis sensitivity, 87 %; CI, 80–93 %) [9]. Even higher sensitivities were found for pheochromocytomas and paraganglioma that are, however, not the focus of this chapter. Performance in abdominal NETs was in general superior when compared to gamma camera-based somatostatin receptor scintigraphy. However, since PET-based somatostatin receptor imaging is superior as well, the question arises whether ^{18}F-DOPA is better and valuable compared to the ^{68}Ga-labeled somatostatin receptor PET tracers. Two studies with a total of 28 patients compared on a head-to-head basis ^{18}F-DOPA with ^{68}Ga-DOTANOC or ^{68}Ga-DOTATOC, respectively. In both studies ^{68}Ga-DOTANOC or ^{68}Ga-DOTATOC visualized considerably more lesions than ^{18}F-DOPA even in pheochromocytomas [29, 40]. One study with 25 patients compared ^{18}F-DOPA with

^{68}Ga-DOTATATE, and also here it was found that ^{68}Ga-DOTATATE had a much higher sensitivity [20].

Taken together, although ^{18}F-DOPA performs pretty well in GEP and bronchopulmonary NETs, when compared with modern PET tracers targeting somatostatin receptors, they are inferior. In general, it therefore seems that this tracer is not necessary for routine use if somatostatin receptor scintigraphy is performed by PET although we cannot rule out that in selected, difficult cases it may be of some value.

9.6.2 $^{123/131}$I-MIBG

A reuptake mechanism exists at the catecholaminergic terminals for noradrenaline. This reuptake mechanism can be visualized using the false neurotransmitter metaiodobenzylguanidine (MIBG) which is also a substrate for the reuptake mechanism. MIBG can be labeled with radioiodine and is most commonly used for SPECT as ^{123}I-MIBG or ^{131}I-MIBG, where the latter may also be used for therapy due to concomitant beta-emission.

For imaging of GEP and bronchopulmonary NETs, the sensitivity of $^{123/131}$I-MIBG gamma camera imaging is somewhat disappointing based on two studies with a total of 125 patients: 63 % (CI, 54–72 %) [9]. For pheochromocytomas, neuroblastomas, and paragangliomas, the same meta-analysis found sensitivities of 79 % (9 studies; $n = 161$; CI, 68–82 %), 84 % (5 studies; $n = 204$; CI, 79–89 %), and 69 % (4 studies; $n = 87$; CI, 58–78 %), respectively. Again, the question arises whether $^{123/131}$I-MIBG imaging has any role when somatostatin receptor imaging and FDG-PET are available. In a recent prospective study, we therefore performed ^{111}In-DTPA-octreotide SPECT, ^{123}I-MIBG, and FDG-PET for head-to-head comparison in a total of 96 consecutive patients [12]. We found an overall sensitivity on a patient basis of 52 %, and ^{111}In-DTPA-octreotide detected more than twice the number of lesions compared to ^{123}I-MIBG. Of the 96 patients, 3 were ^{123}I-MIBG positive and ^{111}In-DTPA-octreotide negative. However, all of these were also FDG-PET positive. Accordingly, when somatostatin receptor scintigraphy and FDG-PET are available, we found no additional value of ^{123}I-MIBG. Therefore, we doubt that there is any future role of ^{123}I-MIBG imaging in NETs apart from companion diagnostics if ^{131}I-MIBG treatment is planned. For this reason, we do no longer offer ^{123}I-MIBG as a routine method for NET at our department.

9.6.3 ^{11}C-5-HTP

The PET tracer and serotonin precursor ^{11}C-5-hyroxytryptophan (^{11}C-5-HTP) is transported into the neuroendocrine tumor cells via LAT, decarboxylated to serotonin, and transported into secretory vesicles by VMAT.

The PET tracer is labeled with ^{11}C, which has a half-life of 20 min. This demands an on-site cyclotron, and that synthesis is made in close relation to the scan. Accordingly, the capacity of ^{11}C-5-HTP PET scans is limited and only available in

few centers. Maybe for this reason, also data on performance is somewhat limited. A total of 54 patients were studied in three different investigations [41–43], and when data were pooled a sensitivity of 87 % (CI, 75–95 %) was found [9]. One of these studies compared the performance with ^{18}F-DOPA and ^{111}In-DTPA-octreotide and found ^{18}F-DOPA best for staging in carcinoids ($n=24$), whereas ^{11}C-5-HTP was best in pancreatic islet cell tumors ($n=23$) [43]. In another study with 42 consecutive NET patients in comparison with ^{111}In-DTPA-octreotide, ^{11}C-5-HTP found more lesions in 58 % of patients, and it was concluded that ^{11}C-5-HTP could be used as a universal imaging method for detection of NETs [42]. However, no other PET-based imaging was included in this study, and the superiority found was probably largely due to difference between SPECT and PET performance. Moreover, no studies until now have compared ^{11}C-5-HTP with PET-based somatostatin receptor scintigraphy and FDG-PET. Accordingly, at present it is unsettled whether ^{11}C-5-HTP would be the method of choice in unclear NET cases. One could speculate if combined somatostatin receptor PET and FDG-PET would not solve most of such cases in an easier way?

9.7 ^{18}F-FDG

^{18}F-2-deoxy-D-glucose (FDG) is a glucose analogue transported into the cells by means of GLUT transporters, in cancer mainly GLUT-1. In the cell, FDG is phosphorylated by hexokinases, and once phosphorylated it is not further metabolized and therefore metabolically trapped in the cell. In this way FDG accumulates to a degree reflecting the glycolytic activity of cancer cells. It should be noted that it visualizes aerobic glycolysis seen in cancer cells, also known as the Warburg effect, indirectly due to the increased glucose uptake. A high uptake of glucose for oxidative phosphorylation, e.g., in the muscles, will also accumulate high amounts of FDG. For comprehensive visualization of the Warburg effect we recently developed the new imaging concept of hyperPET using a hybrid PET/MR scanner [44].

The sensitivity for detection of NETs is rather low for FDG. We found in a prospective study of 98 consecutive NET patients a sensitivity of 58 % [12]. However, the sensitivity depended largely on proliferation index, and accordingly we found the sensitivity to be 41 % for NETs with Ki67 < 2 % but 92 % for Ki67 at or above 15 %. Since most somatostatin receptor-negative tumors have high proliferation index, FDG-PET may be of value in these tumors. In support of this, we found that of the 11 out of 96 ^{111}In-DTPA-octreotide-negative patients, 7 were FDG-PET positive. Therefore, we suggest that FDG-PET could be used in these somatostatin receptor imaging-negative cases. However, the main application may not lie in diagnosing and staging of NETs but in the strong prognostic value of FDG-PET. In line with this, we found that FDG-PET positivity was a stronger prognostic marker than Ki67, presence of liver metastases, and chromogranin A levels [45]. Since aggressiveness of NETs is a major determinant for selection of therapy [6], one could speculate whether FDG uptake could serve as a relevant grouping parameter alone or in addition to proliferation index. Also FDG-PET, as with other cancer forms, may be used as an early indicator for detection of response or failure to therapy in NET patients.

Conclusion

Somatostatin receptor imaging targeting the subtype 2 receptor is a key in the diagnostic workup and staging in NET patients. Recently especially 3 PET-based tracers, ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC, and ^{68}Ga-DOTANOC, were introduced for clinical use. They all perform better than SPECT tracers and whenever possible should be preferred. Comparison between the 3 PET tracers has not shown any clear differences, and choice is at the discretion of the users. FDG-PET may be used for somatostatin receptor PET-negative cases and for prognostication potentially to be used in selecting therapy strategy. With the abovementioned PET tracers available, the need for ^{123}I-MIBG, ^{18}F-DOPA, and ^{11}C-5-HTP seems less obvious.

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Neuroendocrine Tumors of the Lung

10

Dan Granberg

10.1 Introduction

Lung neuroendocrine tumors, which belong to the foregut group of neuroendocrine tumors, have traditionally been divided into typical and atypical carcinoids [1], large-cell neuroendocrine carcinomas (LCNEC) and small-cell lung carcinomas (SCLC), but several other classifications have subsequently been proposed [2–5]. A new WHO classification was introduced in 1999 [6] based on the work by Travis et al. [7]. This classification was revised in 2004 and apart from the grading also includes a staging system. Typical carcinoids contain fewer than 2 mitoses per 2 mm^2 and lack necroses, while atypical carcinoids have 2–10 mitoses per 2 mm^2 and/or necroses. Large-cell neuroendocrine carcinomas and small-cell lung carcinomas have more than 10 mitoses per 2 mm^2. Areas of necroses are common [8]. Typical carcinoids are usually considered as fairly benign tumors, while atypical carcinoids have a considerable malignant potential, and LCNEC and SCLC are highly malignant. Recently, a new grading system has been proposed for lung neuroendocrine tumors, based on the frequency of mitoses, proliferative rate (Ki67), and presence/absence of necroses [9]. In addition, two other conditions, lung carcinoid tumorlets and diffuse idiopathic neuroendocrine cell hyperplasia (DIPNECH), have been recognized. The most common neuroendocrine tumor in the lung is SCLC (20 %) followed by LCNEC (3 %), typical carcinoid (2 %), and atypical carcinoid (0.2 %). This chapter provides an overview of clinical features, diagnosis, and a framework of the currently available therapeutic measures for neuroendocrine tumors of the lung.

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10.2 Carcinoid Tumorlets and Neuroendocrine Cell Hyperplasia

Neuroendocrine cell hyperplasia refers to the hyperplasia of neuroendocrine cells in the airways. The cells may be arranged as scattered single cells, clusters, or linear arrays but are confined to the basement membrane. In contrast, the cells in tumorlets extend beyond the respiratory epithelial basement membrane [8]. Tumorlets are morphologically identical to typical carcinoids but have a maximum size of 5 mm. Neuroendocrine cell hyperplasia may be reactive and found in patients living at high altitudes, in heavy smokers, or in chronic lung diseases such as bronchiectasis or fibrosis. When there is hyperplasia of neuroendocrine cells without predisposing conditions, it is called DIPNECH. Symptoms of DIPNECH include cough, dyspnea, and wheezing. The condition is usually found in middle-age women. Both tumorlets and DIPNECH may be found in patients with lung carcinoids. The preferable treatment of patients with DIPNECH is not well known, but possible alternatives include bronchodilators, inhaled corticosteroids, surgical excision of the dominant lesion, and somatostatin analogues. In about half of the patients, DIPNECH is stable, and in another half, it progresses. Somatostatin analogues have been effective in stabilizing the condition. Metastatic disease has been described [10, 11].

10.3 Lung Carcinoids

10.3.1 Epidemiology and Etiology

Lung carcinoids are rare tumors, although the incidence has increased during the last decades. The reason for this is not known. The incidence is about 0.7/100,000 in Caucasians and 0.5/100,000 in black people. The disease is slightly more common in women than in men [12]. The disease is most frequent in middle ages but occurs in all ages, even in children. Carcinoid is the most common respiratory tumor in children and adolescents. The etiology is unknown, except that patients with multiple endocrine neoplasia type 1 have a higher risk. Smoking is not a proven risk factor.

10.3.2 Pathology

10.3.2.1 Macroscopic Findings

Lung carcinoids may be located centrally (60–84 %) in the main or lobar bronchi or peripherally. Central carcinoids may be seen at bronchoscopy as a polypoid, highly vascular, cherry-red, white-yellow, or brownish-yellow endobronchial tumor (Fig. 10.1). The tumor often infiltrates the surrounding lung parenchyma. Peripheral carcinoids, which are located in segmental bronchi or more distally, are not accessible by bronchoscopy. They may be multiple and surrounded by small satellite

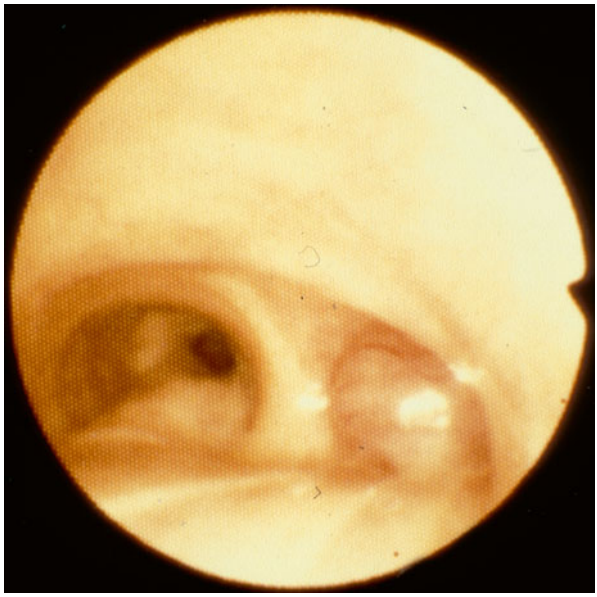

Fig. 10.1 Bronchoscopic visualization of intrabronchial typical carcinoid

lesions, tumorlets. Typical carcinoids may be located anywhere in the lungs, while atypical carcinoids are more often peripheral.

10.3.2.2 Histopathology and Immunohistochemistry

Typical carcinoids are characterized by organoid, trabecular, insular, palisading, ribbon, or rosette-like growth patterns separated by a fibrovascular stroma. The tumor cells are small and polyhedral with small, round, or oval nuclei and eosinophilic, finely granular cytoplasm. Mitoses are rare or absent, and the proliferative rate is usually low. Atypical carcinoids are characterized by nuclear pleomorphism, hyperchromatism, abnormal nuclear-to-cytoplasmic ratio, prominent nucleoli, and areas of increased cellularity with disorganized architecture. Mitoses are more frequent, and necroses may be found (Fig. 10.2a–c). At electron microscopy, typical carcinoids have abundant membrane-bound secretory granules, while atypical carcinoids have fewer granules, distributed in the cytoplasm [13].

Lung carcinoids are epithelial in origin and stain positive for cytokeratin. Most of the tumors also stain positive for the neuroendocrine markers chromogranin A (Fig. 10.2d), synaptophysin, and neuron-specific enolase. In addition, positive immunohistochemistry for various hormones, including serotonin, gastrin-releasing peptide (mammalian bombesin), pancreatic polypeptide, gastrin, human chorionic gonadotropin alpha subunit, leucine enkephalin, VIP, somatostatin, calcitonin, ACTH, and growth-hormone-releasing hormone is found in a majority of tumors. Positive staining for multiple hormones is not uncommon [13, 14]. Expression of S-100 protein may occur, usually in peripheral tumors [15, 16]. The standard (hematopoietic) form of the adhesion molecule CD44 (CD44s) is usually expressed in lung carcinoids and may be used for prognostic evaluation in typical carcinoids

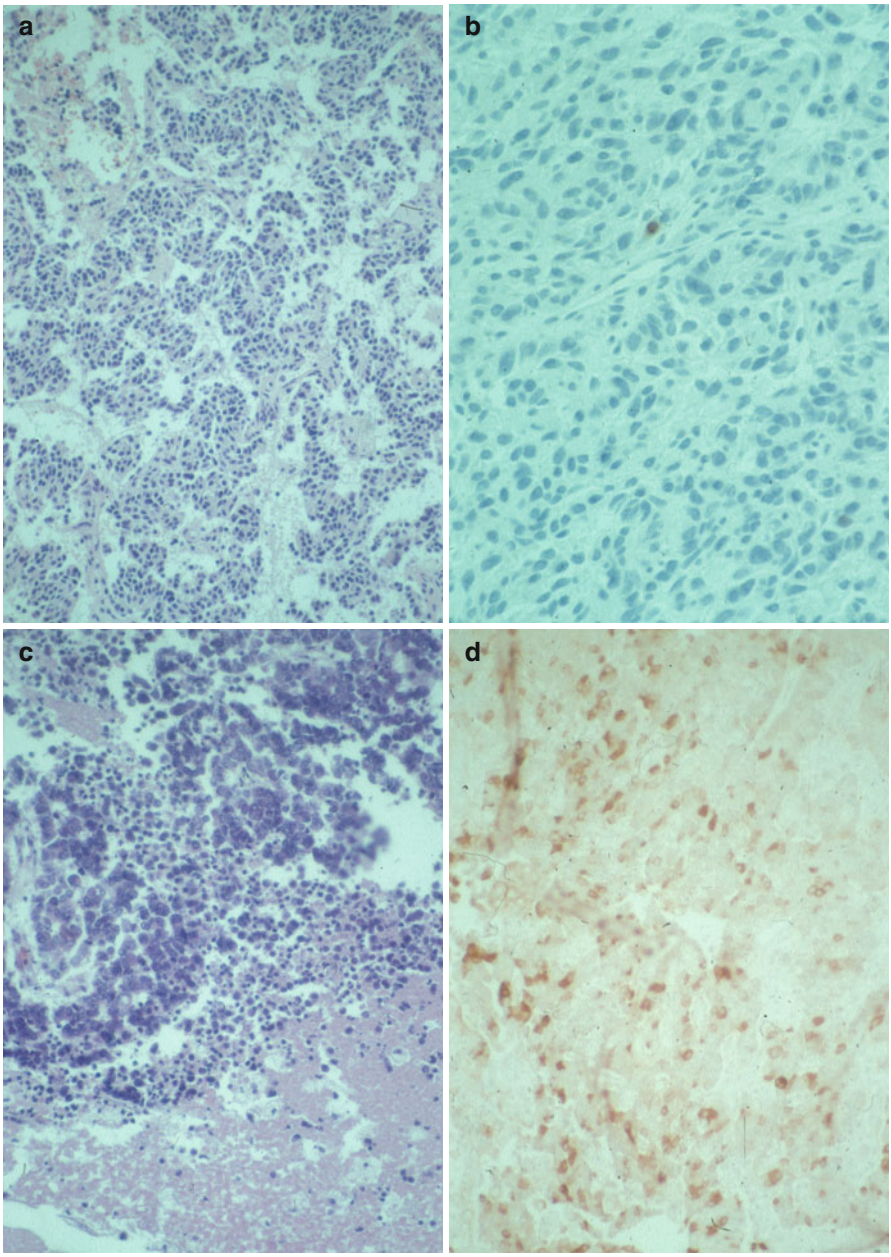

Fig. 10.2 (**a**) Typical lung carcinoid. Hematoxylin-eosin stain. Magnification, ×200. (**b**) Typical lung carcinoid. Ki67 stain, low proliferation. Magnification, ×400. (**c**) Atypical lung carcinoid. Hematoxylin-eosin stain. Magnification, ×200. (**d**) Typical lung carcinoid. Chromogranin A stain, strong positive. Magnification, ×200

[17]. Positive staining for the retinoblastoma gene protein is also common in typical carcinoids [18, 19]. The oncoproteins *p53* and *bcl-2*, however, are usually negative in typical carcinoids but more frequently positive in atypical carcinoids [20, 21]. Thyroid transcription factor-1 (TTF-1) is positive in 28–69 % of lung carcinoids [22, 23] and may help to differentiate a primary lung carcinoid from a metastasis from a neuroendocrine tumor originating elsewhere.

10.3.3 Genetic Alterations

Deletions in the *MEN1* locus at 11q are common in both typical and atypical carcinoids [24]. Homozygous somatic inactivation of the *MEN1* gene is detected in 36 % of sporadic lung carcinoids [25]. Atypical carcinoids frequently have deletions in 10q or 13q [24]. Aneuploidy has been reported in 5–32 % of typical carcinoids and 17–79 % of atypical carcinoids [4, 26–28].

10.3.4 Clinical Presentation

A substantial number of patients (13–51 %) are asymptomatic, and the tumor is diagnosed incidentally on routine chest X-ray or CT scan. Common symptoms on presentation include cough, hemoptysis, wheezing, recurrent pneumonias with or without persisting lung infiltrate on chest X-ray, dyspnea, and chest pain [14, 29–31]. Some patients may have several years' delay in the correct diagnosis due to misdiagnosis as asthma. Like other neuroendocrine tumors, lung carcinoids may secrete hormones. Endocrine symptoms are however rare. Despite serotonin immunoreactivity is present in up to 84 % of the tumors, the classical carcinoid syndrome with flush, diarrhea, bronchoconstriction, right-sided valvular heart disease, and elevated urinary 5-hydroxyindolacetic acid is seen in only about 2–12 % of the patients, usually when liver metastases are present [32–34]. This low frequency may in part be due to the high pulmonary content of monoamine oxidase, which metabolizes serotonin, but also to the fact that most lung carcinoids do not give rise to distant metastases. An atypical carcinoid syndrome with generalized flushing, edema, lacrimation, asthma, and diarrhea may occasionally be seen. This syndrome, which must not be confused with atypical carcinoids, is caused by secretion of histamine. An ectopic Cushing's syndrome, caused by production of adrenocorticotropic hormone (ACTH) or corticotropin-releasing factor, may be seen in 2–6 % of patients with lung carcinoids. These tumors are often small and difficult to detect with conventional radiologic imaging. Acromegaly, caused by secretion of growth-hormone-releasing hormone, is infrequent [35, 36].

Typical carcinoids metastasize in 5–20 % of patients, while up to 70 % of patients with atypical carcinoids develop metastases. Metastases most frequently occur in regional lymph nodes but also distantly to the liver, bones, brain, subcutaneous

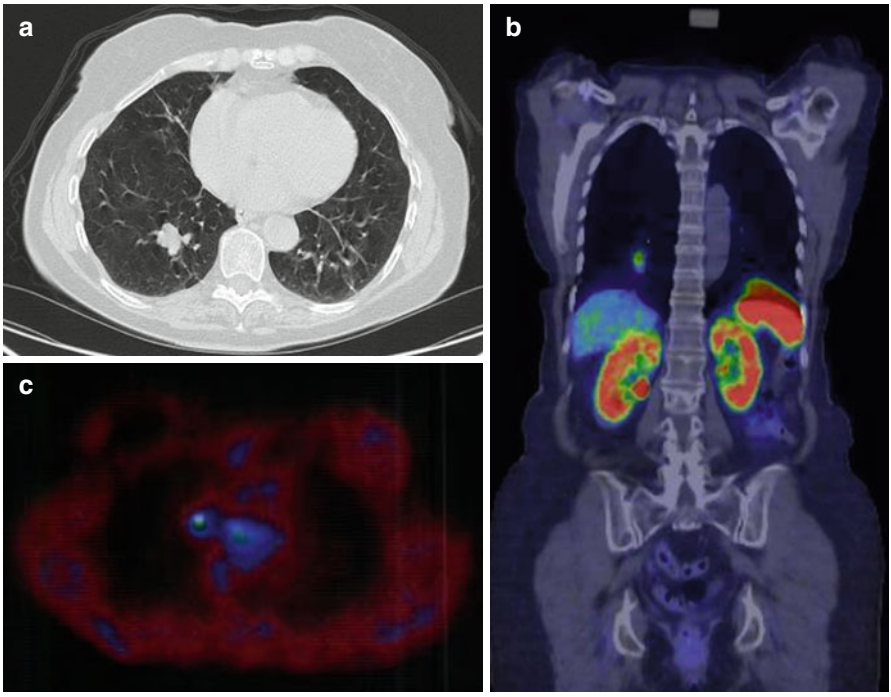

Fig. 10.3 (a) CT scan showing a carcinoid in the right lung. (**b**) PET with ^{68}Ga-DOTATATE showing a carcinoid in the right lung. (**c**) PET with ^{11}C-5-HTP showing a right hilar lymph node metastasis from a typical carcinoid in the right lung, 9 years after primary surgery

tissue, mammary glands, eyes, and adrenals [1, 32, 37, 38]. Metastases may occur late, up to 30 years after surgery for the primary tumor.

10.3.5 Diagnosis

In more than 60 % of the patients, the tumor is visible on chest X-ray. Peripheral tumors are seen as round or oval nodules. Patients with central tumors may have signs of bronchial obstruction and have peripheral atelectasis or pneumonic infiltrates which may be persistent. Computerized tomography (CT) scan (Fig. 10.3a) is more sensitive than chest X-ray and should always be performed in order to identify enlarged lymph nodes, delineate tumor growth, and detect satellite lesions (tumorlets). Magnetic resonance imaging (MRI) may be an alternative to CT scan but is less sensitive in detecting small intrapulmonary lesions.

About 70 % of lung carcinoids express somatostatin receptors and are demonstrable on Octreoscan (scintigraphy with ^{111}In-octreotide) [39]. Positron emission tomography (PET) with ^{68}Ga-DOTATCOC or ^{68}Ga-DOTATATE (Fig. 10.3b) is a newer, more sensitive method to detect somatostatin receptor-positive

neuroendocrine tumors. ^{68}Ga-PET or Octreoscan should always be performed preoperatively for staging of the disease and clarify whether these methods may be used for postoperative follow-up to detect recurrence or metastases. Patients with Cushing's syndrome often have small tumors, which are difficult to detect on CT scan. Up to 12 years delay has been described in localizing the tumor [40]. In these patients, PET with ^{68}Ga or Octreoscan is especially useful to obtain the correct diagnosis. An alternative is PET with ^{11}C-5-HTP (Fig. 10.3c), which is a sensitive tool for detection of neuroendocrine tumors [41]. The value of PET with ^{18}F-fluorodeoxyglucose (FDG) is controversial. Some authors claim that it is of limited value in detecting lung carcinoids, since these tumors often display lower uptake than expected for malignant tumors [42, 43], while others have found this method useful in patients with both typical and atypical carcinoids [44].

Central tumors are accessible via bronchoscopy (Fig. 10.1), which is performed in most patients. Since the tumor is often covered by a layer of normal mucosa, brushing or sputum cytology is frequently negative. To obtain a preoperative diagnosis, it is thus important to take biopsies, despite the risk of bleeding. It is generally safe to take biopsies, both through a flexible and a rigid bronchoscope [45]. Peripheral tumors may be reached by transthoracic CT-guided core-needle biopsy, although misdiagnosis is not uncommon, since the differential diagnosis to small-cell lung carcinoma may be difficult. Staining the tissue sample for the proliferation marker Ki67 may aid in the differential diagnosis between these entities [46, 47].

Plasma chromogranin A should always be measured preoperatively but is usually normal if the tumor is confined to the lung. If chromogranin A is elevated, intense search for distant metastases is warranted. Measurement of urinary 5-hydroxyindolacetic acid, urinary cortisol, and plasma ACTH is not recommended routinely but is only indicated if the patient has endocrine symptoms.

10.3.6 Differential Diagnosis

Alternative diagnoses when a tumor is found on chest X-ray or CT scan include other benign or malignant lung neoplasms, hamartoma, and metastasis from another primary tumor. The histopathological differentiation, both via bronchoscopy and transthoracically, between atypical carcinoid and SCLC may sometimes be difficult, but is important since patients with carcinoids are often cured by surgery, which is generally not performed in patients with SCLC. High proliferative rate, Ki67 > 25 %, favors the diagnosis of SCLC, while a low Ki67 index indicates lung carcinoid [46, 47].

10.3.7 Treatment

The main treatment for patients with lung carcinoids is surgery, which offers the only chance for cure. External radiotherapy is mainly used for palliation of bone or

brain metastases. Patients with metastatic disease or inoperable tumors can be treated with various chemotherapy combinations, peptide receptor radionuclide therapy (PRRT) with ^{177}Lu-DOTA-octreotate or ^{90}Y-DOTA-octreotide, biotherapy (somatostatin analogues, alpha-interferon), or newer targeted therapies such as everolimus or sunitinib. Patients with liver metastases may undergo hepatic arterial embolization with particles, chemotherapeutic agents, or ^{90}Y-labeled microspheres (SIR-Spheres® or TheraSpheres®). Symptomatic treatment for patients with carcinoid syndrome includes somatostatin analogues and alpha-interferon. Patients with metastatic tumors and Cushing's syndrome are generally subjected to bilateral adrenalectomy; symptom relief may be obtained with ketoconazole, metyrapone, somatostatin analogues, or mitotane.

10.3.7.1 Surgery

Radical surgery should be considered in all patients with lung carcinoids confined to the thorax and is in most patients curative. The principles of surgery include complete removal of the primary tumor, preservation of as much healthy lung parenchyma as possible, and a thorough lymph node dissection, aided by frozen sections, with removal of all affected nodes. Possible surgical procedures include bronchotomy with resection of the tumor and bronchoplasty, sleeve resection, wedge or segmental resection, lobectomy, bilobectomy, and pneumonectomy. Wide resection margins are not necessary, but in patients with atypical carcinoids it is recommended to perform at least a lobectomy. Endoscopic removal of the tumor by YAG laser has previously not been recommended, since lung carcinoids often grow deeply into the surrounding tissue (the "iceberg" phenomenon), and radical removal thus is difficult to obtain by laser resection. Two recent studies however questioned this opinion and found initial bronchoscopic laser treatment to be a safe and effective therapy for patients with intrabronchial typical carcinoids. In 36–46 % of the patients, open surgery was however later required [48, 49]. One indication for endoscopic tumor removal by YAG laser is palliation of obstructive symptoms in patients with high cardiopulmonary operative risk and short life expectancy. In a limited number of patients, surgery may be considered after previous laser treatment to reduce the tumor and allow post-obstructive infiltrates to resolve. There are no studies showing the benefit of adjuvant treatment after radical surgery.

10.3.7.2 Radiotherapy

Patients with bone or brain metastases may have palliation from external radiotherapy. This may also be considered in cases with inoperable tumors or incomplete resection. PRRT is an interesting option in patients with metastatic or inoperable tumors and high expression of somatostatin receptors. Two uncontrolled studies have shown promising results. In a small study, 5/9 patients treated with ^{177}Lu-DOTA-octreotate had a radiological response lasting a median of 31 months [50], and in another report, 24/84 patients (29 %) receiving ^{90}Y-DOTA-octreotide responded radiologically [51]. Since there was a high frequency (9.5 % of all patients) of severe permanent decrease in renal function in

patients treated with ^{90}Y-DOTA-octreotide, it may be preferable to choose ^{177}Lu-DOTA-octreotate for PRRT.

10.3.7.3 Chemotherapy

The treatment of patients with metastatic lung carcinoids depends on the histopathology (typical versus atypical carcinoid), proliferative rate (Ki67 index), somatostatin receptor expression, extent of disease, performance status of the patient, and renal and bone marrow function. Various chemotherapy combinations have been tried with limited success, including cisplatin or carboplatin + etoposide, docetaxel or paclitaxel ± doxorubicin, streptozotocin + 5-fluorouracil or doxorubicin, oxaliplatin + capecitabine, 5-fluorouracil + dacarbazine + epirubicin, and 5-fluorouracil + cisplatin + streptozotocin [52–56]. A recent study however confirmed earlier observations that temozolomide as monotherapy may be active in these patients. Significant tumor reduction was seen in 14 % and stabilization of progressive disease in 52 %. All patients with partial response had atypical carcinoids, but stabilization was observed in typical as well as atypical carcinoids [57, 58]. Combining temozolomide with capecitabine and/or bevacizumab may have better effect, but this has not been studied. These combinations are mainly used in patients with higher proliferation or if progression occurs on monotherapy with temozolomide. Patients with high Ki67 often receive platinum-based chemotherapy as first-line treatment.

10.3.7.4 Biotherapy

Biotherapy with somatostatin analogues (octreotide, lanreotide) and alpha-interferon is less effective in patients with lung carcinoids than small-bowel neuroendocrine tumors but may in some patients with low proliferation lead to control of the disease. Another indication for somatostatin analogues is symtomatic relief of a classical carcinoid syndrome or an atypical carcinoid syndrome. Sometimes, the addition of alpha-interferon is necessary to control the carcinoid syndrome.

10.3.7.5 Targeted Therapies

During recent years, new drugs targeting signal pathways or membrane receptors have proven to be active in patients with neuroendocrine tumors. Both everolimus, an mTOR inhibitor, and sunitinib, inhibiting vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and c-kit, have shown to prolong progression-free survival in patients with pancreatic endocrine tumors. In a subanalysis from the randomized, placebo-controlled RADIANT-2 study, everolimus + octreotide LAR was found to prolong progression-free survival in patients with lung carcinoids from 5.6 months to 13.6 months compared with placebo + octreotide LAR. The difference was however not significant, $p = 0.228$. Minor response was seen in 67 % in the everolimus group and 27 % in the placebo group [59]. An ongoing study, which has recently completed the inclusion, is comparing everolimus, pasireotide (another somatostatin analogue), and everolimus + pasireotide in patients with well-differentiated lung carcinoids. The results are awaited with interest. Sunitinib has not been studied in lung carcinoids, but

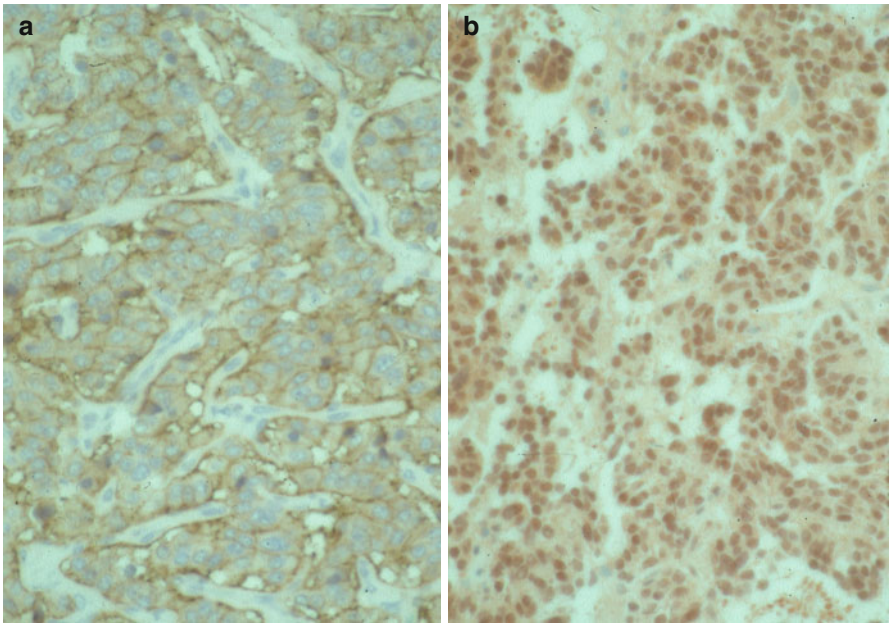

Fig. 10.4 (**a**) Typical lung carcinoid. CD44s stain, strong positive. Magnification, ×400. (**b**) Typical lung carcinoid. *nm23* stain, strong positive. Magnification, ×400

many lung carcinoids express the tyrosine kinase receptors PDGFRα, PDGFRβ, c-kit, and EGFR [60]. This makes it possible that sunitinib and other tyrosine kinase inhibitors may have activity in lung carcinoids.

10.3.8 Prognosis

Most patients with typical lung carcinoids are cured by surgery, even in the presence of lymph node metastases, and have an excellent prognosis. Five- and 10-year survivals are 87–100 % and 82–95 %, respectively [7, 14, 61–63]. Poor prognostic factors include lymph node metastases at diagnosis, presence of tumorlets, and high Ki67 index. On the other hand, positive immunohistochemistry for the standard form of the adhesion molecule CD44 (Fig. 10.4a) and positive nuclear staining for the metastasis suppressor gene *nm23* (Fig. 10.4b) correlate with decreased risk for distant metastases and death [17, 64]. The prognosis for patients with atypical carcinoids is worse, although many of these patients are also cured by surgery. Five- and 10-year survivals are 40–69 % and 31–59 %, respectively [14, 30, 62, 65]. Since metastases may develop late, up to 30 years after primary surgery, patients with lung carcinoids must be followed for long time, at least 10–15 years. The follow-up should include measurement of plasma chromogranin A, CT scan of the thorax and abdomen, and PET with ^{68}Ga-DOTATAOC/DOTATATE (in patients with

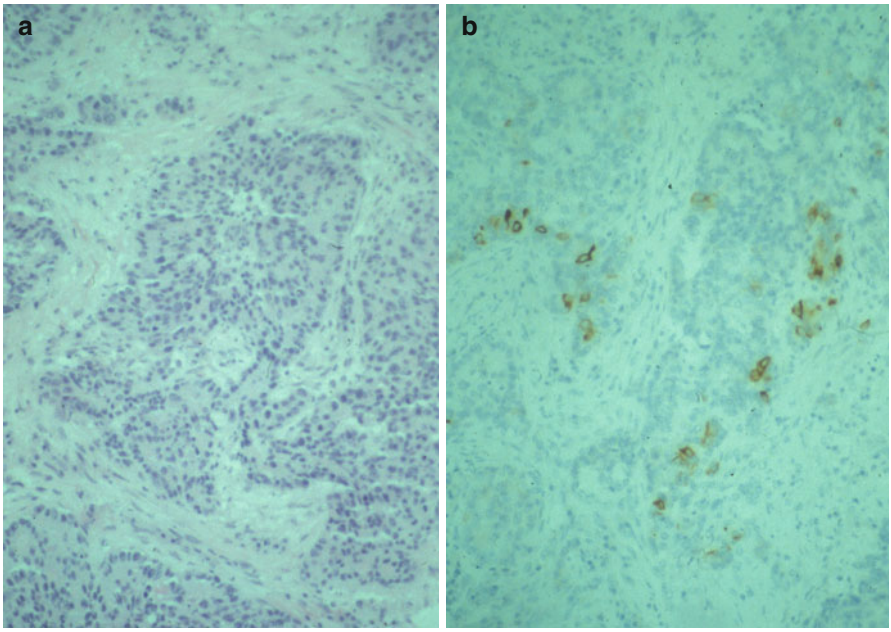

Fig. 10.5 (**a**) Large-cell neuroendocrine carcinoma. Hematoxylin-eosin stain. Magnification, ×200. (**b**) Large-cell neuroendocrine carcinoma. Chromogranin A stain, weak positive. Magnification, ×200

somatostatin receptor-positive tumors) or MRI of the vertebral column. In selected patients, repeated bronchoscopies are indicated. Special attention should be paid to patients with atypical carcinoids or high proliferative rate.

10.4 Large-Cell Neuroendocrine Carcinoma

Large-cell neuroendocrine carcinomas constitute about 3 % of all lung cancers. Almost all patients are smokers. The disease is most frequent in older patients; mean age is between 59 and 66 years. Men are affected more often than women and constitute 55–86 % of the cases [4, 66, 67].

10.4.1 Pathology

LCNEC are usually peripheral and seen as large masses on X-ray. The tumor consists of large cells with moderate to abundant finely granular, eosinophilic cytoplasm and frequent nucleoli. The cells are arranged in organoid, palisading, trabecular, or rosette-like patterns (Fig. 10.5a). The mitotic rate is high, ≥11 (average 75) per 2 mm^2. Large areas of necroses are frequent. The proliferative rate is high.

Aneuploidy is found in 75 % of the tumors. Positive immunohistochemistry for neuroendocrine markers such as chromogranin A (Fig. 10.5b), synaptophysin, and neuron-specific enolase is less frequent than in lung carcinoids. One positive marker is enough for the diagnosis. TTF-1 is positive in about 50 % of the tumors [8]. Positive staining for *p53* and *bcl-2* is common [68], but retinoblastoma protein is usually not present by immunohistochemistry [19]. *K-ras* mutations occur in the same frequency as in other non-small-cell lung cancer [8]. At electron microscopy, the ultrastructural appearance is variable. The majority of tumors have only few cytoplasmic neurosecretory granules.

10.4.2 Clinical Presentation

Common presenting symptoms in patients with LCNEC include cough, dyspnea, hemoptysis, obstructive pneumonias, or constitutional symptoms such as weight loss and malaise. Some patients are asymptomatic and diagnosed incidentally by an abnormal chest X-ray. Endocrine symptoms due to ectopic hormone production have not been described. Metastases occur in between 71 and 100 % of the patients with LCNEC, most often to the hilar or mediastinal lymph nodes. Distant metastases occur to the pleura, liver, brain, bones, adrenal glands, pericardium, and abdominal lymph nodes.

10.4.3 Diagnosis

Many tumors are detectable on chest X-ray. CT scan of the chest and abdomen, as well as PET with FDG, is of value for staging. Histopathological diagnosis may be obtained by surgery or transthoracic core-needle biopsy.

10.4.4 Treatment

There is no consensus regarding the treatment of patients with LCNEC. Surgery is indicated in patients with stage 1. Since the risk for recurrence and distant metastases is high, surgery must be combined with adjuvant chemotherapy. Active combinations include cisplatin + etoposide, cisplatin + irinotecan, and nadaplatin + irinotecan. Several studies have reported the response to cisplatin-based chemotherapy to be comparable in patients with advanced LCNEC and SCLC [69–73]. Amrubicin as monotherapy has also some activity in LCNEC [74]. Patients with widespread disease may receive the same chemotherapy regimens. The role of radiotherapy is more controversial. Patients with EGFR mutations may obtain partial responses with gefitinib [75, 76]. Octreotide, which prolongs progression-free survival in patients with slow-growing neuroendocrine tumors, may be used as adjuvant treatment after surgery for LCNEC [77]. Sunitinib and other agents targeting VEGFR and c-kit are possible new drugs for treatment of LCNEC [78].

10.4.5 Prognosis

The prognosis for patients with LCNEC is dismal. The reported 5-year survival varies between 15 and 57 % [67, 79–85]. Patients with stage 1 tumors have slightly better 5-year survival, 27–67 % [80, 82, 83, 85–87].

10.5 Small-Cell Lung Carcinoma

10.5.1 Epidemiology and Etiology

Small-cell lung carcinoma, which is the most aggressive form of lung malignancy, accounts for 14–20 % of all cases of lung cancer. It is most common in the seventh and eighth decades. There is a clear male preponderance, but this discrepancy may decrease due to the increasing smoking habits among women. Almost all patients with SCLC are smokers, which represents the main risk factor. Other known etiologic factors include ionizing radiation, asbestos, aromatic hydrocarbons (in particular chloromethyl ethers), and various metals [88].

10.5.2 Pathology

A majority of SCLC are centrally located, appearing as hilar or perihilar masses, spreading early to hilar and mediastinal lymph nodes [8]. Only about 5 % are seen as a peripheral solitary mass [89]. Distant metastases occur to the brain, liver, bone marrow, bones, adrenal glands, and pancreas. Brain metastases are seen in 10–20 % on presentation but occur later as the disease progresses in 25 % of patients not receiving prophylactic cranial irradiation [90]. In total, 50–80 % of the patients will develop brain metastases [91].

10.5.2.1 Light Microscopy

Small-cell lung carcinoma consists of small cells, usually less than the diameter of 3 small resting lymphocytes. The cells are round to fusiform and have a high nuclear-to-cytoplasmic ratio and finely granular, hyperchromatic nuclei with inconspicuous or absent nucleoli. Histologic patterns include trabeculae, spindling, nesting, palisading, rosettes, or solid-sheet-like growth, with indistinct cell borders. The mitotic rate is high, $\geq$11 per 2 mm^2, median 80 per 2 mm^2. Necrosis and crush artifacts are frequent. The proliferative rate is high (Fig. 10.6). Combined small-cell lung carcinoma is defined as small-cell carcinoma combined with an additional component, usually adenocarcinoma, squamous cell carcinoma, or large-cell carcinoma [8].

10.5.2.2 Immunohistochemistry

The neuroendocrine markers chromogranin A, synaptophysin, and NSE stain positive in most SCLC, and TTF-1 is positive in up to 90 % of the tumors [92, 93].

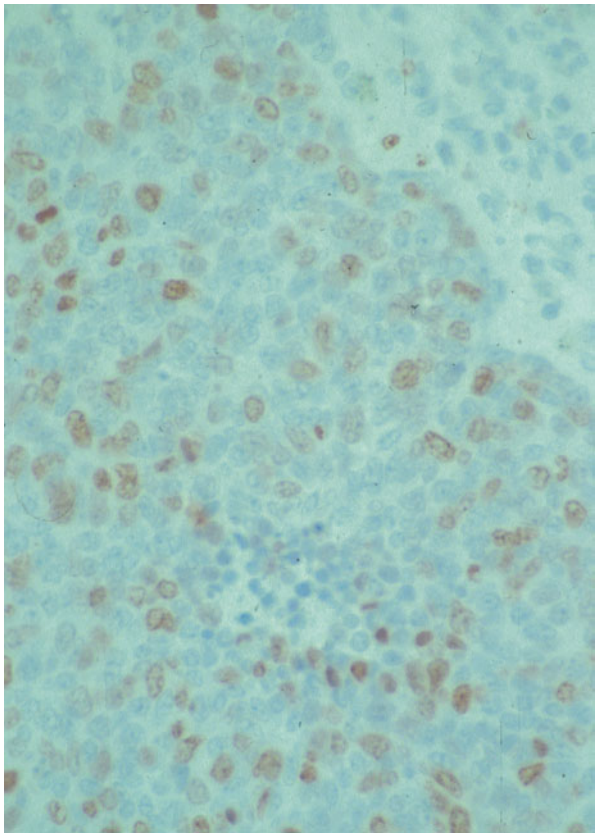

Fig. 10.6 Small-cell lung carcinoma. Ki67 stain, high proliferation. Magnification, ×400

Hormonal immunoreactivity, most often for ACTH, bombesin, and serotonin, may also be found, but staining for multiple hormones is less common than in lung carcinoids [4, 5]. Gastrin-releasing peptide has been shown to function as a growth factor for SCLC [94]. Positive immunostaining for *p53* is common [18, 20, 68] as is *bcl-2* immunoreactivity [68, 95, 96]. Retinoblastoma gene protein is usually negative at immunohistochemistry [18, 19, 97].

10.5.2.3 Electron Microscopy

Neurosecretory granules, about 100 nm in size, are found in at least 60–70 % of cases [8]. Basal laminae are scarce or absent [5].

10.5.3 Genetic Alterations

All SCLCs are aneuploid. The multistep process for development of invasive SCLC encompasses activations of several proto-oncogenes such as *kit*, *SCF*, and *myc* (*c-*, *N-*, and *L-*) and inactivation of several suppressor genes [98]. Some of the most

common allelic deletions are various loci on 3p (present in nearly 100 %), 4, 5q, 10q, 13q, and 17p. DNA gains are seen on 3q, 5p, 6p, 8q, 17q, 19, and 20q [99]. The *p53* gene is homozygously mutated in 90 % of SCLC [100]. The retinoblastoma protein is absent or abnormal in an equally high percentage of tumors. Other characteristic features of SCLC are amplification of M*YC* [101] and methylation of caspase-8 DNA [102]. DNA gain on 17q24–q25 is a potential marker for development of brain metastases [103].

10.5.4 Clinical Presentation

Presenting symptoms include cough, weight loss, dyspnea, chest pain, hoarseness, dysphagia, and superior vena cava syndrome. Endocrine syndromes or paraneoplastic neurologic syndromes are not uncommon on presentation. SIADH (syndrome of inappropriate secretion of antidiuretic hormone) is seen in 11–46 %; ectopic ACTH production leading to Cushing's syndrome, typically causing peripheral edema, proximal myopathy, hypertension, glucose intolerance, and metabolic alkalosis occurs in 2–5 %, and the Eaton-Lambert myasthenic syndrome in 3 % of patients [104, 105].

10.5.5 Staging

Although a TNM classification has been proposed for SCLC [106, 107], since this tumor is not considered as a surgical disease, the TNM classification may not be applicable. Another system is recommended by the Veterans Administration Lung Cancer Study Group based on the feasibility including all known tumor locations in one radiation field. Thus, limited-stage (LS) disease is defined as tumor confined to one hemithorax, with or without hilar, ipsilateral/contralateral mediastinal, and/or supraclavicular lymph node metastases, with/without ipsilateral pleural effusion, while extensive-stage (ES) disease is tumor outside the thorax or in the contralateral lung [108]. About 60–70 % of the patients have extensive disease when diagnosed.

10.5.6 Diagnosis

Apart from chest X-ray, CT scan of the thorax, abdomen, and brain as well as PET with FDG is of value for staging. Octreoscan is of limited value, since metastases are depicted only in about 50 % of the patients, yet nearly all primary tumors are found [109–111]. Although sputum cytology is positive in 75–80 % of patients with SCLC [88], the diagnosis should be based on acquiring enough tissue material for histologic diagnosis. Bronchoscopy, transthoracic core-needle biopsy, mediastinoscopy, or surgical excision of a supraclavicular lymph node can make the diagnosis. It may sometimes be difficult to differentiate between SCLC and atypical carcinoid tumors. Staining of the tissue sample for Ki67 may aid in this differential diagnosis [46, 47].

10.5.7 Treatment

Surgery has little role in the treatment of patients with SCLC, unless in rare patients in whom the diagnosis is made early. The main treatment is chemotherapy and radiotherapy. Earlier schemes included combinations of cyclophosphamide, doxorubicin, and vincristine. Today, platinum-based chemotherapy is used as first-line treatment, with 60–70 % response rates [112]. Patients with limited-stage disease is usually treated with cisplatin or carboplatin + etoposide combined with thoracic radiotherapy. Patients who respond receive prophylactic cranial irradiation. Up to 20 % of patients will have long-term disease control, but a majority will recur. Patients with extended-stage disease are treated with chemotherapy alone, usually cisplatin/carboplatin + etoposide or platinum + irinotecan. Prophylactic cranial irradiation is given to patients who respond. Second-line treatment in relapsing or not responding patients includes topotecan, paclitaxel, docetaxel, irinotecan, vinorelbine, gemcitabine, and temozolomide, but response rates are limited or poor [112].

10.5.8 Prognosis

The prognosis for patients with SCLC is dismal. Overall 5-year survival is <7 % [112]. Patients with limited-stage disease have significantly better prognosis (median survival of 16–22 months, 5-year survival around 25 %, and about 20 % cured) than patients with extensive-stage disease (median survival 10 months, 2-year survival <5 %) [112, 113]. The main poor prognostic factors are more widespread disease (even within the stages), poor performance status, male gender, and old age [114].

10.6 Concluding Remarks

Lung carcinoids are generally slowly growing tumors, yet harboring malignant potential. The main treatment is surgery with resection of the primary tumor and affected lymph nodes. Maximal preservation of normal lung parenchyma is warranted. There is no scientific evidence for postoperative adjuvant treatment. Long-term follow-up is necessary, since recurrences may occur late after several decades. Atypical carcinoids have a more malignant behavior than typical. Large-cell neuroendocrine carcinomas are highly malignant tumors with poor prognosis. If possible, radical surgery should be performed, followed by adjuvant chemotherapy. Small-cell lung carcinomas are highly aggressive tumors, rapidly spreading to distant organs. Intensive platinum-based chemotherapy, in patients with limited-stage disease combined with concurrent thoracic radiotherapy, should be started as soon as possible. Patients who respond to initial treatment should be offered prophylactic cranial irradiation. The prognosis is nevertheless dismal, with short expected survival (Fig. 10.7).

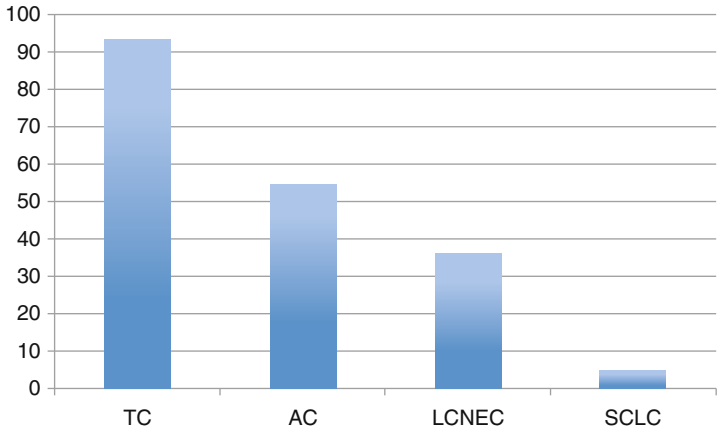

Fig. 10.7 Median 5-year survival in patients with typical carcinoids (*TC*), atypical carcinoids (*AC*), large-cell neuroendocrine carcinomas (*LCNEC*), and small-cell lung carcinomas (*SCLC*)

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Overview of Gastrointestinal Neuroendocrine Tumours

11

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Neuroendocrine cells are distributed throughout the body with different potentials of action; neoplasms of these neuroendocrine cells may arise in various tissues. There are differences in terminology, classification and grading of these neuroendocrine neoplasms, mostly the organ or tissue of origin leads to a specific nomenclature and grading.

Neuroendocrine tumours (NETs) in the digestive system are a group of neoplasms related to tumours arising from tubular gastrointestinal organs and pancreas. GI-NETs are rare neoplasms. During the last few years, there have been important advances in the management of NETs as several treatment options have been made available for even patients with advanced disease, including cytotoxic therapy and targeted agents. In this chapter, a brief overview of gastrointestinal NETs is given as an introduction.

11.1 Epidemiology

GI-NETs are relatively rare, accounting for approximately 0.5 % of all human cancers. The incidence of GI-NETs is reported to be 1–2 case per 100,000 individuals per year, although there is evidence for a remarkable increase in the incidence of these tumours, probably due to increased awareness as well as increased detection

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through new endoscopic and imaging techniques [1, 2]. In 1997, Modlin et al. summarised the results from the SEER registry and pointed out to a rise in the incidence patterns over the two time periods (1973–1991 and 1992–1999). The age-adjusted incidence rates of GI-NETs increased from 0.84 to 1.77 for white males and from 0.79 to 1.49 per 100,000 person per year for white females. The rates increased in a similar fashion for African-Americans. In the SEER database from the USA, the increase of incidence of carcinoids was 3 %, annually [3]. Metastatic disease accounts for 12–22 %. Most NETs are mainly sporadic, but clustering within families and association with the genetic syndromes are also possible, and an increased risk of secondary cancers has been reported [4].

11.2 Classification

Traditionally, GI-NETs have been called as carcinoids. However, the term carcinoid may lead to a misunderstanding of a benign lesion, although carcinoid on its own does not classify a lesion as benign or malignant. Nevertheless, this term remains in widespread use, both in the official WHO classification of NETs of the lung and as a synonym for NETs arising in the tubular GI tract.

Clinically, these tumours are defined as functioning or non-functioning depending on the presence of a syndrome related to increased peptide or hormone secretion. Such a functional distinction, however, does not provide prognostic information [1], although in non-functioning or non-syndromic cases, the diagnosis can be delayed for up to 5 years [5]. This causes significant numbers of patients admitting to hospitals in metastatic stage (up to 25 %) or unresectable disease (60 %) [2, 6, 7].

In 1963, William and Sandler divided the GI-NETs into three categories based on an embryogenic basis: foregut carcinoids (lung, stomach, duodenum, upper jejenum, and pancreas), midgut carcinoids (lower jejenum, ileum, appendix, caecum) and hindgut carcinoids (colon and rectum) [8] (Table 11.1). However, this classification was not practical in the diagnosis and clinical workout of patients. At the end of 2010, a new classification system, the WHO 2010, was developed by European Neuroendocrine Tumor Society (ENETS)/American Joint Cancer Committee (AJCC). The new system is an integration of fundamental criteria of former WHO classification (differentiation and proliferation) together with tumour node

Table 11.1 Embryologic classification of carcinoids

Foregut carcinoids	Midgut carcinoids	Hindgut carcinoids
Thymus	Jejunum	Transverse colon
Bronchus	Ileum	Descending colon
Stomach	Ascending colon	Sigmoid colon
Duodenum	Caecum	Rectum
	Appendix	Genitourinary tract

metastases (TNM) staging and grading systems. In WHO 2010 system, former criteria such as tumour site, size, extent and vascular invasion were changed with widely used criteria of TNM staging.

The ENETS classification 2006/2007, the 7th American Joint Cancer Committee/Union Internationale Contre le Cancer (AJCC/UICC) classification 2009 and the current WHO classification 2010 all equally define a grading system based upon the proliferation index Ki-67 and/or mitotic index with low-grade G1 tumours (Ki-67 $\leq$2 %, mitotic count <2 per 10 high-power field; HPF) (9). Well-differentiated NETs are classified together as neuroendocrine tumours (NETs) grade 1 (G1) or G2. The carcinoid term is sometimes used instead of NET G1. The term neuroendocrine carcinoma refers to all poorly differentiated NETs and high-grade (G3) malignant neoplasms. NEC is further subdivided into a small-cell and a large-cell subtype. Mixed adenoneuroendocrine carcinomas (MANEC) and hyperplastic and preneoplastic lesions are termed as special groups. Tumour characteristics as localisation, size, composition, relationship to anatomic structures, resection margins and the presence of metastases should be assessed in order to classify the tumour according to the TNM staging system [9].

11.3 Oesophageal NETs

Oesophageal neuroendocrine tumours are extremely rare. In 1969, an oesophageal carcinoid case was reported [10]. The age of oesophageal NET patients ranged from 44 to 82 (median 64 years). The lesions predominate in males. Dysphagia, weight loss, pain and reflux oesophagitis are the most common symptoms. The diagnosis is based on classical approach as to other oesophageal masses.

Reported cases are poorly differentiated tumours. Immunohistochemical studies revealed positivity for NSE and serotonin, but these tumours lacked argentaffin and argyrophilic staining. Barrett's oesophagus may be a potential factor for development of oesophageal NETs.

The stage is an important prognostic factor for oesophageal NETs. Stage I (localised disease to mucosa, lamina propria or submucosa) and stage II (involving only the muscularis propria) NETs have a good prognosis after resection. However, patients with stage III (extending through the muscularis propria into adventitia) and IV grade tumours (invading the adventitia, as well as involving a mediastinal structure, most commonly pulmonary vessels) typically succumb to the disease, as such lesions have a propensity for significant local spread and relatively poor outcome. In one series, most of the tumours had already invaded the oesophageal wall and local lymph node metastasis was present [11]. However, there are also few cases reported to be successfully treated with oesophagogastrectomy [12].

The small size of reported patients limits to develop a general therapeutic approach, but most reports indicate oesophagogastrectomy and subtotal excision with gastro-oesophageal anastomosis are the preferred methods [13].

11.4 Gastric NETs

Gastric neuroendocrine tumours are rare neoplasms arising from enterochromaffin cells of the stomach. Gastric NETs are heterogenous neoplasms consisting of 8.7 % of all GI-NETs. The wide application of upper gastrointestinal endoscopic techniques and broader use of proton pump inhibitors lead to increased diagnosis of gastric NETs [14, 15].

Gastric NETs are heterogenous neoplasms with varying histopathology and biological behaviour. A classification system was proposed which distinguishes gastric NETs as four different groups. This classification system which based on mainly clinicopathological features, such as hypergastrinaemia, has prognostic and management implications.

Type 1 NETs are the most frequent type and comprise approximately 70–80 % of all cases. The lesions are usually less than 10 mm, multifocal and localised in the gastric fundus or body [16]. Type 1 is mostly related to atrophic gastritis. Gastrin is the key molecule in pathological process of gastric NETs developing from enterochromaffin-like (ECL) cells. Generally this type has an excellent prognosis after resection; metastatic potential of type 1 tumours is low (5 % to local lymph nodes, <2 % distant metastasis) [14, 17]. Histopathologically these tumours are regular-shaped cells with round nuclei. Mitotic index is low; tumour spread is limited to the submucosa and mucosa. Mostly type 1 gastric NET cells are positive with chromogranin A (CgA) and neuron-specific enolase (NSE). Determination of CgA expression is helpful but neither pathognomonic nor diagnostic since enterochromaffin cells of the stomach express high amounts of CgA.

Type 1 gastric NETs are diagnosed incidentally during upper GI endoscopy. Chronic atrophic gastritis may lead to reduced intrinsic factor secretion, achlorhydria, vitamin B12 deficiency and macrocytic anaemia.

In diagnosis and staging, endoscopic ultrasonography is helpful for local staging and for resection of lesions. Abdominal computed tomography scan can aid to diagnosis of metastatic stage [18]. Netazepide is a potent and selective cholecystokinin 2 receptor antagonist which has an oral antagonistic effect on gastrin receptors. Netazepide was reported to regression of tumour and decrease in chromogranin A levels [19, 20].

Annual endoscopic surveillance along with annual abdominal ultrasonography is recommended in type 1 GNET patients [21]. In patients with lesions of >10 mm, CT and somatostatin receptor scintigraphy may be offered.

Type 2 gastric NETs are less common tumours with similar characteristics of type 1 tumours with increased gastrin levels but no achlorhydria or atrophic gastritis. Type 2 tumours are usually associated with multiple endocrine neoplasm (MEN-1) type 1 or Zollinger-Ellison syndrome [22, 23]. Peak incidence of type 2 tumours is 45 and there is no gender predominance. Type 2 lesions are small (10–20 mm) multiple well-differentiated lesions scattered multifocally throughout the stomach, limited to the mucosa and submucosa. Type 2 tumours also express CgA, but levels do not correlate with malignancy [23]. A moderate level of atypia and a slight increased of mitotic index may be encountered in type 2 lesions when compared to

type 1 lesions. A practical test to differentiate type 1 GNET with type 2 is to determine fasting gastric PH. It is high in type 1 and low in type 2 as expected.

Type 2 tumours are diagnosed endoscopically, if amenable endoscopic resection is curative. But due to the multifocality, endoscopic resection may not be optimal. In such cases, somatostatin analogues may be used, to induce tumour regression, decrease plasma gastrin levels and relieve symptoms [24]. Besides removal of the gastric lesions, the source of hypergastrinaemia should be considered, such as gastrinoma according to the location as defined in the related chapter of the book.

Type 3 NETs are usually sporadic; 14–20 % of gastric NETs are classified as type 3. Type 3 gastric NETs are observed more frequently in men with a peak incidence at the age of 50. They are usually solitary and large tumours occurring in any part of the stomach. They are usually well differentiated, larger than 1 cm in size but with invasion of the muscularis propria. Mitotic index is higher (>2 %) and focal necrosis is common. These types of tumours are not gastrin dependent [25]. Type 3 tumours are more aggressive and usually negative for CgA and have a poor prognosis. These tumours are likely to have lymph node involvement and liver metastases at the time of diagnosis [26]. If amenable, surgical resection with lymph node dissection is necessary; depending on local growth features, radical gastric resection may be included [27].

Type 4 NETs are solitary tumours with larger size which are poorly differentiated neuroendocrine carcinomas. They can occur in any part of the stomach with a peak incidence over age 60. These tumours are more frequently seen in men. They are gastrin-independent tumours. Histopathology is characteristic of a grade 3 carcinoma with abundant atypia, mitosis and necrosis. Chromogranin A is absent, whereas neuron-specific enolase is strongly expressed [28]. Type 4 NETs have a poor prognosis; at the time of diagnosis, lymph node invasion, angioinvasion and deep wall invasion usually exist in type 4 tumours. Therefore, type 4 tumours are likely to be diagnosed at the advanced stage [29]. Surgical resection is the most appropriate treatment for this type of tumour. Type 4 gastric NET demands an aggressive surgical approach followed by chemotherapy and multimodality adjuvant therapy [30].

A brief overview of characteristics and treatment strategies of gastric NETs is done in Table 11.2.

11.5 Duodenal Neuroendocrine Tumours

Foregut NETs include those arising in the oesophagus, stomach, pancreas and duodenum [31]. Primary duodenal NETs account for less than 2 % of all gastrointestinal NETs [32].

Duodenal NETs are usually solitary, small lesions restricted to duodenal submucosa at the time of diagnosis. They are usually located in the first and second part of the duodenum [33]. They are relatively benign lesions with slow growth pattern and low metastatic potential which lead to broad classification of duodenal NETs as carcinoids [34, 35].

Table 11.2 Classification and characteristics of gastric neuroendocrine tumours

	Type 1	Type 2	Type 3	Type 4
Incidence (among gastric NETs)	70–80 %	5–6 %	14–25 %	Rare
Pathological features	Multiple, small, intramucosal lesions	Multiple, small, intramucosal lesions	Single, large lesion, more extensive stage lesions	Single, large
	<1 % MI	<1 % MI	>2 % MI	Severe, grade 3 histology
	CgA and NSE positive	CgA and NSE positive	CgA positive	>30 % MI
				CgA negative
				NSE positive
Prognosis	Very good	Good	Less good	Poor
	Metastatic potential very low	10–30 % metastatic potential	50–100 % metastatic potential	100 % metastatic potential
Treatment strategy	Endoscopic resection	Endoscopic resection or limited surgery	Radical surgery	Radical surgery if amenable
Chemotherapy	NN	NN	Usually NN	Combination chemotherapy:
				Cisplatin-etoposide
				Carboplatin-octreotide/ pasireotide
				Somatostatin analogues
Radiotherapy	NN	NN	Usually NN	Targeted radiotherapy with 177Lu-octreotate (177Lu) and 90Y-labelled somatostatin analogue
Biological treatment and targeted treatment	NN	NN	Usually NN	Interferon
				Sunitinib
				Everolimus

MI mitotic index, *CgA* chromogranin A, *NN* not necessary, *NSE* neuron-specific enolase

Five major types of NETs may be seen in the duodenum:

Type 1 tumours are gastrinomas which are the most frequent form and usually seen in the proximal duodenum. One third of duodenal gastrinomas are associated with MEN-1 and Zollinger-Ellison syndrome (ZES) [36, 37]. They are small lesions rarely exceeding 10 mm.

Type 2 duodenal tumours are somatostatinomas. They are the second common duodenal NETs which may be periampullary located. They may be associated

with von Recklinghausen disease [38]. They are often fairly large, deeply invasive and metastatic to local lymph nodes at the time of diagnosis.

Type 3 tumours are benign gangliocytic paragangliomas which are found at the ampulla or periampullary region.

Type 4 tumours are rare. These tumours may secrete serotonin and calcitonin.

Type 5 tumours are extremely rare tumours located at the ampulla of Vater. They are highly aggressive, malignant tumours [39].

Duodenal NETs are mostly asymptomatic and non-functional. They are usually detected incidentally during upper GI endoscopy. And also upper GI endoscopy is the preferred method of detection of duodenal NEs. In retrospective analysis of duodenal NETs, tumour size (>20 mm), tumour depth of invasion (muscularis propria) and presence of mitotic figures have been defined as independent risk factors [40, 41].

Duodenal NETs can be considered as early lesions if tumours are less than 10 mm in size with grade 1 features and without lymphovascular invasion and no muscularis propria invasion [29]. There is no consensus on optimal management strategy of duodenal NETs. According to retrospective case series, tumours with "early lesions" characteristics can be safely removed endoscopically. Treatment of locally advanced and metastatic lesions does not differ from other intestinal NETs.

11.6 Neuroendocrine Tumours of the Gall Bladder and Extrahepatic Bile Ducts

Gall bladder neuroendocrine tumours (GB-NETs) represent only 0.5 % of all NETs [42]. GB-NETs were hypothesised to derive from multipotent stem cells or from neuroendocrine cells of intestinal or gastric metaplasia of gall bladder epithelium [43, 44]. They are usually poorly differentiated, aggressive, large-cell or small-cell NECs [42]. The symptomatology of GB-NETs is nonspecific, and their diagnosis is often made at the cholecystectomy for cholecystolithiasis or polyps. For well-differentiated tumours, simple cholecystectomy may be adequate. The vast majority of GB-NETs, however, require cholecystectomy and regional lymphadenectomy combined with a hepatic resection to obtain adequate clear margins [42].

Primary neuroendocrine (carcinoid) tumours of the extrahepatic biliary ducts are extremely rare. Well-differentiated NETs behave less aggressively; only 23 cases were reported as grade 3 NET [45]. The origin of neuroendocrine neoplasms of the biliary tracts is unclear. They may arise from metaplastic epithelia. Metastasis rate is less than 40 %. Clinical symptoms due to hormone production are extremely rare. Tumour size (<2 cm or not) was hypothesised to be the independent risk factor. Accurate preoperative diagnosis is important and can be made by examining brush cytology specimens obtained during endoscopic retrograde cholangiopancreatography and/or endoscopic ultrasound-guided fine-needle aspiration. Immunocytochemical staining properties are important in differential diagnosis especially before operation. Surgery is the mainstay of treatment in well-differentiated, localised tumours. In general, prognosis is poor.

11.7 NETs of the Jejenum and Ileum

NETs of the small intestine are rare tumours in general, but particularly the ileum is the most common NET site in human body. These tumours have a slight male predominance with a peak incidence at the sixth and seventh decades of life [46]. A potential risk factor associated with increased risk of development of NEN of the small bowel is a family history of first-degree relatives with cancer, suggesting a genetic component independent of MEN-1, which is not associated with jejunoileal NEN [46]. Patients with small intestinal NETs may develop symptoms due to effects of the primary tumour, due to enlarging metastases or secondary to hormonal secretion. Small intestinal NETs originate most commonly in the distal ileum, within 60 cm of the ileocaecal valve [47].

Jejunoileal NETs are frequently diagnosed while on a screening colonoscopy. The most frequent symptom is abdominal pain along with other constitutional symptoms. From the clinical point of view, it is important to distinguish between hormonally active and non-active NETs, because the active ones which secrete peptides and hormones cause a characteristic syndrome known as carcinoid syndrome. Tumour-specific, hormone hypersecretion symptoms from carcinoid syndrome in metastatic patients include secretory diarrhoea, flushing and importantly valvular heart disease [48–50]. It is important to note that carcinoid syndrome occurs in patients with liver metastasis, but in rare cases, excess serotonin production/tachykinin production from extraperitoneal metastasis or ovarian metastases can result in carcinoid syndrome without evidence of liver metastasis.

In diagnosis cross-sectional imaging with CT and/or MRI should be followed by somatostatin receptor scintigraphy (SRS), ideally in combination with SPECT/CT. ^{68}Ga-DOTATOC-PET in combination with contrast-enhanced CT may be a more sensitive alternative, and this technique is getting more widely used globally in the last decade.

In 2012, ENETS renewed the guideline for tumours of the lower jejunum and ileum [46]. Jejunoileal NETs are highly prone to metastasise; they tend to be fairly slow growing and are associated with relatively favourable survival durations compared to other metastatic carcinomas. A single-institution analysis of overall survival stratified by TNM stage revealed that 5-year survival rates were 100 % for stage I and II tumours vs. 91 % for stage III (locoregionally advanced) and 72 % for stage IV tumours. The median overall survival for stage IV tumours was 103 months. Among stage III patients, survival differed significantly between patients with resectable mesenteric tumours (95 % 5-year survival) and patients with unresectable mesenteric tumours (78 % 5-year survival) [51].

In localised disease, surgery is the mainstay of treatment. But usually, due to lack of early diagnosis, locally advanced disease or metastatic stage is the area of concern. Therefore, in symptomatic cases, somatostatin analogues are effective. They are also proven to be useful for tumour growth stabilisation. Liver-directed therapies and systemic cytotoxic drugs and targeted agents will be necessary in selected cases.

11.8 Appendix NETs

The appendix is the third common site for development of gastrointestinal tract NETs (small intestine 44.7 %, rectum 19.6 %, appendix 16.7 %) [1, 52]. These tumours develop primarily in the distal part of the appendix and invade deeply into the appendix wall, causing local symptoms and requiring surgical removal of the tumour. Appendix NETs, mostly limited to the appendix, show a benign behaviour and produce serotonin but are non-functional and are usually discovered as an incidental finding at appendectomy for appendicitis. Most appendix NETs are diagnosed in earlier stages. They are usually grade 1 tumours, rarely grade 2, but NECs of the appendix are extremely rare reported only in few case reports [53].

Histology is always necessary to establish the diagnosis. Cytology may be helpful, particularly in the rare metastatic setting. Immunohistochemistry for CgA and synaptophysin may guide the pathologist in differential diagnosis. Serum CgA levels can be used as a surrogate marker although not particularly validated for diagnosis and follow-up.

At the limited stage survival is good, but in few cases presented with metastasis, survival is relatively poor (5-year survival rate <25 %) [46]. From retrospective studies it can be postulated that an appendiceal NEN with a size <1 cm, with invasion up to the subserosa or mesoappendiceal invasion up to 3 mm and clear surgical margins, poses no further risk of recurrence after appendectomy. However, tumours >2 cm, deep invasive tumours and tumours located at the base of appendix may confer a relevant risk for metastasis. According to ENETS guideline, right hemicolectomy is justified only in those rare tumours 1–2 cm but with positive or unclear margins or with deep mesoappendiceal invasion (ENETS T2), higher proliferation rate (G2) and/or angioinvasion. Tumours with a diameter >2 cm should be treated by right hemicolectomy [46, 54].

Goblet cell carcinoids are a rare histological type of neuroendocrine tumours which may be located in the appendix. They occur during the fifth decade, more often in the Caucasian population than any other group, with an equal distribution between the sexes [46]. They are incidentally found in the appendix, but they have a dismal prognosis. In limited stages survival is better. For locoregional disease the 5-year survival rate is 50–80 %, while in cases with distant metastasis, the 5-year survival rate is less than 20 % [46, 55].

11.9 Colonic NETs

Neuroendocrine tumours of the colon are rare but are increasing in incidence as a result of greater investigation with endoscopy and improved histological reporting. Neuroendocrine tumours rarely occur in the colon with many previously reported caecal NET representing appendiceal tumours. The mean age of diagnosis is approximately 55–65 years. Non-appendiceal colonic NETs have a slight preponderance

for a Black ethnic background in the USA; however, there is no gender prominence in general.

The Pan-SEER analysis from 1973 to 2007 found that colonic NETs made up 7.21 % of all NETs [56]. Right colonic lesions were more frequent than left colonic lesions. In the gastrointestinal tract tumours can arise from enterochromaffin or Kulchitsky cells while those in the distal colon and rectum arise from L cells. These tumours are generally synaptophysin positive and may also have scattered serotonin- and somatostatin-positive cells. It is common for isolated neuroendocrine groups to be present in random colonic biopsies performed for other reasons. This may be an incidental finding or may be a response to inflammation and these are not usually tumours. In addition, small polyps containing small NETs can be found and removed routinely at colonoscopy.

Most NETs of the colon arise in the caecum, followed by the ascending colon. Like in adenocarcinomas, due to high capacitance of the right colon, patients only become symptomatic once they reach a large size (>2 cm). Colonic NETs usually present late, as large tumours, often with extensive metastatic disease when the diagnosis is made. The majority of colonic NETs are asymptomatic at the time of diagnosis. Only in a small percentage of patients (10 %), classical carcinoid symptoms may occur [57]. Most of the time diagnosis is incidental. If present any, common symptoms are pain, bleeding, altered bowel habits, weight loss and anorexia. With advanced disease they may present with symptoms of bowel obstruction, anaemia or a palpable liver. Due to the late presentation, 90 % of tumours will be greater than 2 cm in size, and up to two thirds may have regional or distant metastases at presentation [58]. A frequent presentation is of liver metastases at routine ultrasound of the liver. Ulcerative colitis and Crohn's disease may associate with colonic NETs [59].

Colonic NETs have the worst survival of any GEP-NETS with overall 5-year survival reported to be 61.8 % (between 40 and 70 % depending on the specific site and stage) [2]. Poorly differentiated NETS (high grade), both small and large cell, do not have separate survival data rates available, but prognosis is more likely to represent that of colon adenocarcinoma. In the caecum, the rate of localised disease only has been reported to be as low as 16 % [60]. Metastases are usually to the liver, lymph nodes, mesentery and peritoneum [61]. Survival for sigmoid and other distal colonic NETs is better, probably due to earlier diagnosis and treatment with easier access to high-quality endoscopy [62].

Colonoscopy is the gold standard technique in evaluation and local treatment of colonic NETs. In suspected metastatic cases, cross-sectional imaging (CT/MRI) is required. Indium-111 octreotide scanning or gallium-68 octreotide PET are useful in diagnosis of metastatic well-differentiated NETs; in poorly differentiated tumours their diagnostic significance is poor. In such poorly differentiated NETs, FDG PET may be required.

Treatment strategies are recommended in ENETS guidelines according to the disease stage. Colonic tumours >2 cm, tumours with muscularis invasion and G3 tumours are recommended to be treated according to the surgical guidelines for adenocarcinoma [62]. For metastatic patients, palliative surgery against bowel

obstruction may be offered. The management of advanced disease is controversial, but new treatments such as peptide receptor-targeted therapy and molecular targeted agents may offer improved progression-free survival in non-resectable disease.

11.10 Rectal NETs

Rectal NETs represented the most frequent of GEP-NETs with 17.7 % of all NETs in the US-based SEER 17 (2000–2007) registry, with an annual percentage increase of 8.2 % [63]. The mean age of presentation is 56 and there is no sex difference, but the incidence is higher in Asia-Pacific patients [64]. In Asia, rectal carcinoids in Japanese studies accounted for 60–89 % of all gastrointestinal carcinoids. Well-established endoscopic screening guidelines in Japan may be the reason of higher incidence; however, some evidence exists that there is a definite ethnic association with rectal carcinoids [64].

If any, symptoms may include rectal bleeding, constipation, rectal pain or tenesmus. The incidence of functioning tumours in the rectum is extremely low. These tumours rarely secrete serotonin; therefore, carcinoid syndrome is very rare even in metastatic cases. Overall, no particular hormone preponderance has been described.

Rectal NETs are diagnosed incidentally on screening or surveillance colonoscopies [65]. They are usually small polypoid lesions located above the dentate line. Most of the rectal NETs are diagnosed in localised stage (>82 %) [57]. Metastatic disease may present with right upper quadrant abdominal pain and hepatomegaly, lethargy, wasting, anorexia or generalised symptoms of carcinomatosis.

Rectal NETs have an overall 5-year survival of 88.3 %, with localised disease having a rate of 90.8 %, regional disease at 48.9 % and those with distant metastases 32.2 % [57]. Size of the tumour is closely related to metastatic potential with greater risk in >2 cm tumours. Rectal NETs below 1 cm in size have a very low risk of lymph node metastasis, while those between 1 and 2 cm in size have a risk of 5 %. Other prognostic markers are invasion depth and lymphovascular invasion of detection which is improved with the use of endoanal ultrasound [61].

Rectal NETs are diagnosed similarly to adenocarcinomas of the rectum. Colonoscopy and CT/MRI are usually necessary. Indium-111 octreotide scanning or gallium-68 octreotide PET in addition to FDG PET may be required to diagnose the metastatic cases.

The only definitive curative treatment for rectal NETs is complete resection. Transanal resection using a variety of techniques and equipment offers the ability to resect higher lesions and a full-thickness mucosal-muscular resection. Aggressive surgery such as low anterior resection may be offered for intermediate-risk and high-risk patients. Lesions >2 cm have a significantly higher metastatic risk; they will have major surgery with total mesorectal excision due to high metastatic potential for curative reason. There is no evidence for adjuvant medical therapy after surgery in any of these tumours. For metastatic patients, palliative surgery against bowel obstruction may be offered. There is currently only limited evidence to suggest the use of somatostatin analogues as antitumour agents for non-functioning

colorectal NETs [62]. Peptide receptor radionuclide therapy (PRRT) can be considered in patients with inoperable metastatic disease and a positive indium-111 octreotide scan. The yttrium-90 or lutetium-177 labelled to octreotide or octreotate are the other therapeutic options.

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Insulinoma

12

Simona Grozinsky-Glasberg, Petachia Reissman, and David J. Gross

12.1 Introduction

Insulinomas, the most common functioning pancreatic neuroendocrine tumors (PNETs), represent a rare group of neuroendocrine tumors (NETs) developing from pancreatic beta cells, with an incidence of about 1–3 per million per year [1, 2]. The age of the patient at the time of diagnosis is 47 ± 16 years (mean ± SD) (range, 18–85 years), with a female predominance (66 %) [3].

Specifically, insulinomas are small tumors (82 % <2 cm and 47 % <1 cm) [4], and most (~90 %) are single and located in the pancreas, equally distributed in the pancreatic head, body, and tail. The vast majority (>90 %) of insulinomas are solitary and nonmetastatic at presentation and can be therefore surgically cured [5]. In about 10 % of patients, the insulinoma is a component of multiple endocrine neoplasia type 1 (MEN-1): these patients can present with multiple insulinomas as well as with other secreting/functioning or nonsecreting/nonfunctioning neuroendocrine tumors [6]. Finally, a rare condition is malignant insulinoma, also found in about 10 % of insulinoma patients [2]. Extrapancreatic insulinoma occurring in ectopic pancreatic tissue has also been reported [7].

The aim of this chapter is to summarize the existent literature with respect to the approach to a patient with insulinoma with regard to diagnosis (clinical, biochemical, and radiological) and treatment (surgery, medical therapies), including long-term medical strategies in patients with disseminated or inoperable disease.

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12.2 Clinical and Laboratory Manifestations of Insulinomas (Endogenous Hyperinsulinism-Related Hypoglycemia)

12.2.1 Clinical Manifestations

The clinical symptoms of insulinomas are heterogeneous and related to hyperinsulinemic hypoglycemia.

Hypoglycemia results in autonomic and neuroglycopenic symptoms [8, 9].

Autonomic symptoms comprise of *adrenergic symptoms*, such as palpitations and tremor, and *cholinergic symptoms*, with sweating, hunger, and/or paresthesias [10].

Neuroglycopenic symptoms comprise of severe weakness as well as a wide variety of psychiatric and neurological manifestations (most commonly behavioral changes, confusion, agitation or slow reaction patterns, blurred vision, seizures, transient loss of consciousness or hypoglycemic coma) [8]. Hypoglycemic coma is usually sudden, with agitation, hypothermia, and occasionally pyramidal signs; it may be profound and prolonged and therefore may cause neurologic and cognitive sequelae, leading occasionally to brain death. The combination of autonomic and neuroglycopenic symptoms strongly suggests the diagnosis of hypoglycemia [11]. As autonomic symptoms may often be lacking in patients with prolonged hypoglycemia, it is important to remember that neuroglycopenic symptoms are more specific of hypoglycemia related to insulinoma. Therefore, measurements of plasma glucose concentrations are recommended in all patients with such symptoms. Hypoglycemic spells typically occur after a period of fast, several hours after the last meal, or after physical exercise. However, sometimes, hypoglycemia may occur within hours following a meal, or even without any relationship with meal time, and therefore, it is important to remember that symptoms occurring in nonfasting patients cannot rule out the diagnosis of insulinoma. In a series of 214 insulinoma patients, symptoms of hypoglycemia were reported exclusively in the fasting state in 73 %, postprandial state in 6 %, and both fasting and postprandial state in 21 % [12]. Weight gain is also reported in 25 % of patients.

In the rare pregnant patient with insulinoma, symptoms may mimic those of emesis in early pregnancy, may disappear in late pregnancy as a consequence of insulin resistance, recurring after delivery [13–15].

Because insulinoma symptoms are not specific and can mimic other pathological conditions, a broad differential diagnosis should be considered, a major distinction being made from patients with noninsulinoma pancreatogenous hypoglycemia (NIPHS) [16, 17], hypoglycemia related to oral hypoglycemic agents and exogenous insulin administration (no circulating insulin antibodies) [18].

12.2.2 Laboratory/Biochemical Diagnosis of Insulinomas

12.2.2.1 Whipple's Triad

The combination of symptoms of hypoglycemia, plasma glucose level ≤2.2 mmol/l (≤40 mg/dl), and symptom relief after administration of glucose (known as *Whipple's triad*) is fundamental for the diagnosis of insulinoma.

12.2.2.2 Plasma Glucose and Insulin Concentrations

Glucose

Since the lack of diagnostic specificity of the clinical symptoms in patients with insulinomas, measurement of plasma concentration of glucose is mandatory, ideally during clinical symptoms, and must be performed on venous blood samples with a reliable laboratory method.

The threshold to define hypoglycemia is controversial: according to recent expert recommendations [18], a 0.55 g/L threshold is mandatory. However, the glycemic threshold that can cause clinical symptoms is very heterogeneous among individuals, and a plasma concentration of glucose <0.7 g/L at the time of clinical symptoms is believed to be enough to warrant further investigation. Since even values below 0.55 g/L can be found in some healthy individuals, young women in particular, it is also recommended by the expert consensus that such levels should be taken into account for further evaluation only in patients who had presented with Whipple's triad. Only on extremely rare occasions insulinoma patients are asymptomatic and do not fulfill Whipple's triad [3]. In many insulinoma patients, the clinical symptoms may appear only at very low glucose levels, so that they may be asymptomatic at the time of plasma glucose concentrations even below 0.45 g/L [19].

Insulin

Another basic requirement for the diagnosis of insulinoma is to provide evidence for inappropriate insulin secretion at the time of hypoglycemia. The diagnosis of hypoglycemia related to endogenous hyperinsulinism can be made when the serum insulin concentration is ≥3 mIU/L, serum C-peptide concentration is ≥0.6 ng/mL (0.2 nmol/L), and serum proinsulin concentration is ≥5 pmol/L at the time of venous plasma glucose concentrations <0.55 g/L. During a fast test, beta-hydroxybutyrate levels of ≤2.7 mmol/L and an increase in plasma glucose of at least 0.25 g/L after intravenous glucagon indicate mediation of hypoglycemia by insulin [3].

A mandatory prerequisite for a valid diagnosis of insulinoma is a careful processing of the blood samples for insulin in order to obtain reliable biochemical data for evaluation; these data are important especially when the diagnosis of endogenous hyperinsulinemia/insulinoma seems to be less questionable despite the patient's clinical presentation. Identical serum and plasma samples measured in different laboratories may produce widely disparate insulin values, being many times unacceptable for comparisons [20]. Assay characteristics such as linearity, recovery, accuracy, and cross-reactivity to proinsulin vary among the laboratories, and therefore, a rigorous quality control and continuous quality improvement are needed to maintain reliability of the insulin measurement [21].

Insulin concentrations have been measured for many years with nonspecific assays that yielded cross-reactions with proinsulin. The insulin assays that are now in use have negligible cross-reaction with proinsulin. Though the 3 mIU/L threshold (which replaces a 6 mIU/l threshold previously established with less specific insulin assays) was established for insulin-specific assays, it is known now that the finding of low insulin levels with such assays cannot rule out the diagnosis of insulinoma [22]. Up to 35 % patients with insulinoma were found to have insulin levels below

the recommended diagnostic threshold (3 mIU/L) at the time of hypoglycemia, depending on the assay employed [3].

12.2.2.3 Other Biomarkers

C-Peptide, Proinsulin, and Beta-Hydroxybutyrate

C-peptide and proinsulin levels at the time of hypoglycemia have better diagnostic accuracy than insulin levels: a serum C-peptide level of ≥0.6 ng/mL with a plasma glucose of ≤0.55 g/l is one of the criteria for defining endogenous hyperinsulinism hypoglycemia [3, 12, 23].

Another valuable tool for the diagnosis of insulinoma is measurement of fasting plasma beta-hydroxybutyrate levels. As insulin is known to suppress ketogenesis, a progressive rise in beta-hydroxybutyrate of >2.7 mmol/L was found to rule out the diagnosis of insulinoma when blood glucose levels were 0.50–0.60 g/L during a 72-h fast test (with high, but still controversial, sensitivity and specificity) [12, 23].

Most of the insulin metabolism takes place in the liver and in the kidney; moreover, the kidney also removes 70 % of C-peptide by renal filtration. Therefore, measuring insulin and C-peptide levels in patients with severe renal insufficiency is not helpful; in such patients, it has been recommended to use beta-hydroxybutyrate levels and glucose response to glucagon at the end of a fast test to provide evidence for insulin-related hypoglycemia [24].

12.2.2.4 The Prolonged (72 h) Fasting Test

One of the most robust standard tests used for establishing a biochemical diagnosis of insulinoma is the prolonged (72 h) fast, which is recommended if a venous sample cannot be collected when hypoglycemia occurs spontaneously. Currently, it is recommended that a prolonged, supervised, hospital setting fast be performed, at least for 48 h if not for 72 h; the absence of hypoglycemia after a 72-h fast should exclude the diagnosis [10, 25]. However, although the sensitivity of the 72-h fast is high and the test plays an important role in the diagnosis of an insulinoma, a "normal" test result should be interpreted in the light of clinical symptoms [26]. There is a detailed protocol for the fast test [9, 16, 18]. Whereas the patient is allowed to drink calorie-free beverages, blood samples are collected every 6 h until the plasma glucose concentration is less than 0.6 g/L; if the patient remains asymptomatic, then the frequency of sampling is increased to every 1–2 h. Serum insulin, C-peptide, proinsulin, and beta-hydroxybutyrate are to be measured in all the samples taken at the same time when plasma glucose concentration drops below 0.6 g/L. It is recommended that at the end of the fast test, a sample should be collected to measure oral hypoglycemic agents. Insulin antibodies should also be measured. The fast test can be terminated (1) when Whipple's triad is observed, (2) when plasma glucose concentrations drop below 0.55 g/L in a patient who had previously experienced Whipple's triad, and (3) if plasma beta-hydroxybutyrate levels rise above 2.7 mmol/L [18]. In most insulinoma patients, plasma glucose concentrations below 0.45 g/L are usually reached within the first 48 h of the test [3, 27], though the 72-h fast test, followed sometimes by a brief period of exercise (e.g., 5-min running up and down

one flight of stairs), was reported to be necessary to provide a clear diagnostic conclusion in a few patients [16, 17, 26].

12.2.2.5 C-Peptide Suppression Test

It was Service et al. [28] who first published data for the C-peptide suppression test, a simple, outpatient, less expensive, and less time-consuming procedure, whose rationale is based upon the physiological suppressibility of endogenous insulin secretion by means of an exogenous insulin infusion. With a specificity of 96 % and a sensitivity of 100 %, the C-peptide suppression test (C-peptide cutoff levels above 0.2 nmol/l) identifies those patients who definitely will require a further standard in-hospital fasting test [3, 29].

12.2.2.6 Glucagon Test

Glucagon and insulin are part of a feedback system that keeps blood glucose levels at a stable level, as glucagon raises blood glucose levels, opposite to insulin [30].

An increase in plasma glucose of at least 25 mg/dL (0.25 g/L, 1.4 mmol/L) after intravenous glucagon suggests insulin-induced hypoglycemia [18]. The test should be performed at the end of a prolonged fast test: at the time of symptomatic hypoglycemia with concomitant plasma glucose concentrations of ≤0.45 g/L (2.5 mmol/L) or after 72 h of fast and measuring plasma glucose at 10-min intervals for 30 min showing higher glucose in insulinomas (with a range of maximal increase in plasma glucose concentrations of 1.4–5.4 mmol/L in insulinoma patients and of 0.1–1.3 mmol/L in controls) [23, 31, 32].

In summary, clinical and laboratory diagnosis of insulinoma patients, as recommended also by European Neuroendocrine Tumor Society (ENETS) guidelines [4], can be established using the following 6 strict criteria: documented blood glucose levels ≤2.2 mmol/l (≤40 mg/dl), concomitant insulin levels ≥6 U/l (≥36 pmol/l; ≥3 U/l by ICMA), C-peptide levels ≥200 pmol/l, proinsulin levels ≥5 pmol/l, hydroxybutyrate levels ≤2.7 mmol/l, and absence of sulfonylurea (metabolites) in the plasma and/or urine.

Further testing includes the 72-h fast, which is the gold standard for establishing the diagnosis of insulinoma [33]. When the patient develops symptoms and the blood glucose levels are ≤2.2 mmol/l (≤40 mg/dl), blood should be drawn for C-peptide, proinsulin, and insulin: presence of detectable insulin during hypoglycemia is highly suggestive for insulinoma [8, 9, 16].

12.3 Differential Diagnosis of Insulinoma [34–36] (Table 12.1)

12.3.1 Reactive "Functional Hypoglycemia"

Reactive "functional hypoglycemia" is a controversial entity, and it is now known that many organic disorders may result both in fasting and/or reactive hypoglycemia. Many patients present with dizziness or other minor autonomic symptoms within the hours following a meal, but most do not meet the criteria for

Table 12.1 Differential diagnosis of endogenous hyperinsulinemic hypoglycemia

Congenital

- *Transient neonatal hyperinsulinism (mechanism not known)*
- *Focal hyperinsulinism (K_{ATP} channel disorders)*
  - Paternal SUR1mutation with clonal loss of heterozygosity of 11p15
  - Paternal Kir6.2 mutation with clonal loss of heterozygosity of 11p15
- *Diffuse hyperinsulinism*
  - K_{ATP} channel disorders (SUR1 or Kir6.2 mutations)
  - Glucokinase gain-of-function mutations
  - Hyperammonemic hyperinsulinism
  - Short chain acyl coenzyme A dehydrogenase deficiency
  - Carbohydrate-deficient glycoprotein syndrome (Jaeken disease)
  - Beckwith-Wiedemann syndrome (suspected due to hyperinsulinism but pathophysiology uncertain: 11p15 mutation or IGF2 excess)

Acquired

- *Postprandial hyperinsulinemic hypoglycemia*
  - Dumping syndrome
  - Post-gastric bypass surgery
  - Insulin autoimmune syndrome
- *Insulinoma*
  - Sporadic
  - Multiple endocrine neoplasias type 1
- *Adult nesidioblastosis*
- *Drugs (quinine etc.)*
- *Factitious use of oral anti-hyperglycemic drugs (sulphonylurea etc.)*
- *Adrenal insufficiency*
- *Unusual (generally transient)*
  - Maternal diabetes mellitus
  - Perinatal asphyxiation
  - Intrauterine growth restriction
  - Rhesus isoimmunization

hypoglycemia, or for Whipple's triad. Rarely, insulinoma can present as reactive hypoglycemia [37].

12.3.2 Hyperinsulinemic Hypoglycemia

12.3.2.1 Drug-Induced Hypoglycemia

The first step in the evaluation of hypoglycemia is to check if the patient is treated with medications that can cause hypoglycemia. There is a large variety of drugs implicated in hypoglycemia (e.g., aspirin, pentamidine, quinine, disopyramide, etc.) and listed elsewhere) [38, 39].

12.3.2.2 Factitious Hypoglycemia Related to Insulin and Insulin Analogues (High Insulin Levels with Low C-Peptide and Proinsulin Levels, Exogenous Hyperinsulinism)

The finding of high insulin levels with concomitant low C-peptide and proinsulin levels is very specific for the diagnosis of surreptitious insulin injections [40].

Importantly, insulin assays that can identify the presence of insulin analogues are to be preferred [41, 42].

12.3.2.3 Factitious Hypoglycemia Related to Sulfonylureas or Glinides (High Insulin, C-Peptide, and Proinsulin Levels) (Factitious Endogenous Hyperinsulinism)

At the time of symptomatic hypoglycemia, in order to distinguish factitious (or accidental) hypoglycemia from the endogenous abnormal insulin secretion (observed in patients with insulinomas and nesidioblastosis), it is mandatory to collect a plasma sample or a urine collection to measure oral hypoglycemic agents (sulfonylureas and glinides). This diagnosis is difficult and usually suspected in patients whose relatives are diabetic or health professionals [43].

12.3.2.4 Idiopathic Noninsulinoma Pancreatogenous Hypoglycemia Syndrome (Endogenous Hyperinsulinism)

The noninsulinoma pancreatogenous hypoglycemia syndrome (NIPHS) identifies a group of hyperinsulinemic hypoglycemic patients with unique clinical, diagnostic, surgical, and pathologic features [17, 44]. These patients experience predominantly postprandial hypoglycemia and have nesidioblastosis with islet cell hypertrophy, findings different from those in patients with insulinomas.

12.4 The Histopathological Diagnosis of Insulinomas

The latest 2010 WHO classifies NETs of the pancreas into three categories: (1) well-differentiated endocrine tumors, with benign or uncertain behavior at the time of diagnosis; (2) well-differentiated endocrine carcinomas with low-grade malignant behavior, and (3) poorly differentiated endocrine carcinomas, with high-grade malignant behavior. Most insulinomas are well-differentiated endocrine tumors, WHO group 1; however, occasionally they belong to the WHO 2 or 3 group [45].

For the appropriate histological diagnosis of insulinoma, a detailed macroscopic and microscopic description and an immunohistochemical staining for neuroendocrine tumor markers (chromogranin and synaptophysin), as well as for insulin, proinsulin, and amyloid, are all recommended. However, the immunohistochemical determination of insulin expression by tumor cells does not appear to be an absolute need for diagnosis, as some insulinomas do not stain positively for insulin due to a rapid turnover of insulin secretion from the insulin-producing cells [46]. Evaluation of the mitotic index and Ki-67 index is required to define the grade of the tumor and the related patient prognosis and treatment approach [4].

In 5–10 %, insulinomas may be malignant [47, 48]. Whereas the clinical and biochemical diagnostic criteria of insulinoma do not differ from those of benign insulinomas, the histological diagnosis of malignancy is difficult, and actually the only definite criterion for malignancy is the presence of metastatic disease. However, in clinical practice, a malignant insulinoma is generally found to be a single large tumor, and in most cases, there are synchronous metastases, generally located in regional lymph nodes or in the liver. Suspicion of aggressive tumor behavior, apart

from metastases, includes invasion of adjacent organs, a tumor size >2 cm, angioinvasion, and high proliferative activity [49–51].

Rarely, metastatic nonfunctioning pancreatic NETs may change their biological behavior in parallel with tumor progression, with transformation to insulinoma, manifested by a clinical picture of endogenous hyperinsulinemic hypoglycemia (*personal experience, unpublished*).

12.5 The Role of Anatomical and Functional Imaging in the Diagnosis of Insulinoma

As a rule, imaging should be performed only after the biochemical diagnosis of insulinoma has been established. The role of imaging is to detect the anatomical localization and to stage the tumor prior to surgery [11]. It is known that the majority of insulinomas are solitary and located in the pancreas. They are characteristically small (most being ≤2 cm at presentation) and therefore extremely difficult to localize radiologically [52]. Intraoperative palpation and ultrasound examination of the pancreas are thought to be the best methods to detect insulinomas [53]. Using such methods intraoperatively remains mandatory. However, careful imaging procedure should be performed in an attempt to localize the tumor before surgery, allowing preoperative planning of the surgical procedure according to the size and precise location of the tumor (Whipple's procedure or middle or distal pancreatectomy, enucleation vs. resection).

The imaging techniques have greatly improved, and the most useful modalities are 3-phase computed tomography (CT), gadolinium-enhanced dynamic magnetic resonance imaging (MRI), and endoscopic ultrasound. Invasive techniques such as selective celiac and mesenteric arteriography, venography, and venous sampling are rarely used, and together with somatostatin receptor imaging and positron emission tomography (PET) with [11]C-5-hydroxytryptophan (5-HTP) as tracer (HTP-PET) or [11] C-l-DOPA (DOPA-PET), they represent complementary techniques in specific situations, such as tumor localization in patients with MEN-1. Unfortunately, there is a wide discrepancy in the use of localization techniques between different centers, as result of the specialist expertise and the availability of imaging equipment [53].

12.5.1 Computed Tomography (CT)

High-definition multi-detector helical CT scan is the first examination recommended to be performed to localize insulinomas; meticulous technique is mandatory [54, 55]. Most insulinomas are small, isodense with the pancreas on pre-contrast images, and then hypervascular on arterial phase images but sometimes are more easily detected on portal venous phase images. More rarely, the tumors are hyperdense in comparison to the pancreas, or have nodular calcification, or appear hypovascular, cystic, or hypodense after injection of contrast medium. Exceptionally, ectopic insulinomas are located in the proximity of the pancreas or of the liver. While the sensitivity of non-helical CT had been 29 %, multi-detector triphasic helical CT scan technique can result in 94 % sensitivity [54, 56].

As the majority of benign insulinomas tend to be small at presentation and, therefore, seldom alter the contour of the pancreas, 3-phase CT should be used to maximize detection. Insulinomas are typically hypervascular, and their appearance is that of a hyper-attenuating lesion in both the arterial and portal venous phases. Liver metastases also tend to be hypervascular, and, therefore, the arterial phase shows the number and size of liver metastases better than the venous phase. The reported sensitivity of CT for the detection of insulinomas is in the range of 30–85 %, depending on tumor size [56], whereas combined 3-phase CT and endoscopic ultrasound may further increase this sensitivity up to 100 % [57].

12.5.2 Magnetic Resonance Imaging (MRI)

MRI has high sensitivities (85–95 %) for the detection of insulinomas and/or insulinoma-related metastases. MRI is superior to CT for the detection of small lesions: the enhancement pattern of insulinomas on MRI is related to their hypervascularity; usually, they are low in signal intensity on fat-suppressed T1-weighted images and moderately high in signal intensity on fat-suppressed T2-weighted images [58–61].

12.5.3 Transabdominal Ultrasound

Transabdominal ultrasound examination is considered to be of low sensitivity in the detection of pancreatic neuroendocrine tumors like insulinomas. Recently, contrast-enhanced ultrasonography has been reported to detect insulinomas in a higher number of patients [62].

12.5.4 Endoscopic Ultrasound

Endoscopic ultrasound (EUS) is currently considered the best preoperative procedure to localize insulinomas with high sensitivity (of 94 %) [63], mainly for lesions located in the head and body of the pancreas and less for tail lesions. Even if recent CT scan techniques lead to localization of most insulinomas, EUS permits a better evaluation of the lesions (especially if they are multiple) and of their proximity to pancreatic ducts and vessels, and it also enables the operator to obtain biopsies [64]. The high spatial resolution of this technique allows the precise anatomical localization of very small lesions. Combined with 3-phase CT, the sensitivity rises to 100 % [64].

12.5.5 Intraoperative Ultrasound and Laparoscopic Intraoperative Ultrasound

Intraoperative ultrasound (IOUS) is mandatory during surgery of insulinomas in order to localize non-palpable tumors. IOUS is also highly useful in defining the relationship of the tumor to the adjacent pancreatic and bile ducts and blood vessels.

Intraoperative localization techniques (both careful palpation of the pancreas and IOUS) represent the most reliable way for tumor localization and for proceeding with the appropriate surgical approach (tumor enucleation vs. middle pancreatectomy). Moreover, it is indispensible in patients in whom multiple lesions are suspected, e.g., in MEN-1 patients [65, 66]. In experienced hands, laparoscopic IOUS can identify more than 85 % of insulinomas [67, 68].

Despite the high sensitivity of IOUS, a detailed preoperative examination is necessary to localize insulinomas, to minimize the risk of reoperation, to help in the choice of the surgical technique, and to render laparoscopic surgery as the preferred technique when it appears to be the possible choice to remove the tumor [69].

12.5.6 [111]In-Pentetreotide Scintigraphy (OctreoScan) and Positron Emission Tomography (PET)

OctreoScan is only positive in up to 46 % of benign insulinomas because of the low expression of somatostatin receptor (SSTR) type 2 by insulinomas [70]. In malignant insulinomas, however, the relative distribution of SSTR subtypes is different from benign tumors with a higher rate of scan positivity [71–73].

PET imaging of insulinomas with [18]F-fluorodeoxyglucose ([18]F-FDG) is disappointing, presumably because of their low proliferation rate. Promising results, however, have been obtained using [11]C-5-HTP, [18]F-DOPA, and [68]Ga-DOTA-DPhe [1]-Tyr [3]-octreotide ([68]Ga-DOTATOC) [74, 75].

Recently, there are promising results using a radiolabeled glucagon-like peptide 1 (GLP-1) analogue. Insulinomas are characterized by a very high expression of GLP-1 receptors [70]. Using a radiolabeled GLP-1 analogue ([111]In-DOTA-exendin-4) in six patients with insulinomas, GLP-1 analogue scintigraphy correctly localized the tumor in all patients, whereas CT scan was positive only in 1, MRI scan only in 1, and EUS in 4 [76].

12.5.7 Angiography

Angiography combined with calcium stimulation and transhepatic portal venous sampling (THPVS) was considered the gold standard for insulinoma localization [77]. This technique combines both anatomic and functional localization and screens for an insulin concentration gradient during insulin levels measurement in the right hepatic vein; it may be used in difficult cases [53].

In a recent study [78], the diagnostic accuracy of most methods employed to localize insulinomas in the years 1990–2009 was compared, demonstrating that the multi-detector CT scan employed by an experienced radiologist (with MRI as complementary technique) predicts tumor localization with the highest accuracy, whereas OctreoScan and EUS can be valuable in selected cases, and calcium stimulation may provide an additional functional perspective in those cases not localized by the previously mentioned procedures.

12.6 Therapy of Insulinomas

12.6.1 Surgical Therapy

Surgery remains the only curative treatment of insulinomas, and long-term remission can be achieved by surgery in 95 % of patients [79]. The type of surgery depends on the size and the location of the tumor and on its proximity to anatomical structures (pancreatic duct, common bile duct, splenic and superior mesenteric vessels, and adjacent organs). The entire pancreas should be exposed and explored, using inspection and intraoperative ultrasound, as multiple tumors need to be excluded.

Usually, tumor enucleation is preferred to minimize risks of postoperative pancreatic exocrine deficiency and diabetes mellitus. It should be performed only to excise small (approximately 1 cm in diameter) expectedly benign small tumors on the surface of the pancreas [5]; provided that the surgery is performed by experienced surgeons, the risk of pancreatic fistula after enucleation is not higher than that observed in larger resections of the pancreas [80]. However, when the tumor is anatomically unsuitable for enucleation, central or distal pancreatectomy is safe and effective alternative [68, 81, 82], intraoperative ultrasound IOUS remains mandatory, and rarely, bidigital palpation is required [83]. Importantly, an extensive resection is always the rule for larger size or locally advanced insulinomas that are suspected to be malignant.

Except for pancreaticoduodenectomy (Whipple's procedure), laparoscopic surgery can be successfully and safely utilized in specialty centers for the majority of pancreatic procedures, with the many advantages in comparison to open surgery [84], although the rate of pancreatic fistulas is not reduced, as it reduces the duration of the hospitalization and the associated morbidity. Laparoscopy must be performed only by experienced surgeons in both advanced laparoscopy and pancreatobiliary surgery. Noteworthy, as intraoperative bidigital palpation of the pancreas is not possible in this situation, laparoscopic ultrasound by an experienced surgeon is mandatory (Fig. 12.1).

When no insulinoma is detected at operation, it is recommended to end the procedure and to perform additional investigations in order to localize the tumor. Blind distal pancreatectomy is not recommended [85], due to its short- and long-term morbidity and its frequent failure to achieve disease cure.

12.6.2 Medical Therapy

Dietary consultation is important to prevent prolonged periods of fasting and the development of hypoglycemia and its associated symptoms. Self-monitoring of glucose levels is recommended to insulinoma patients in order to detect asymptomatic hypoglycemia and to prevent occurrence of hypoglycemic spells [86]. The patients should be advised regarding everyday personal safety to avoid loss of consciousness and its possible consequences.

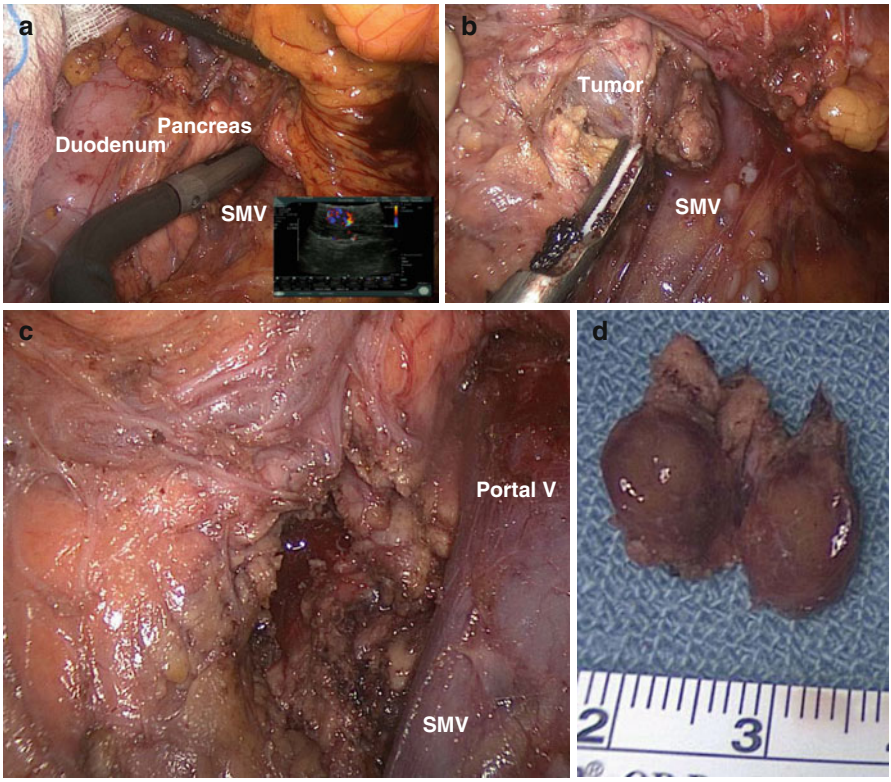

Fig. 12.1 Head of pancreas insulinoma enucleation. (**a**) Intraoperative laparoscopic ultrasound of the head of the pancreas using a flexible 10-mm probe, showing a 1.2-cm insulinoma. Note the common rich vascularity of the tumor as seen on the Doppler ultrasound in the lower right of the picture. (**b**) Enucleation of the insulinoma using an ultrasonic energy dissector; SMV, superior mesenteric vein. (**c**) Post-enucleation, note the tumor bed and the minimal defect in pancreatic tissue. (**d**) Specimen, the tumor is well encapsulated and completely excised

Medical management is reserved only for preoperative control of blood glucose levels, for patients who are unable or unwilling to undergo surgical treatment, or for unresectable metastatic disease.

Continuous glucose infusion may be necessary in some patients, while waiting for surgery or for the effect of other treatments. Vitamin B1 supplementation is recommended for patients with severe recurrent hypoglycemia requiring large infusions of glucose, in order to prevent Wernicke's encephalopathy [87]. Diazoxide is the most effective drug for controlling hypoglycemia; at a dosage of 50–300 mg/day (up to 600 mg/day), it suppresses insulin secretion by direct action on the beta cells and by enhancing glycogenolysis [88]. However, the disturbing side effects of diazoxide (e.g., fluid retention and edema, weight gain, nausea and digestive intolerance, renal impairment, skin rashes, and hirsutism) limit its use. Verapamil and diphenylhydantoin have also been reported to be helpful in the control of hypoglycemia [89–91]. Glucocorticoids can be effective in

refractory cases [92], by decreasing insulin secretion and increasing peripheral insulin resistance. However, their association with well-known adverse effects precludes long-term use of these drugs.

Somatostatin analogues (SSAs) inhibit insulin secretion mainly through their effects on SSTR2A and SSTR5 subtypes, which were found in 70 % of insulinomas [73]; however, in patients with tumors that do not express these receptor subtypes, SSAs may worsen hypoglycemia, possibly by suppression of glucagon secretion [89, 93, 94], and therefore, this therapy should be started in an in-hospital setting. SSAs can achieve normalization of plasma glucose levels in up to 60 % of the patients [95], with few adverse effects, mostly digestive intolerance with diarrhea and steatorrhea that can be managed by pancreatic enzyme supplementation. The dose of octreotide that was found to control the hypoglycemia varied between 50 and 2,000 μg per day, and it had to be determined on an individual basis. A short 100-μg octreotide test, not OctreoScan uptake, was predictive of the long-term efficacy of octreotide treatment on hypoglycemia. This could be explained by the differing affinities of OctreoScan and octreotide for SSTR2 [95, 96].

Interferon alpha may also be beneficial in some selected cases [97].

12.6.3 Specific Treatments for Patients with Malignant Insulinoma

Malignant insulinomas present a dual therapeutic challenge, in terms of both the control of tumor progression and the control of symptomatic hypoglycemia. The course of the metastatic disease is very heterogeneous, with a reported 10-year survival of about 30 % [25]. However, some patients may present with a very slow progression rate, whereas others present with rapid tumor progression and poor survival rate.

Symptomatic treatment of metastatic insulinomas aims at achieving short-term control of the hypoglycemia while awaiting the effects of antitumor treatment or when antitumor treatments do not prove to be effective. All the medications used in benign insulinomas can be employed, but usually, a combination of several hyperglycemic drugs is needed [95].

Since malignant insulinomas are rare, and there is no prospective study to date on this disease specifically, the therapeutic approach is as for other pancreatic neuroendocrine carcinomas. Whenever it is possible, surgery has to be initially considered, aiming at a total excision of all detectable lesions. However, as recurrence is frequent (about 60 %) [47], other treatments should be considered, such as chemoembolization of liver metastases [98], radiofrequency ablation [99], cytotoxic chemotherapy (traditionally with a combination of streptozotocin, doxorubicin, and 5-fluorouracil and, recently, with capecitabine and temozolomide) [100, 101, 102], peptide receptor radionuclide therapy [103–105] (PRRT, especially with ^{177}Lutetium-DOTATATE), or targeted therapies with tyrosine kinase inhibitors (sunitinib) or mTOR inhibitors (everolimus) [106, 107].

The mTOR inhibitor everolimus (Afinitor, Novartis, Switzerland) deserves special mention as it seems to possess a profound effect for the control of both hypoglycemia and tumor progression [108, 109]. Everolimus has recently been shown to improve progression-free survival of patients with well-differentiated progressive metastatic pancreatic NETs and has been approved as a new antitumor therapeutic option for this indication [107]. Long-term administration of everolimus may alter insulin secretion, as well as insulin-mediated peripheral glucose utilization and insulin-mediated suppression of hepatic glucose production, thereby resulting in normo- or hyperglycemia in most of the patients with malignant insulinomas [110]. A recent, largest to date, study in patients with malignant insulinomas [111] clearly demonstrated that 11/12 patients (91 %) experienced a complete resolution of hypoglycemia on everolimus, despite failure of other previous therapeutic modalities. Cardiac and pulmonary tolerance should be carefully monitored in patients treated with everolimus [111].

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Gastrinoma

13

Janice L. Pasieka and Anthony J. Chambers

13.1 Introduction

Gastrinomas are rare tumors of the neuroendocrine system, occurring within the pancreas and duodenum. The annual incidence is estimated at 0.5 per million of the population [1]. Overproduction of gastrin by these tumors produces a sustained increase in gastric acid secretion, leading to clinical manifestations of complicated peptic ulcer disease known as the Zollinger-Ellison syndrome (ZES) [2]. Although most gastrinomas grow slowly, over 60 % are malignant and 25 % show rapid aggressive behavior [3–5]. Most commonly gastrinomas metastasize to regional lymph nodes and the liver, and it is this malignant potential which has become increasingly important since the introduction of effective medical therapy to control gastric acid secretion. Gastrinomas can occur sporadically or in a familial pattern as a component of the multiple endocrine neoplasia type 1 (MEN1) syndrome [3, 6, 7]. Given that the endocrinopathy associated with these tumors can be well controlled medically, the role of surgical resection in the setting of advanced disease and in patients with MEN1 syndrome is the subject of continued debate.

13.2 History

The first case of a gastrinoma was described by Sailer in 1946, when he reported the case of a patient with a large, pancreatic islet cell tumor without clinical or biochemical evidence of hyperinsulinemia [8]. At that time, insulinomas were the only recognized functional islet cell tumors, and although a duodenal ulcer was present,

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Sailer did not associate the two findings. It was not until Robert M. Zollinger and Edwin H. Ellison presented their experience of two cases before the American Surgical Association in 1955 that it became apparent a relationship existed [2]. It was during the discussion that followed that another seven cases of the same clinical manifestations were described. The two cases presented by Zollinger were unique in that they presented with marked gastric hypersecretion, complicated refractory peptic ulcer disease, and non-insulin-secreting islet cell tumors of the pancreas. This clinical triad associated with gastrin-secreting tumors now bears the authors' names [9]. They concluded in their report that excessive amounts of a substance produced by the islet cell tumors were likely responsible for the increased gastric acid secretion in these patients.

The role of gastrin in the causation of ZES was first proposed by Gregory in 1960, who injected an extract derived from a pancreatic islet cell tumor from a patient with refractory peptic ulcer disease into an animal model and demonstrated a dramatic increase in gastric acid secretion, concluding that a "gastrin-like substance" was secreted by these tumors [10]. Gastrin was first isolated from antral mucosal cells by Gregory in 1964 and later found to be nearly identical in composition to an extracted hormone from gastrinoma [11]. The development of a radioimmunoassay for the measurement of serum gastrin concentrations by McGuigan and Trudeau in 1968 enabled the diagnosis of gastrinoma and ZES to be made preoperatively [12]. However, elevated gastrin levels can also be seen in atrophic gastritis, gastric outlet obstruction, retained antrum, post-vagotomy, G-cell hyperplasia, and the use of proton pump inhibitors [13] (Table 13.1). It was recognized by Isenberg in 1972 that there was a paradoxical rise in gastrin with the administration of secretin in patients with suspected ZES allowing for a more specific delineation of the disease [14].

The initial experience of 260 cases of ZES laid the foundation for the current understanding and management of this disease at that time [3]. Based on the data collected in this registry, Ellison and Wilson recommended total gastrectomy as

Table 13.1 Causes of hypergastrinemia

Inappropriate hypergastrinemia – gastric pH <2
Gastrinoma/Zollinger-Ellison syndrome
Helicobacter pylori antral predominate
Retained gastric antrum
Gastric outlet obstruction
Short bowel syndrome
Appropriate hypergastrinemia – associated with hypochlorhydria
Pernicious anemia
Chronic atrophic gastritis
Previous vagotomy
Gastric acid-suppressing medications (PPI, H2 antagonist)
Renal impairment

the definitive initial operation. The first report of a successful resection of gastrinoma to treat ZES was by Rawson in 1960, where a patient with peptic ulcer disease and hypersecretion of gastric acid underwent removal of a pancreatic islet cell tumor by Professor Stammers at the University of Birmingham [15]. The patient did not undergo gastrectomy or vagotomy yet had a complete relief of symptoms and a marked reduction of gastric acid secretion after resection of the tumor. Consequently the recommendation from these authors was that surgical management be directed at "eradication of the tumor and its local metastases rather than total gastrectomy" [16]. Although it is now recognized that duodenal gastrinomas are fairly common, in some series as high as 60 % [1, 4, 17], it was in 1961 when Oberhelman first reported the occurrence of duodenal gastrinomas in patients with ZES [18]. Since Oberhelman's publication, duodenal gastrinomas were described more frequently and subsequently changed the operative approach. Both NW Thompson and JA Norton recognized the necessity of performing a duodenotomy in sporadic gastrinoma patients when no pancreatic tumor was found [17, 19, 20].

Interestingly, in the discussion section of the paper by Oberhelman, Dr Victor Richards is quoted as saying "the demonstration by Gregory that these tumors produce either gastrin or a gastrin-like substance has led many people to work experimentally on the concept of producing an antihormone. I do not know of any clinical experience, however, with the use of antigastrin in the management of these cases, but it is an idea that is worth thinking about for the future." A decade later, this idea would conceptually come to fruition with the development of H2-receptor antagonists followed by proton pump inhibitors (PPIs) [9].

In 1953, Underdahl et al. published their experience at the Mayo Clinic of eight cases of multiple adenomas involving a combination of two or all three of the pituitary, parathyroid glands, or pancreas and a review of the literature. Parathyroid tumors were present in all cases, pituitary tumors were present in four, and five had islet cell tumors. They commented that "multiplicity of tumors was a striking feature and occurred both in relation to the parathyroids and in relation to the pancreatic islets" [21]. It is interesting to note that 2 cases of the 14 reviewed and 2 of their own 8 cases suffered from duodenal ulcers as well as one of their cases having had a peptic ulcer. In the following year, Wermer stated that "this complex clinical pathologic picture has now been seen so often that the possibility of mere coincidence can be dismissed." He went on to say that "we are dealing here with an example of dominant hereditary transmission and it appears probable that one single autosomal gene is responsible for the whole pathologic picture" [22]. From the five cases which he presented in his 1954 paper, he astutely observed that there were two instances of gastric ulcer and two of duodenal ulcers—a finding which he did not believe to be mere coincidence, but another manifestation of the abnormal gene. In the review article by Ellison and Wilson from 1964, they identified an association with other endocrine diseases in patients with ZES in 21 % of cases [3]. We now recognize that MEN1 syndrome is found in approximately 20 % of patients with ZES.

13.3 Clinical Features and Diagnosis

When ZES was first described, the gastrinomas were mainly pancreatic tumors, and patients suffered from refractory peptic ulcer disease, vomiting, diarrhea, and weight loss (Table 13.2). Treatment was based on controlling the acid production (total gastrectomy) and resection of the pancreatic tumor if possible. Nowadays, gastrinomas are often small and up to 60 % are located in the duodenal wall. Symptoms are often those of peptic ulcer disease without diarrhea and weight loss, as PPIs are very effective at controlling the gastric acid secretions. Delay in the diagnosis of ZES has increased as a result of the effective use of PPIs [5, 23–25].

Gastrinomas are rare tumors, occurring with an incidence of 0.5 cases per million population per year [23, 26]. Gastrinomas appear to be more frequently in males, with a male to female ratio of 1.3:1–1.5:1 [4, 24, 27]. The tumor typically presents in late adulthood with a mean age at diagnosis between 41 and 54 years, earlier for patients with MEN1 (33 years) [1, 23, 28, 29]. Historically, patients with gastrinoma were typically diagnosed late in the course of their disease with complications of severe refractory peptic ulcer disease. In the modern era of the use of PPI therapy, a history of peptic ulceration is absent in 29 % of patients at the time of diagnosis [23]. Although many patients with ZES present with more severe manifestations of peptic ulcer disease, the vast majority of ZES patients are indistinguishable from idiopathic peptic ulcer disease [23]. As a result, many patients are initially treated with PPIs early in the course of their disease, leading to an improvement or resolution of their symptoms and a potential development of metastatic disease before a diagnosis is established [1, 5, 23, 24]. Ellison reported an increase in advance stage at presentation when patients were diagnosed between 1986 and 1998. Patients diagnosed up to 1985 had an incidence of metastatic disease of 19 % and a 5-year disease-free survival (DFS) of 29 % compared with an incidence of metastatic disease of 55 % and 5-year DFS of 2 % after 1986 [30].

ZES patients will present primarily with pain (83 %) and diarrhea (71 %) in a background of long-standing history of reflux (GERD) and recurring peptic ulcer disease (PUD) (Table 13.2). Only 11 % of patients have a single symptom. Complicated PUD is decreasing; in 1964, 45 % of patients presented with life-threatening bleeding, whereas in the NIH report from 1999, only 24 % presented with bleeding [1, 3]. Up to 20–25 % of gastrinomas occur in the setting of multiple endocrine neoplasia type 1 (MEN1). The diagnosis of ZES should be considered in any patient with PUD who is *H. pylori* negative, is refractory to standard treatment, has ulcers in unusual locations, or has MEN1 syndrome [5].

The diagnosis of gastrinoma is based on biochemical testing, demonstrating an elevation of fasting serum gastrin (FSG) level (>150 pg/ml) in the presence of raised gastric acid secretion [28]. In patients receiving acid-suppressing therapy with H2-receptor antagonists or PPIs, FSG is secondarily raised by these medications, and as such, these should be discontinued prior to testing [5, 23]. Gastric secretory studies showing a BAO of more than 15 mEq/h (more than 5 mEq/h in the presence

Table 13.2 Clinical presentation of Zollinger-Ellison syndrome

Symptom	Percentage of patients (%)
Abdominal pain	78–94
Peptic ulcer disease	74–96
Duodenal ulcer	60–96
Gastric ulcer	24
Jejunal ulceration	29
Bleeding	27–42
Diarrhea	72
Gastroesophageal reflux	42
Esophageal ulceration or stricture	4–6
Weight loss	7–18

of previous gastric resection) or a pH of less than 2 are the most commonly used criteria to determine acid hypersecretion [28]. FSG can also be elevated in other disease states that must be differentiated from ZES (Table 13.1). Most patients with gastrinoma have highly elevated gastrin levels, with 36 % having levels more than 10 times normal [31]. When the results of FSG and acid secretory studies are borderline or nondiagnostic in patients where a clinical suspicion for ZES exists, provocative testing of FSG after administration of intravenous secretin (2 mg/kg) or calcium is indicated. Because most of these patients are on PPIs, these drugs must be stopped a week before provocative testing. H2-receptor antagonists may be utilized for symptom control, but also must be stopped 48 h before [23]. There is a risk for developing rebound hypersecretion following PPI withdrawal. The day of the secretin test is the ideal time to get a gastric pH and upper endoscopic ultrasound (EUS) to avoid having to withdraw PPIs again at a later date. Following secretin injection, FSG increases from baseline levels within 2–3 min by more than 100 pg/ml in 94 % of patients with gastrinoma and rises by more than 50 % in 84 % of cases [32]. Similarly, provocative testing after calcium infusion results in a rise in gastrin of more than 50 % from baseline levels in 84 % of cases [5]. Combining these two investigations lead to an increased sensitivity for confirming the diagnosis of a gastrinoma. Synthetic secretin has replaced native secretin. An analysis of 293 NIH ZES patients and 537 patients from the literature has established the criteria of a rise ≥120 pg/mL (sensitivity 94 %, specificity 100 %) replacing the old criteria of >200 pg/mL [5, 31, 33].

Chromogranin-A (CgA), a protein contained within the secretory granules of neuroendocrine cells, is a nonspecific marker for neuroendocrine tumors (NET) and is elevated in 90 % of patients with gastrinoma [34]. Chromogranin-A levels tend to correlate with those of serum gastrin; however, higher levels are not associated with an increased risk of advanced or metastatic disease. However, in a series of 72 patients with serial measurements of gastrin and CgA, these markers did not have sufficient sensitivity to replace serial imaging for the detection of small but important changes in tumor burden [35].

13.4 Location of Primary Tumors

Although sporadic ZES was first described in association with neuroendocrine tumors of the pancreas (pNETs), the primary tumor can be located in the duodenum in an equivalent proportion of cases and may also arise uncommonly from other sites [1, 4, 36]. In 90 % of cases, the primary tumor can be localized within the "gastrinoma or Stabile's triangle," a region encompassing the proximal duodenum and head of the pancreas [37]. This triangle is defined by (1) the junction of the cystic and common bile ducts (superiorly), (2) the junction of the second and third parts of the duodenum (inferiorly), and (3) the junction of the neck and body of the pancreas (medially). Duodenal gastrinomas are small tumors arising within the submucosa and are found in the first and second parts of the duodenum in 83–92 % of cases and rarely distal to this [20, 29, 38] (Fig. 13.1). Mean size ranges from 0.6 to 1.3 cm in diameter and 49–80 % of the tumors are less than 1 cm in size, and for this reason, they are rarely seen on imaging studies [4, 17, 38–40]. The pancreas is the site of gastrinoma in 17–55 % of patients with sporadic ZES, and the tumor is located proximally in the head of the pancreas in 33–53 % of cases [1, 4, 36, 41]. Gastrinoma is the second most common functional neuroendocrine tumor of the pancreas, accounting for 36 % of lesions [42, 43]. Pancreatic gastrinomas tend to be larger than their duodenal counterparts with a mean diameter of 2.7–3.2 cm [4, 38, 39, 41, 42]. Sporadic gastrinomas rarely present in multiple sites in 11–16 % of cases, and pancreatic and duodenal gastrinomas may coexist in 9–11 % of cases [4, 27]. In contrast, greater than 75 % of patients with ZES/MEN1 have multiple pancreatic and duodenal neuroendocrine tumors [44, 45].

Ectopic gastrinomas arising outside the gastrinoma triangle are rare accounting for only 6 % of reported ZES cases [46]. Primary ectopic gastrinomas have been reported in the liver, biliary tree, ovary, kidney, heart, stomach, jejunum, and greater or lesser omentum [1, 5, 47, 48]. The occurrence of primary lymph node gastrinomas (primary LN) is controversial. A primary LN gastrinoma is defined by the following conditions: (1) removal of an involved lymph node in the absence of a gastrinoma primarily found elsewhere and (2) normal postoperative FSG and a negative secretin stimulation test [1]. In a large series from NIH, 26 % of patients with ZES undergoing laparotomy that had only involved lymph nodes removed without identification of a primary tumor of the pancreas or duodenum resulted in normalization of the gastrin postoperatively [36]. Whether these nodal lesions represent metastases from a small primary tumor that evaded detection at the time of initial operation or are a true primary remains controversial. In a long-term follow-up from this NIH series, 69 % of the primary LN gastrinomas remained biochemically cured leading these authors to conclude that lymph nodes can be the primary site of gastrinoma in 10 % of patients with ZES [49]. It has been demonstrated that primary LN do not occur in ZES/MEN1 and that the designation as a lymph node primary should be tentative until 10 years after surgery [1, 50].

Metastatic disease affecting regional lymph nodes or the liver can be demonstrated in 52–69 % of patients with gastrinoma on initial imaging studies or at the time of laparotomy [36, 51, 52]. Involvement of regional lymph nodes, particularly peripancreatic and periduodenal nodes, can be demonstrated in 40–75 % of patients with duodenal gastrinoma and 50 % for pancreatic [5, 51, 53]. In contrast to

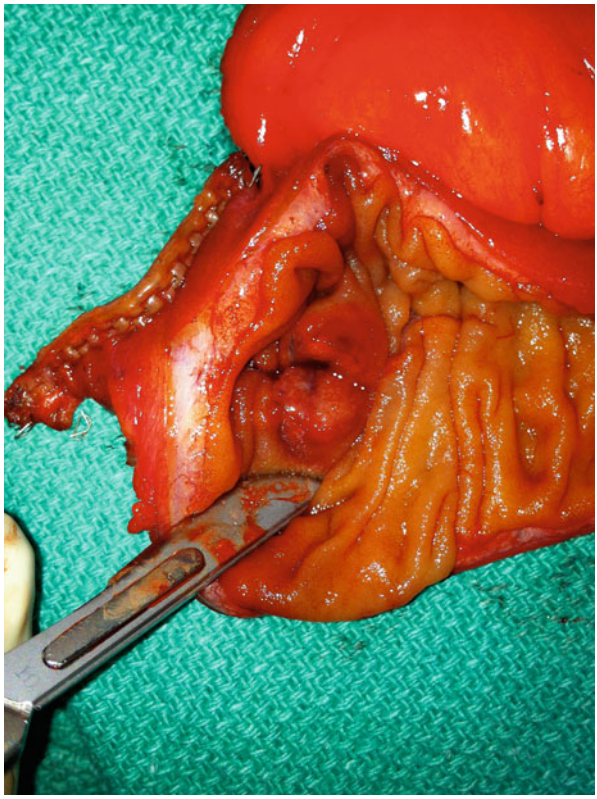

Fig. 13.1 Photograph showing a 10 mm gastrinoma found within the fourth part of the duodenum at laparotomy in a patient with Zollinger-Ellison syndrome. The lesion was resected with a small bowel resection including the positive lymph nodes found in the adjacent mesentery

metastatic liver disease, regional lymph node involvement alone has not been associated with a reduction in survival, with an overall 10-year survival approaching 100 % [4, 53, 54]. Hepatic metastases are present in 8–22 % of patients with gastrinoma at the time of initial assessment [51, 53, 54]. Several authors have found that pancreatic gastrinomas are more frequently associated with hepatic metastases than were duodenal gastrinomas (52 % vs. 5 %) [4, 53]. The size of the primary tumor was associated with an increased risk of hepatic metastases, with tumors smaller than 1 cm having a 4 % risk of hepatic metastases, tumors 1–3 cm in size having metastases in 28 % of cases, and 61 % of tumors greater than 3 cm [53, 55]. The development of hepatic metastases significantly worsens survival and is associated with a 10-year survival rate of 26–30 % [53, 54]. Other areas of metastatic disease include peritoneal disease in 6 %, bony metastases in 3–7 %, and lung metastases in 2 % [27, 56].

13.5 The Genetics of Gastrinomas

Germ-line mutation of the MEN1 gene, a tumor suppressor gene located on the short arm of chromosome 11 (11q31), is responsible for the MEN1 syndrome [6]. The MEN1 gene codes for the nuclear protein *menin* which is involved in cell

growth regulation. Twenty-five percent of gastrinomas are associated with the MEN1 syndrome [1, 7]. Mutations of the MEN1 gene are inherited in an autosomal dominant pattern, and between 36–42 % of patients with this mutation will develop gastrinoma [44]. Mutation of the MEN1 gene has also been implicated in the pathogenesis of sporadic gastrinoma. Mutations of this gene have been found between 17 and 58 % of gastrinomas in patients who do not possess germ-line MEN1 mutations or a family history of MEN1-related tumors [57, 58]. It has been proposed that MEN1 gene mutation is an early event in the pathogenesis of both sporadic and familial gastrinomas, with mutations occurring equally in duodenal and pancreatic gastrinomas [7]. The biological behavior of gastrinomas displaying MEN1 gene mutation is similar to those where this mutation is not found, with no increased risk of metastatic disease or of recurrence after surgical resection [57, 58].

A number of other genetic mechanisms are involved in the pathogenesis of gastrinoma in addition to mutations affecting the MEN1 gene. Chromosomal deletions causing loss of heterozygosity of chromosomes 1q and 22q have also been identified in gastrinomas [59, 60]. Inactivation or deletion of the p16 tumor suppressor gene at chromosome 9p21, coding for the p16 protein involved in the regulation of cell growth and cell cycle inhibition, has also been demonstrated in 52 % of gastrinomas [61]. Gene expression using microarrays has identified numerous additional gene alterations in pancreatic NETs; however, there is no clear concordance between the studies, and none have established as clearly important for pathogenesis [5, 7, 55, 62].

13.6 Imaging

Cross-sectional imaging with computed tomography (CT) and magnetic resonance (MRI), somatostatin receptor scintigraphy (SRS), and endoscopic ultrasound (EUS) have been utilized for the localization and staging of patients with ZES and to detect recurrent disease. NETs are hypervascular and therefore demonstrate a greater degree of enhancement than the normal pancreas [1]. Cross-sectional imaging with CT and MRI does not possess the resolution to detect duodenal primary tumors small but can detect pancreatic gastrinomas with a sensitivity of 54–71 % and metastatic regional lymph nodes [51, 63, 64] (Fig. 13.2). Somatostatin receptors are expressed by 85–100 % of gastrinomas, and for this reason, SRS using radiolabeled octreotide can be used to detect primary gastrinomas and nodal or distant metastatic lesions [63] (Fig. 13.3). SRS has a sensitivity of 11–30 % for the identification of duodenal lesions, 25–77 % for pancreatic lesions, 72–82 % for nodal metastases, and 67–100 % for hepatic metastases from gastrinoma [1, 63, 65]. In a study of 122 patients with ZES by Termanini et al., SRS was positive for disease in 61 % of patients and was the only imaging modality that localized tumor in 12 % of cases, changing the management of 47 % of cases [66]. In another study, SRS was able to detect primary tumors and metastatic lesions with a sensitivity superior to that of CT, MRI, and angiography combined; however, it was unable to identify 33 % of the primary tumors subsequently found at laparotomy [63]. These results correlated

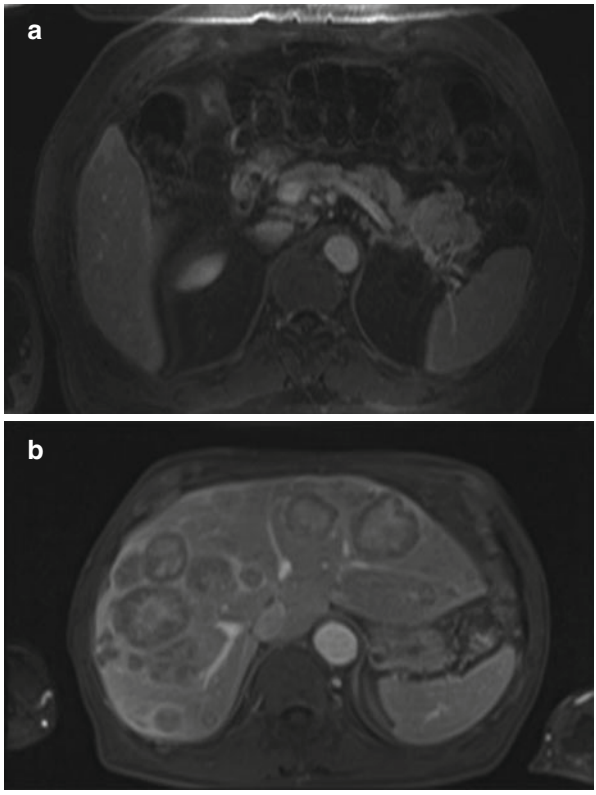

Fig. 13.2 (**a**) Computed tomography showing peripancreatic lymph node. The primary 8 mm duodenal gastrinoma was not seen, yet the lymph node gave us a clue as to its location. (**b**) MRI demonstrating metastatic liver disease in a patient with Zollinger-Ellison syndrome from a large pancreatic gastrinoma

with the tumor size and location as SRS detected only 30 % of gastrinoma <1 cm, 64 % of those between 1.1 and 2 cm, and 96 % of those >2 cm and missed small duodenal lesions.

EUS has been shown to be useful in the detection of pancreatic neuroendocrine tumors including gastrinomas, identifying even small lesions with a sensitivity of 82 % and a specificity of 95 % [67]. This technique also has the advantage of defining the relationship of primary tumors to pancreatic ductal and vascular structures, and needle biopsy of pancreatic lesions can also be performed. EUS was able to detect up to 91 % of pNETs missed by CT [67]. EUS unfortunately is less sensitive in the detection of duodenal gastrinomas due to their small size.

Invasive testing involving arteriography or selective venous sampling has been utilized to assist in the localization of gastrinoma in patients where CT, MRI, and SRS do not localize the tumor. Selective angiography with secretin injection (SASI) involves cannulation of the right or left hepatic veins and sampling of gastrin levels from this location after intra-arterial injection of secretin or more commonly calcium into the gastroduodenal, hepatic, splenic, left gastric, and superior mesenteric arteries [68]. Secretin stimulates gastrin release from the tumor present in the vascular distribution of the arterial injection, producing an increase in gastrin levels as measured in the hepatic veins. Utilizing the same principles, intravenous calcium

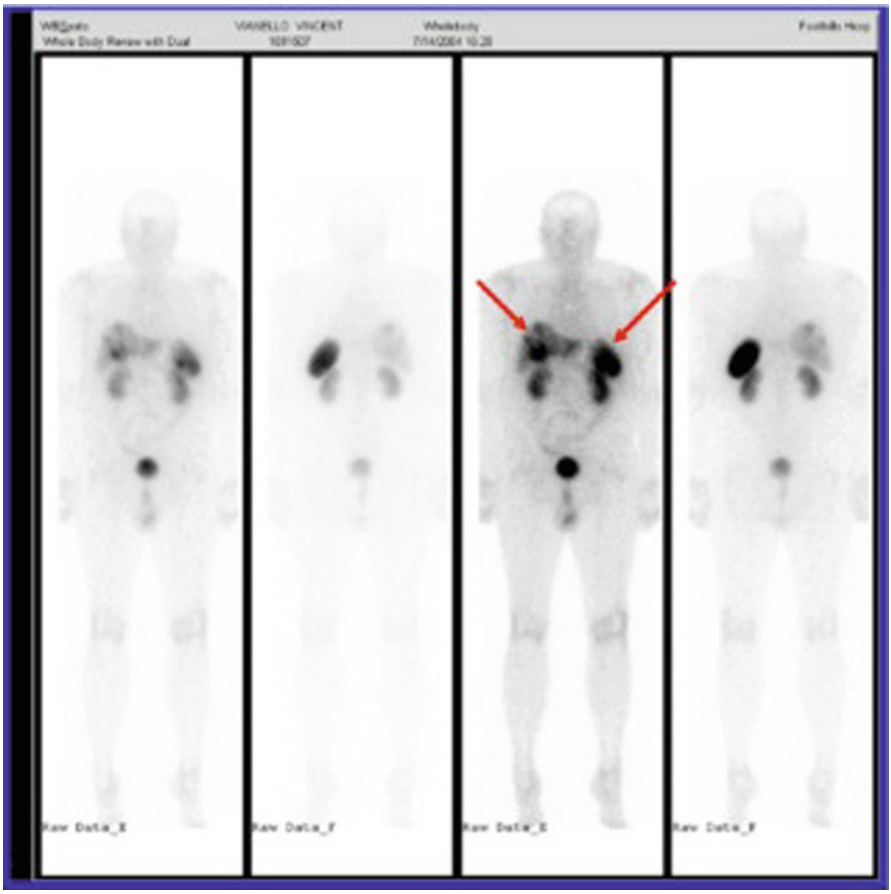

Fig. 13.3 Octreotide scan demonstrating positive uptake in a patient with metastatic Zollinger-Ellison syndrome

injection has also proven to be effective in localizing the gastrinoma [69]. The proportion of patients with gastrinoma where the tumor can be regionalized by SASI varies from 71 to 89 % [1].

Whole body positron emission tomography (PET) using 18-Fluorodeoxyglucose (FDG) is a powerful modality for oncological imaging, yet unfortunately does not accumulate in most well-differentiated NET including gastrinomas. 11C-5-hydroxytryptophan has been shown to be superior to CT and SRS in the detection of neuroendocrine primary tumors or their metastases [70]. PET using 68-gallium-labeled DOTA-TOC, a somatostatin analogue with a greater affinity for somatostatin receptors than octreotide, has been associated with a sensitivity of 97 % for the detection of NETs and metastases [71, 72]. In one study of 52 NET patients, 68-gallium DOTA-TOC proved to be

superior to CT and/or MRI for detection and staging. This modality impacted the therapeutic decision making in almost all patients [72].

In patients with negative imaging, an experienced surgeon becomes an invaluable localizing tool. In a recent report, Norton et al. were able to find the gastrinoma in 57/58 patients with negative imaging. These tumors were small (mean 0.8 cm) and found in the duodenum 67 %, pancreas 17 %, and primary lymph nodes (10 %) [40]. Immediate biochemical cure was established in 35/58 (60 %), with long-term biochemical cure (mean 9.4 years) in 27 (46 %) patients.

13.7 Prognosis and Management of Localized Sporadic Disease

With the introduction of effective medical therapy for the control of gastric acid hypersecretion in patients with ZES, the role of surgery in the management of patients with gastrinoma has shifted from the control of gastric acid secretion to the requirement for oncological resection of the primary tumor to prevent disease progression and metastatic spread [30, 43, 73]. Surgical resection is the only modality capable of achieving cure in patients with ZES. Given the malignant potential of the primary tumor, surgery also has an important role in preventing the progression to metastatic disease. In their study comparing the long-term follow-up of 160 ZES patients undergoing surgical resection of gastrinoma with 35 ZES patients managed nonoperatively, Norton et al. found that significantly fewer patients undergoing surgical resection developed hepatic metastases during long-term follow-up (5 % vs. 29 %) [36]. More importantly this group demonstrated that surgery can increase survival. The 15-year disease-specific survival was 98 % in the surgery group versus 74 % in the nonoperative group. In those patients who did not undergo surgery, 90 % of deaths were secondary to progressive metastatic disease. Similarly at Ohio State University, Ellison et al. found that patients undergoing R0 resections had a significantly greater long-term survival than those who had incomplete resection of disease or who did not undergo surgery (10-year survival 85 % vs. 30 %) [4].

Surgical resection of the primary tumor and involved regional lymph nodes is curative in patients with gastrinomas that have not metastasized to distant sites. Inspection and palpation of the structures in the gastrinoma triangle is performed after kocherization of the duodenum and can successfully detect 61–71 % of primary gastrinomas [20, 38, 39]. Intraoperative ultrasound (IOS) has been shown to improve the ability to localize primary tumors of the pancreas and identify metastatic disease within the liver, but is not more sensitive than palpation in the detection of duodenal primaries [17, 39]. Small primary tumors within the duodenum are best identified by inspection and palpation of the mucosal surface via a longitudinal duodenotomy of the second part of the duodenum and inversion of the mucosa [19, 20]. After introducing routine duodenotomy to the operative exploration of patients with ZES, Norton et al. demonstrated a significant improvement in the ability to localize the primary tumors from 76 to 98 % of cases [17]. Routine

Table 13.3 Surgical strategies for Zollinger-Ellison syndrome

Clinical scenario	Strategy
Sporadic localized disease	
Pancreatic gastrinoma	
Size <3 cm	Enucleation (open or laparoscopic)
Size >3 cm	En bloc resection
	Whipple or distal pancreatectomy + lymph node resection
Duodenal gastrinoma	
Solitary/small	Local resection
Multiple ± large ± local invasion	Pancreaticoduodenectomy + lymph node resection
Metastatic gastrinoma	Consider surgical cytoreduction of hepatic metastases if >70–90 % of tumor volume removable
	and/or
	Multimodality medical therapy: PPI, octreotide, radionuclide therapies, hepatic artery embolization, mTOR inhibitors
ZES/MEN1	
Biochemical or image detected <2 cm	Duodenotomy, 80 % distal pancreatectomy and enucleation of NETs (head of the pancreas) plus lymph node resection
	or
	Duodenotomy, local resection of pNETs
	Consider lymph node resection
	or
	Treat medically with PPIs
Image detected >2 cm	Duodenotomy and en bloc pancreatic resection with regional lymph nodes

MEN1 multiple endocrine neoplasia type 1, *mTOR* mammalian target of rapamycin, *NET* neuroendocrine tumor, *PPI* proton pump inhibitor, *ZES* Zollinger-Ellison syndrome

duodenotomy also had significantly higher rates of long-term biochemical cure postoperatively occurring in 52 % of patients undergoing duodenotomy versus 26 % of patients who did not.

Options for resection of the primary gastrinoma are dependent on the location and size of the tumor (Table 13.3). The majority of duodenal lesions are small and can be resected locally, either removing from the submucosa or with an elliptical resection of the full thickness of the duodenal wall (Fig. 13.1). Lesions of the pancreas can be enucleated or formally resected depending on their size and location within the gland. Lesions within the head of the pancreas smaller than 2 cm can be enucleated in most cases. Pancreaticoduodenectomy is required for large or locally invasive tumors of the head of the pancreas, whereas a distal pancreatectomy is the procedure of choice for the rare gastrinoma located in the body or tail of the pancreas [51, 74] (Fig. 13.4a, b). Spread to regional lymph nodes can be demonstrated in 36–67 % of patients with gastrinoma, and therefore resection of regional lymph

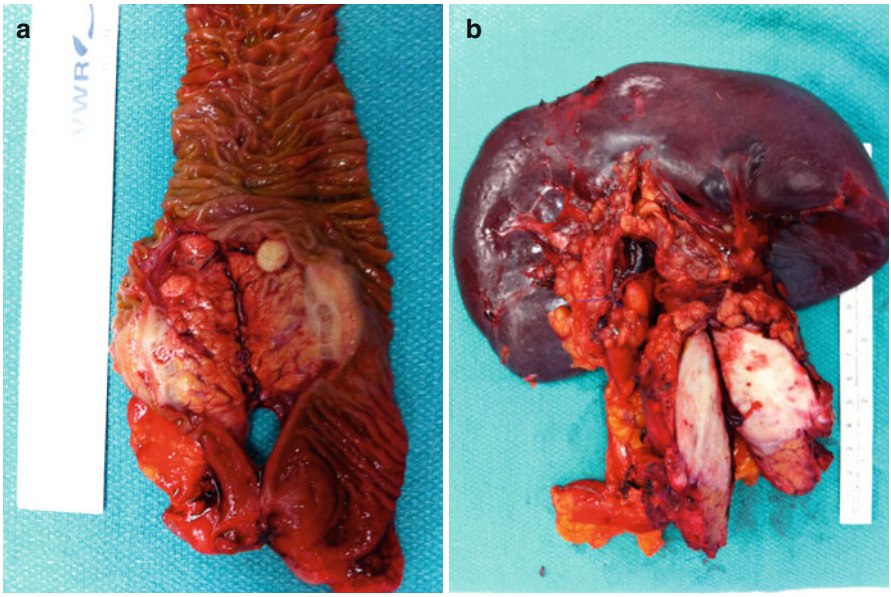

Fig. 13.4 (**a**) A surgical specimen following a pancreatoduodenectomy (Whipple procedure) for an invasive gastrinoma in the head of the pancreas. (**b**) Surgical specimen following a distal pancreatectomy for a rare distal pancreatic gastrinoma

node groups should occur at the time of resection of the primary tumor [43, 52, 54]. A recent retrospective study in 41 ZES patients found higher rates of biochemical cure and a prolonged disease-specific survival in those ZES patients undergoing systemic lymphadenectomy compared to those who did not [75]. The authors concluded that in sporadic gastrinoma, systemic lymphadenectomy during the initial surgery may reduce the risk of persistent disease and improve survival.

The definition of biochemical cure following surgery for ZES is a normal FSG and negative secretin stimulation test. Initial postoperative cure rates are reported to be greater than 60 %; however, this decreased to 23–50 % at 10 years [4, 36, 75]. Although recurrences after potentially curative surgery are common, disease-specific survival is excellent with 15-year rates between 74 and 98 % [4, 36, 75]. Natural history studies demonstrate that 25 % of sporadic ZES patients have an aggressive clinical course [53, 54]. In one retrospective study, negative prognostic factors for disease-specific survival were pancreatic location, tumor size >2.5 cm, Ki-67 index >5 %, preoperative FSG >3000 pg/ml, and liver metastases [75]. Interestingly sex, age, type of surgery, and presence of lymph node metastases had no influence on disease-free or disease-specific survival. Others have found that female gender, ectopic ACTH production, and bone metastases also are negative prognostic factors [5, 54].

13.8 Management of Advanced and Metastatic Disease

Synchronous liver metastases occur in 20–30 % of ZES patients. In addition up to 40 % of patients will develop disease progression following an R0 resection [1, 76, 77]. Medical therapy with PPI is highly effective in the control of gastric hypersecretion, and for this reason, the role of surgical palliative debulking for the control of the endocrinopathy is somewhat controversial. As a general principle, multimodality, including radiofrequency ablation, cryotherapy, and surgical resection liver-directed therapy of functional NETs, does appear to provide a palliative benefit in patients with endocrinopathies and improve survival in carefully selected patients [76–82]. In a series of 213 ZES patients, hepatic metastases occurred in 32 % of cases, and diffuse involvement of the liver was present in 75 % of these cases [81]. The remaining 19 patients had all gross disease in the liver resected. Patients undergoing resection of hepatic metastases had a 5-year survival rate of 85 %, which was superior to those patients with hepatic disease who were not resectable. Although surgical selection bias likely played a significant role in these results, others have also demonstrated a survival benefit associated with the aggressive surgical debulking of neuroendocrine tumor metastases [80, 83, 84]. A 2009 Cochrane Database review concluded, despite no randomized trials, that liver resection was the mainstay of survival-prolonging therapy for Stage IV NETs [85]. More recently a multi-institutional analysis of the surgical management of hepatic NET metastasis found a prolonged 5- and 10-year survival of 74 and 51 %, respectively [80]. Although only 12/339 (4 %) of the patients had ZES, patients with hormonally functioning NETs benefitted the most from surgery.

Hepatic artery chemoembolization (HACE) and bland embolization (HAE) offer options for cytoreduction of bilobar disease not amenable to surgical resection or RFA. Response rates after embolization vary between 50 and 96 % depending upon the criteria utilized such as biochemical, radiographic, symptomatic control [78]. Median duration of response extends up to 18 months (4–51 months) [78, 86–88].

Radioembolization of the hepatic artery using yttrium-90-bound resin (SIR-Spheres [Sirtex Medical LTD, New South Wales, Australia]) or glass microspheres (TheraSphere [MDS Nordion, Ottawa, Canada]) has been used in the treatment of hepatic NET metastases including gastrinomas. In a multicenter retrospective review of 148 NET patients undergoing radioembolization, Kennedy et al. found partial response rate of 61 %, stable disease 23 %, complete response 3 %, and progression 5 % at 6 months [89]. King et al. found similar results in 34 NET patients treated with SIR-Spheres. The radiological response was 50 %, and the symptomatic response was 55 % at 3 months and 50 % at 6 months with an overall median survival of 29.4 months [90]. Unlike hepatic artery embolization, this technology requires proper patient selection and pre-procedural evaluation for hepatopulmonary shunts. It is recommended that no more than 20 % of blood flow is diverted to the lung and some patients may require pre-procedural occlusion of these shunts to minimize extrahepatic delivery of yttrium [91].

Radionuclide therapy with 111-indium-labeled octreotide has been associated with a symptomatic response in 53 % of patients with metastatic NETs including gastrinomas and stabilization of disease in 35 % of cases [92]. The somatostatin analogue DOTA-TOC radiolabeled with either yttrium-90 or 177-lutetium has recently been shown to have a superior tumor response than the 111-indium-labeled octreotide and has become the radionuclide therapy of choice in selected NET patients [93]. Although not specific for gastrinomas, these compounds have been shown to have a partial tumor response in 25 % of NET patients and stabilization of disease in 55 % [94].

Systemic therapy with cytotoxic chemotherapy has a limited role in the management of disseminated metastatic gastrinoma. Grama et al. treated 18 patients with metastatic gastrinoma with a combination of 5-fluorouracil, streptozocin, doxorubicin, and IFN-A and documented a radiological response of only 17 % [95]. In a study of 84 patients with locally advanced or metastatic pNETs treated with a combination of 5-fluorouracil, doxorubicin, and streptozocin, Kouvaraki et al. reported a partial or complete radiological response in 39 % of the patients; however, none of the 11 patients with metastatic gastrinoma displayed a response to the therapy [96].

Medical treatment with somatostatin analogues including octreotide and lanreotide is utilized in metastatic gastrinoma both to reduce tumor gastrin production and for their antiproliferate properties [55]. In a small series of 15 patients with metastatic gastrinoma treated with subcutaneous octreotide, 53 % of cases demonstrated a radiological response, with stabilization of progressive disease in 47 % and partial tumor regression in 6 % [97]. The mean duration of the clinical response was 25 months.

Several medical agents targeting growth factor receptors and related signaling pathways have recently been studied for the management of metastatic NETs. Everolimus is an inhibitor of mammalian target of rapamycin (mTOR), a serine-threonine kinase involved in cell cycle and growth regulation and apoptosis via the PI3K/AKT/mTOR signaling pathway. Sunitinib is a tyrosine-kinase inhibitor that inhibits vascular endothelial growth factor receptors (VEGFR) and platelet-derived growth factor receptors (PDGFR), both of which are expressed on pNETs. Two phase 3 multicenter, double-blinded, randomized, placebo-controlled trials utilizing these therapies in the treatment of progressive pNETs demonstrated promising results [98, 99]. Sunitinib, as compared to placebo, causes more than a doubling in progression-free survival (11.4 vs. 5.5 months ($p < 0.001$)), an increase in the rate of objective tumor response and an increase in overall survival [98]. Everolimus caused a 65 % reduction in the estimated risk of progression (progression-free survival 11 vs. 4.6 months ($p < 0.001$)) [99]. Although both treatments caused adverse drug-related events in significant numbers compared to placebo, most events were grade 1 or 2. These studies provide optimism for the systemic treatment of NETs, however, the choice of which drug and the optimal sequence of therapeutic options for metastatic NETs remains [43, 73, 100–103].

13.9 Management of ZES/MEN1

The management of the gastrinomas occurring as part of MEN1 syndrome remains controversial [4, 5, 7, 55, 104, 105]. The role of surgery in the management of ZES/MEN1 is less clearly defined than for sporadic tumors due to the excellent long-term survival associated with this condition, the low rates of long-term cure of ZES after surgical resection, and the effectiveness of medical control of ZES with PPI therapy [4, 5, 36]. ZES occurring in the context of MEN1 displays important differences in biological behavior compared with sporadic gastrinomas. The mean age of onset of ZES in patients with MEN1 is younger (34–42 years) and rare before the age of 24 years. The duodenum is the most common site of gastrinoma in patients with MEN1, occurring in up to 88 % of cases, with synchronous tumors of the duodenum and the pancreas occurring in 24 %. ZES is the most common functional endocrinopathy in patients with MEN1 (42–60 %), followed by insulinoma (19 %), vasoactive intestinal polypeptide-producing tumors (4 %), and nonfunctional NETs (35 %) also occurring [7, 106]. The long-term survival of patients with ZES/MEN1 is excellent, and there is evidence that gastrinomas occurring in the context of MEN1 have a lower malignant potential than sporadic tumors. Hepatic metastases are present in only 3–4 % of MEN1 patients with gastrinoma at the time of diagnosis but will develop in long-term follow-up in 14–23 % of MEN1 patients [107]. A number of studies have found that the long-term survival of patients with MEN1 developing ZES is superior to that seen in sporadic forms of the tumor [7]. Utilizing the long-term prospective NIH database, Ito et al. found that the 30-year overall survival for ZES/MEN1 was 85 % and disease-related survival 88 % [108]. Factors predicting a poorer prognosis were high gastrin levels, development of other endocrinopathies, the need for >3 parathyroid operations, and distant metastases.

Since the introduction of effective medical therapy for the control of gastric acid hypersecretion, patients with ZES/MEN1 no longer die from complications of severe PUD, and long-term survival can be expected [107–109]. The rate of biochemical cure of ZES/MEN1 is rare except when either a Whipple or total pancreaticoduodenectomy has been preformed. Patients with MEN1 display a field defect with hyperplasia and multiple micro-tumors of gastrin-staining cells in the duodenum [110]. Most surgeons who believe surgery is beneficial to the MEN1 patient advocate the *Thompson procedure* [104, 105]. This involves a distal pancreatectomy, an enucleation of NETs in the head of the pancreas, a duodenotomy, and a resection of any duodenal tumors, along with a lymph node dissection around the porta hepatis [111]. Unfortunately, long-term biochemical cure after this operation for ZES/MEN1 patients is rarely achieved [51, 112]. Initially, postoperative normalization of FSG occurred in 68 % of ZES/MEN1 patients without hepatic metastases, but this rate fell to 33 % with provocative testing with intravenous secretin, implying that most patients harbor microscopic foci of residual disease [112, 113]. Many of the original ZES/MEN1 patients operated on by Thompson have had to undergo a completion Whipple operation [113]. A more focused approach to resection of pancreatic NETs, leaving a larger pancreatic remnant and enucleating lesions in the body of the gland, has been adopted by the University of Michigan due to the high

incidence of recurrent disease and surgical challenges associated with completion Whipple procedures.

Since biochemical cure is rare and symptomatic control is easily achieved with PPIs, surgical resection has been advocated to reduce the risk of developing metastatic disease [105, 114]. Akerstrom et al. utilize both biochemical and EUS screening to identify patients early before the development of metastases [105]. Once the patient develops an endocrinopathy such as ZES, the risk of lymph node metastases is >50 %. Therefore, these authors do not wait for anatomical imaging to demonstrate NETs. Thompson on the other hand suggested that surgery should be done when any tumor was visualized by cross-sectional imaging [115]. Others have taken a more conservative approach, utilizing surgical resection for tumors of more than 2–3 cm in size, citing the low risk of hepatic metastases with smaller tumors [107, 114, 116]. From the NIH database, Norton et al. demonstrated that patients with tumors <2.5 cm can be surveyed without the risk of liver disease or mortality [116]. Triponez et al. showed that the metastatic risk increased in MEN1 patients for tumors >2 cm [114]. Finally in a study of 55 patients with MEN1 and pNETs, Kouvaraki et al. found that patients who underwent surgical resection had significantly higher overall 10-year survival compared with those who did not (81 % vs. 34 %) [117]. As there is an absence of randomized controlled data examining the role of surgery, the indications for surgical resection for patients with ZES/MEN1 remain institution specific.

13.10 Summary

The management of ZES has evolved due to advances in medical therapies and diagnostic imaging and improvements in surgical technique. Surgical resection is the procedure of choice for sporadic gastrinomas and has an important role in the ongoing management of selected patients with ZES/MEN1 syndrome. A multimodality approach to the treatment of advanced and disseminated disease in patients with ZES is crucial. These patients benefit from aggressive cytoreductive approaches to the metastatic disease with the addition of systemic therapies to control both tumor growth and the endocrinopathy.

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VIPoma and PPoma

14

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14.1 VIPoma

14.1.1 Epidemiology

VIPomas represent 1.4 % of Gastro entero pancreatic (GEP) neuroendocrine tumours (NETs), corresponding to an incidence of 0.01/1,000,000 yearly. About 85 % are pancreatic, and amongst islet cell tumours, they are about 2 % [1]. Rare extrapancreatic types exist including oesophagus, liver and retroperitoneum, but these forms are mainly present in paediatric age and are attributable to neuroblastomas, which produce also VIP. In adults the extrapancreatic types have low malignant phenotype.

In the pancreas VIPoma usually arises as a unique tumour. It may reach great dimensions, 3 cm as diameter (range of 1–7 cm) on the average, but one case report describes also a 20 cm VIPoma [2]. The main location is (75 %) within the pancreatic body and tail. It is more frequent in female (about 56 %) and the mean age at diagnosis is 49 years.

The pure secreting VIPoma is rare, because about 75 % of VIPomas produce also pancreatic polypeptide (PP), neurotensin, gastrin and GIP. However, it is nearly

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always biologically active [3, 4]. Four to ten percent of VIPomas belong to the MEN syndrome. Wermer syndrome, induced by VIP production, arises in 1–7 % of islet cell tumours.

14.1.2 Molecular Mechanisms

To date we can find in the literature 133 case reports and 161 patients grouped in 8 cohorts on VIPoma. Amongst all publications, a small number of them report the investigation about the molecular mechanisms for the development of VIPoma.

In particular, a study investigated benign and malignant NETs for microsatellite loss of heterozygosity (LOH) analysis and fluorescence in situ hybridization (FISH) in order to evaluate the importance of chromosome 3p deletions in the molecular pathogenesis and biological behaviour. Amongst these patients a VIPoma showed LOH at all informative microsatellite markers. This finding could indicate that the tumour cells initially had a 3p deletion which was followed by duplication of the remaining allele. Such endoreduplication was also reported for colorectal cancer [5, 6]. In a similar case series, chromosome 6q losses were observed. Some tumour suppressor genes are located in this region of deletion. These genes include "absent in melanoma 1" (AIM1), "cyclin C" (CCNC) and "receptor-type protein-tyrosine phosphatase kappa" (PTPRK). AIM1 and CCNC were mapped to 6q21 and PTPRK to 6q22.2-q22.3. The first one exerts its effects through interactions with the cytoskeleton. The CCNC is upregulated by 1α,25-dihydroxyvitamin D3, inhibits cellular growth and induces apoptosis. The PTPRK gene seems to be involved in the regulation of cell adhesion by dephosphorylation of β-catenin and γ-catenin/plakoglobin or cadherins, thereby contributing to the formation and maintenance of intact adherens junctions [7].

Another report of pancreatic NETs including VIPomas evaluated the putative tumour suppressor gene DPC4 located at the chromosome 18q21.1. This gene, also indicated as Smad4, is a member of the highly conserved family of Smad proteins that are involved in the transduction of signals from the transforming growth factor family of cytokines. This study found out no mutations in this gene for VIPomas [8].

A study about the role of sex chromosome in NET development included 1 VIPoma. This analysis showed a loss of chromosome X in 40 % of female patients whereas loss of chromosome Y in 36 % of male patients without loss of the X chromosome. Sex chromosome loss is associated with aggressiveness of pancreatic NETs and may also predict a poorer clinical outcome. This phenomenon could be explained by the role of genes on the X chromosome in the induction of senescence and control of cell proliferation [9].

BRAF mutations were also investigated in NET, but these genetic alterations were really low (3 %). However, none of the two VIPomas of this case series bore BRAF gene impairment [10].

Recently, in a patient with VIPoma, LOH downregulation was observed by the microarray analysis for the mismatch repair gene MSH2 in the primary tumour. Besides, in the same case, a strong overexpression of the chemokine CXCR4 gene

was found in the liver metastases. On the basis of these findings, the authors argued a role for MSH2 gene impairment in carcinogenesis and for CXCR4 dysregulation in metastasis development [11].

14.1.3 Clinical Manifestations

In 1958 Verner and Morrison described in two patients a syndrome including diarrhoea, hypokalaemia, hypochlorhydria and metabolic acidosis [12, 13]. For this syndrome an acronym was used: WDHH (watery diarrhoea, hypokalaemia, hypochlorhydria). It was also defined as "pancreatic cholera", but this definition is not proper when it arises from an extrapancreatic origin. The term VIPoma was suggested by the elevated plasma levels of the vasoactive intestinal polypeptide (VIP), which are associated with this kind of tumour. VIP is a 28-amino acid polypeptide which is distributed throughout the body and normally functions as a neurotransmitter. In the gut it regulates the blood flow, smooth muscle activity, pancreatic and intestinal secretions and inhibits gastric acid production. Excessive circulating VIP concentrations induce secretion in all intestinal segments of Na+, K+, Cl- and HCO3- as well as water, but also gastric acid secretion, bone resorption, glycogenolysis and vasodilation.

The Verner-Morrison syndrome is characterized by sizeable diarrhoea (1–6 l/die), which is watery and increasing. The diarrhoea associated with VIPoma is characterized by its persistence for 48–72 h after fasting and by great faecal volumes of 6–8 l/die. The secretory nature of the diarrhoea is confirmed if it is refractory to fasting. The hydro-electrolytic disequilibrium is a consequence of diarrhoea and includes hypokalaemia, hypomagnesaemia, hypovolaemia and metabolic acidosis. These events induce lethargy, muscular weakness, cardiac conduction disturbances, weight loss, abdominal pain, paralytic ileus, dyspepsia and gallbladder hypotonia. Flushing could be found in about 20 % of VIPomas, and rarely acute kidney tubular necrosis was reported. In 75 % of VIPoma patients, hypercalcaemia was described, and hypophosphataemia could be associated because of hyperparathyroidism. Hypercalcaemia could be a consequence of paraneoplastic hormonal secretion. The development of hypercalcaemia was attributed to the release of PTHrp by tumour cells. Half of VIPoma patients develop hyperglycaemia because of reduced glucose tolerance, and an increased hepatic glycogenolysis has been hypothesized as a possible explanation for this finding. In 40 % of those patients, hypochlorhydria has been reported. Hypochlorhydria is due to the inhibition of gastric acid production by VIP. Hyperchloremic acidosis can also develop because of low bicarbonate levels as a consequence of severe intestinal loss [14].

In earlier stages the Verner-Morrison syndrome could be confused with diarrhoeas from different origin, including those related with bacterial, viral and parasitic infections and with inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. Since also other neuroendocrine tumours could induce diarrhoea, the one that is related to VIPoma needs to be distinguished. VIP plasma levels allow to recognize those patients affected by this NET. However, VIP could be present in

various molecular forms, and its levels could exceed 170 pg/ml. These high VIP levels are often associated with high plasma levels of other diarrhoea-inducing molecules, such as pancreatic polypeptide (PP), gastric inhibiting polypeptide (GIP) and prostaglandin E2 (PGE2) [15]. For this reason the already-known role of VIP as the main inducer of diarrhoea has been limited. Besides some VIPoma patients without diarrhoea showed high VIP plasma levels.

Of malignant VIPomas clinical onset is usually accompanied by advanced stage of neoplastic disease. In these patients the clinical management of hydro-electrolytic disequilibrium needs to precede any other treatment. Since the past decades, the use of prednisone has been revealed as a quite efficacious way to manage diarrhoea. Anyway this symptom could be properly contrasted by the treatment with anti-diarrhoeal drugs, prostaglandin inhibitors and indole derivatives, since early phases. The treatment with octreotide, a somatostatin analogue, achieved the best control of diarrhoea with subsequent improvement of hydro-electrolytic disequilibrium. In some patients in whom octreotide was delivered for diarrhoea control, a benefit in metastases regression was also obtained [16]. These results have been subsequently implemented by the findings from the PROMID trial about the antitumour benefit on efficacy end point by octreotide in GEP NET patients.

14.1.4 Biochemical Indicators

VIPomas are characterized by hypokalaemia in 100 % of patients and hypochlorhydria in 70 %, as a consequence of electrolytic loss by secretory diarrhoea, hypercalcaemia in 40 % and hyperglycaemia in 20 %. However, this biochemical changes are not sufficient for diagnosis, because only high VIP plasma levels are really mandatory for VIPoma's diagnosis. Since fasting normal VIP values are ranged between 150 and 170 pg/ml, in VIPoma patients hugely higher levels are found in plasma [14]. The VIP levels are usually found elevated in almost all cases, but it could also be found normal between the episodes of diarrhoea. However, the evaluation of VIP plasma levels needs some particular technical devices to guarantee its accuracy and its right interpretation. In fact VIP is a highly unstable protein which easily undergoes a proteolytic degradation [17].

Anyway high VIP plasma levels could be found in patients affected by liver and kidney failure, myocardial infarction, intestinal ischemia, AIDS and laxative-induced diarrhoea.

Besides VIPomas are able to produce other peptides such as PP, calcitonin, gastrin, neurotensin, gastric inhibitory peptide (GIP), serotonin, glucagon, insulin, somatostatin, growth hormone-releasing hormone and peptide histidine-methionine [18].

14.1.5 Diagnosis

When a NET has a dimension less than 1 cm, CT scan reaches a sensitivity less than 10 %. However, VIPomas are usually found when they are greater than 3 cm, so that CT sensitivity could reach 100 % [19]. The magnetic resonance imaging (MRI) could help differentiating the small pancreatic tumours from the surrounding

normal pancreatic tissue. This imaging technique has reached a sensitivity of 85 and 100 % specificity [20]. On the basis of the high expression of somatostatin receptors in VIPomas, OctreoScan has been considered as a reliable evaluation for this kind of tumours. Somatostatin receptors are expressed in 80–90 % of VIPomas.

The histological examination of VIPoma show some molecular patterns, such as solid, acinar or trabecular tissue architecture with scant mitoses. The cells are cytologically bland, with modest amount of cytoplasm and round nuclei without visible nucleoli. Few mitotic figures can be found. However, since 60–80 % of VIPomas are metastatic diagnosis, locoregional lymph nodes and suspicious distant metastases could confirm that those histological features are associated with a malignancy [21].

14.1.6 Treatment Options

The suspicion of this neuroendocrine tumour in patients with chronic diarrhoea could help to diagnose early this rare disease. Before any curative or palliative treatment is started, the life-threatening hydro-electrolytic disorders must be corrected. Some patients could require a massive intravenous potassium replacement because a consistent potassium deficit induced by chronic gastrointestinal losses.

More than half of VIPomas are resectable at diagnosis. Curable rate by resection reached 10 % [22]. The localization of VIPomas is relatively easy because 80 % have a single site and range between 1 and 7 cm. Pancreatic forms are usually localized in the pancreatic tail. For this reason in these cases, the surgical treatment is represented by a distal pancreatectomy. When pancreatic lesions could not be identified, the exploration of adrenal glands and sympathetic plexus for extrapancreatic forms is necessary. Cytoreductive surgery includes palliative debulking of the primary tumour. This treatment achieves a tumour bulk reduction, the improvement of hormone-mediated symptoms and the prevention of local and systemic tumour effects. A complete resection of the primary tumour improves the prognosis greatly.

In metastatic patients with low risk, the surgical option could be taken into account for symptoms control, which could be reached in 40 % of patients. In high-risk patients with multiple metastatic sites, a systemic treatment is overriding. However, in these patients a palliative debulking surgery should be considered, since patients' outcomes could be improved.

The medical treatment of VIPoma is mainly directed to symptom control. Somatostatin analogues achieved diarrhoea improvement in 56 % of patients. Besides a VIP plasma level reduction was also observed in 60 % [23]. Octreotide was delivered at doses ranging between 50 and 1,500 mcg/die. The best response rate was 83 % with median duration of 6 months. However, octreotide 250–450 mcg/day induced 60 % of symptom control, 70 % of biochemical changes and 5–10 % of tumour responses. The stabilization of neoplastic disease was documented by CT scan in 30–50 % of patients [24]. Some authors reported a marked decrease in the tumour blood flow in two patients with VIPomas. This finding allowed to hypothesize a direct vasoactive effect of octreotide on the tumour blood supply or an indirect effect of reduced hormonal secretion on blood flow [25].

Twenty percent of patients experienced resistance to octreotide and 16 % showed a direct antitumour activity.

After a prolonged treatment with octreotide, tachyphylaxis could be observed. For this reason dose escalation or combination with other agents such as interferon-alpha could be considered to overcome resistance. In the next future newer somatostatin analogues will be available, such as pasireotide or SOM-230, with prolonged half-life and higher affinity for somatostatin receptors.

As alternative treatment options both chemotherapy and radiotherapy were tested. The former showed no significant benefits and the latter obtained anecdotal benefit but shortly lasting.

14.2 PPoma

PPoma belongs to those non-functioning NETs, which are not associated with a clinical syndrome. This group include also tumours secreting neurotensin or calcitonin. This lack of function may be due to the inability of these peptides in the induction of physiopathological changes and subsequent clinical symptoms. In these tumours the symptoms are usually a consequence of compression, if tumour is greater than 5 cm or of metastases development. About 65 % of these NETs are malignant [26].

PPoma cells show a differentiation that makes them similar to the gamma cells of the Langerhans islets, which represent 10 % of the pancreatic islet cells and secrete >90 % of total PP [27, 28].

PPoma is characterized by higher plasma levels of pancreatic polypeptide (PP, also reported as growth inhibiting factor, GIF, or human pancreatic polypeptide, hPP). This peptide was identified in 1972 by two independent laboratories [29]. Its function has not been yet clarified. However, we know that it weakly inhibits pancreatic enzymes and gastric acid secretion and contrasts gallbladder contraction. PP could also be found in islet cell tumours, like 75 % of VIPomas and 25 % of gastrinoma, in multiple endocrine neoplasias (MENs) and in other intestinal and bronchial tumours.

Pure PPomas are rare. They represent less than 1 % of NETs. Until now the literature reports 24 patients with sporadic pancreatic (non-MEN) and 6 with extrapancreatic PPomas. The age of these patients ranged from 20 to 74 with a mean age of 51. The incidence was similar for females and males. It develops more frequently in the head of pancreas and could reach dimensions greater than 5 cm because of the lack of specific symptoms. Metastases developed in 8 out of the 24 patients reported, but this evolution seems to be not associated with poor survival [30–43].

PPomas are diagnosed by ultrasound and CT scan. These tumours are mostly found incidentally in patients undergoing diagnostic tests for non-specific

symptoms. The association of high basal plasma PP levels with highly vascular tumours on CT scan should induce the suspicion for PPoma. Other investigations, including endoscopic ultrasound, somatostatin receptor scintigraphy, PET, transhepatic portal venous sampling, selective angiography with secretin injection or surgical exploration, could help for diagnosis.

Sometimes symptoms and clinical signs could be described such as watery diarrhoea, mild hypergastrinaemia, hypokalaemia, higher basal acid production and peptic ulcer, which help to differentiate it from VIPoma. In some patients skin rash, similar to that observed in patients with glucagonoma, was reported, and it disappears as a consequence of tumour removal. However, high PP plasma levels (>100 pg/ml) are the most relevant feature [31]. Plasma samples for PP evaluation should be taken in the rested fasting state, since PP values were found increased after a meal and exercise, but also in patients with hypoglycaemia and cholinergic stimulation [28]. It is important to highlight that the finding of high PP levels is not sufficient for PPoma diagnosis. In fact, it was found higher also in other NETs (25–70 %) and in non-neoplastic patients including chronic pancreatitis, kidney failure, diabetes, hypoglycaemia, duodenal ulcer, alcoholism, advanced age and medications (erythromycin, cisapride, laxative abuse) [17].

A method exists to distinguish the neoplastic from the non-neoplastic production of PP. It consists of a test using atropine. When 1 mg intramuscular atropine is delivered, PP secretion is normally suppressed by the vagal cholinergic regulation [44]. PP produced by a tumour is not inhibited by atropine. However, this mechanism is not absolute, because in some patients with PP-secreting tumours, PP levels could be reduced by atropine. This phenomenon was explained by the presence of PP-secreting benign hyperplasia, which responds to atropine delivery [45]. A stimulation test was also developed using secretin infusion, which induces more significant PP level increase in patients with PPoma than in non-neoplastic patients [46]. However, these dynamic tests have not been yet indicated for clinical practice because a wide case series does not exist to confirm their validity.

To date no histological features have been identified to distinguish PPomas from other pancreatic NETs. In fact they show all the typical neuroendocrine characteristics. Electron microscopy could show secretory granules. Only immunohistochemistry could confirm PP expression by tumour cells. A cut-off of more than 50 % of tumour cells expressing PP was established to classify a tumour PPoma, since this peptide could be also expressed by other islet cell tumours.

Since PPoma is biologically inactive, its diagnosis is usually late, when it has already become malignant and of greater dimensions. For this reason often surgery is not radical for this NET. When dimensions are limited, a careful enucleation with a limited normal tissue margin can be considered for selected patients. Cytoreductive surgery for metastases was proposed, but a clear advantage for patients has not yet been demonstrated.

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Glucagonoma

15

Suayib Yalcin, Cenk Sokmensuer, and Ece Esin

15.1 Introduction

Glucagonomas are neuroendocrine tumours of the pancreas (pNET) originating from alpha islet cells, which synthesize and secrete glucagon or other peptides derived from the preproglucagon gene. These tumours are associated with a well-described glucagonoma syndrome characterized by hyperglucagonemia, skin rash, glucose intolerance, hypoalbuminemia, weight loss, and anemia [2].

15.2 History

The glucagonoma syndrome was first reported by Becker et al. in a patient with skin rash associated with pancreatic tumour in 1942 [3]. However, the syndrome initially was not attributed to hypersecretion of glucagon until McGavran et al. reported the first case of a patient with the glucagonoma syndrome, who had elevated plasma immunoreactive glucagon level, diabetes mellitus, skin rash, and pNET in 1966 [1]. In 1974, Mallinson et al. reported the association of skin rash with hyperglucagonemia, describing that nine cases with clinical glucagonoma syndrome consisted of dermatitis, diabetes mellitus, unexplained weight loss, hypoaminoacidemia,

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anemia, and a glucagon-producing tumour of the pancreas [4]. In 1984, Wilkinson described necrolytic migratory erythema (NME) for the skin rash associated with pancreatic tumours [5].

15.3 Epidemiology

Representing 1 % of all pancreatic NETs, the incidence of glucagonoma is about 1 in 20 million [6]. Patients usually present between the ages of 19 and 72 years, typically around 50's. No glucagonoma case has been reported in children yet [7]. Although initially a female tendency was suggested in the incidence [6], in the most recent series, an even distribution among men and women has been reported [7]. No race predilection is known for glucagonoma either.

15.4 Molecular Pathogenesis

The molecular pathogenesis of glucagonomas is still largely unknown. Glucagonomas may be associated with the MEN-1 characterized by a family history of pituitary, pancreatic islet cell, or parathyroid tumours in 3 % of the cases, and glucagonoma is associated with 13 % of MEN-1-related tumours. The malignant progression of glucagonoma may be associated with complex genetic mechanisms, but no specific molecular markers of malignancy have been defined with certainty so far [8].

15.5 Histopathology

Histologically glucagonomas are classified as well-differentiated functioning pNETs since these tumours demonstrate the typical morphological characteristics of other pNETs. Glucagonomas usually present as encapsulated firm nodules varying in size from several mm's up to 25 cm (Fig. 15.1) [2, 7]. The majority of glucagonomas are 5–10 cm large in size at the time of diagnosis. The tail of the pancreas is predominantly involved. In one study slightly more than 50 % of glucagonomas occurred in the tail and 80 % in another study. But, in the largest study, 22 % of glucagonomas were in the head region of the pancreas, 14 % in the body, and 51 % in the pancreatic tail. Most glucagonomas are within the pancreas; however, two glucagonomas with the clinical syndrome were found in the proximal duodenum and kidney. Glucagonomas usually occur as a single tumour, although 10–12 % patients have been reported as multicentric tumours or diffuse lesions throughout the pancreas.

Immunocytochemical and histologic studies show typical findings of pNETs. The tumours consist of cords and nests of well-differentiated islet cells (Fig. 15.2). Despite their benign histologic appearance and slow growth rate such as low mitotic index and uncommon nuclear atypia, most pancreatic glucagonomas are malignant with high metastatic potential. Sixty percent of glucagonomas were reported as

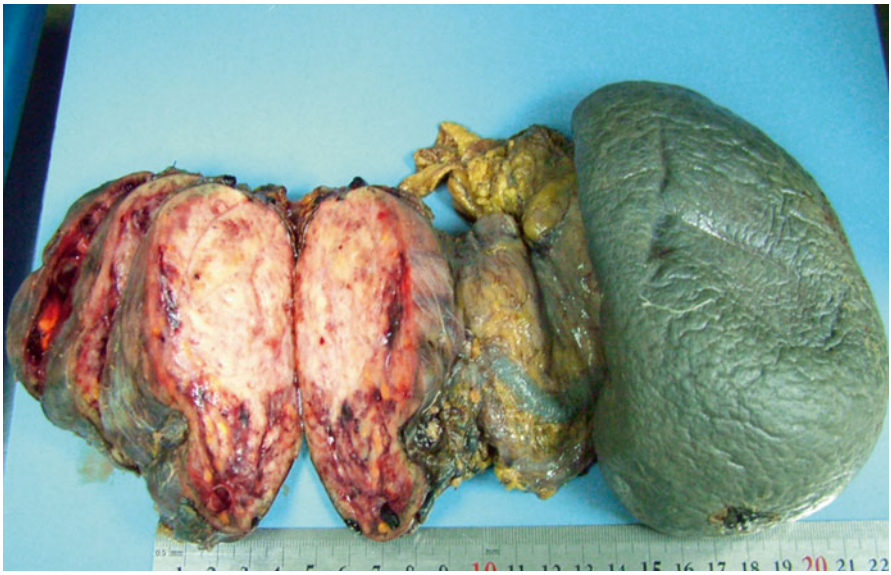

Fig 15.1 Glucagonoma presenting as a firm nodule on macroscopic examination

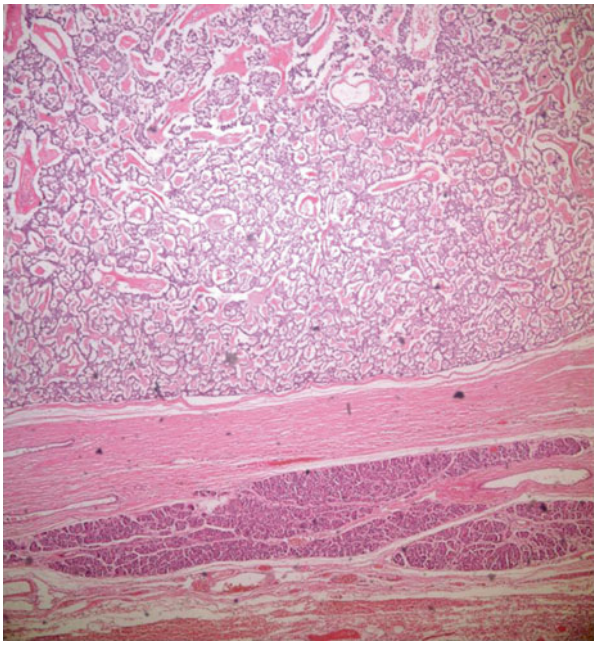

Fig 15.2 Tumor cells forming cords and nests in glucagonoma on microscopic examination

malignant, and 51.4 % of all malignant glucagonomas were metastatic at the time of diagnosis.

Glucagon is usually detectable within the tumour cells by immunoperoxidase staining and glucagon mRNA may be detected by in situ hybridization. Glucagonomas

are often mixed with pancreatic polypeptide-containing cells or less often with insulin- or somatostatin-containing cells. Characteristic alpha cell granules may be seen on electron microscopy. Alpha cell tumours can be divided into two distinct types. Those tumours associated with the glucagonoma syndrome are usually solitary and large, with undescriptive microscopic pattern, atypical granules ultrastructurally, and few cells positive for glucagon immunohistochemically. These ultrastructural and immunohistochemical aberrations may be the result of abnormalities in the biosynthesis and secretion of glucagon, sometimes with alternative production of the related precursor peptides glycentin and oxyntomodulin. The alpha cell tumours which are not associated with the glucagonoma syndrome are often multiple and small, have gyriform pattern of growth, are strongly immunoreactive for glucagon, exhibit typical α cell granules ultrastructurally, and are nearly always benign. In one series of 1,366 autopsy cases, the frequency of adenomas was reported to be 0.8 %, and all contained glucagon-producing cells and argyrophilia can be demonstrated with Grimelius technique [7, 9–12]. In contrast, detection of atypical granules is typical in patients with tumours associated with hyperglucagonemia syndrome.

Skin biopsies are not diagnostic in patients with glucagonoma. The histopathology of NME changes can be numerous as much as the clinical presentation. Typical histological findings include epidermal necrosis, parakeratotic hyperkeratosis, irregular epidermal hyperplasia, papillary dermal angiodysplasia, subcorneal pustules, and suppurative folliculitis. None of these findings indicate a pathognomonic finding; in contrast histologically and clinically, it is similar to the rash of zinc deficiency, acrodermatitis enteropathica. In its classic form, early lesions demonstrate superficial spongiosis or necrosis with subcorneal and midepidermal bullae. Fusiform keratinocytes with pycnotic nuclei are often present with mononuclear inflammatory infiltrates. This characteristic histologic pattern is best seen in early lesions. The most specific feature on skin histological examination is necrolysis of the upper epidermis with vacuolated keratinocytes, leading to focal or confluent necrosis, but this histopathologic feature may be seen in other deficiency states like pellagra, necrolytic acral erythema or zinc deficiency [9].

15.6 Pathophysiology

The etiology and pathogenesis of clinicopathologic characteristics of glucagonoma have not been enlightened yet. But the pathophysiology of glucagonoma syndrome is probably related to the known actions of glucagon. Glucagon stimulates glycogenolysis, gluconeogenesis, ketogenesis, lipolysis, and insulin secretion; alters intestinal secretion and inhibits pancreatic and gastric secretion and gut motility. Hyperglycemia in the glucagonoma syndrome results from the increased glycogenolysis and gluconeogenesis. Because glucagon increases secretion of insulin, which prevents lipolysis and maintains normal free fatty acid concentrations, ketonemia usually does not develop.

Venous thromboembolism, NME, and angular cheilitis were observed in patients treated with continuous intravenous glucagon for refractory tumour-induced

hypoglycemia. This observation indicates that glucagon may have a direct, causative role in diabetico-dermatogenic syndrome (DDS) [13]. Normalization of glucagon concentrations by surgery results in a rapid disappearance of the skin rash. Further evidence for the role of preproglucagon-derived peptides in the pathogenesis of necrolytic migratory erythema comes from its rare association with other diseases, notably hepatic cirrhosis and celiac disease. The patient with celiac disease had marked elevation of circulating enteroglucagon levels. In those patients with cirrhosis, glucagon values were not invariably elevated, but hepatocellular dysfunction may result in increased levels of other preproglucagon-derived peptides, such as enteroglucagon. In one case of cirrhosis, the rash was successfully treated with fatty acid and zinc supplementation. However, it is not clearly established that the skin rash is associated with hyperglucagonemia since numerous patients that have been given large doses of glucagon for long periods did not develop skin rash. Hypoaminoacidemia, nutritional lack of zinc and fatty acids, and hepatocellular dysfunction are all considered as possible triggering factors of NME. Hyperglucagonemia provokes multiple nutrient and vitamin B deficiencies, which in turn are the probable cause of this typical skin disorder [14].

15.7 Clinical Features

Skin rash and mild diabetes are the predominant signs of the glucagonoma syndrome. The incidence of this DDS was reported to be as 57.2 % [14]. Glucagonomas predominantly metastasize to the liver and adjacent lymph nodes, less frequently to the vertebra, ovary, peritoneum, and adrenals. Unlike carcinoid syndrome, liver metastases are not a prerequisite for the clinical syndrome to occur. The rash may occasionally appear prior to the onset of systemic symptoms. Most patients with rash usually also have weight loss, diarrhea, sore mouth, weakness, mental status changes, or diabetes mellitus.

The characteristic feature of the glucagonoma syndrome is the rash, which is called as NME. It occurs in 90 % of the cases and is usually the presenting feature. The rash exhibits a 7- to 14-day sequence of erythema, blister, secondary breakdown, infection, healing, and hyperpigmentation. The initial manifestation of the rash is well-demarcated symmetrical areas of erythema (an annular or arciform erythema) at intertriginous and periorificial sites, usually in groins, which migrates principally to the limbs, buttocks, and perineum. These lesions may become vesicopustular or bullous and coalesce, and eroding and encrusting may occur. The rash may be mildly pruritic and occasionally painful. The extent and severity of lesions may wax and wane. The healing process commences after 10–15 days; lesions clear centripetally and leave indurated and hyperpigmented areas, which are usually permanent. The rash remits and relapses unpredictably, and this has made it difficult to assess treatment efficacy. On the basis of this morphology, differential diagnosis includes pemphigus foliaceus, pemphigoid, vasculitis, psoriasis, herpes, seborrheic or contact dermatitis, eczema, pellagra, zinc deficiency (inherited, acrodermatitis enteropathica, or acquired), extensive candidiasis, or even chemical burn. Usually

NME rapidly disappears after successful surgical removal of the glucagon-secreting tumour. Although this eruption is a very specific cutaneous marker for underlying glucagonoma, in a few affected patients, no evidence of associated malignant disease has been found. Glucagon itself may act directly on the skin to cause the rash, perhaps by increasing arachidonic acid levels, which it does in human keratinocyte cultures. The rapid response of the rash to octreotide, before changes in circulating amino acids or glucagon, suggests a direct action on the skin, consistent with the inhibition of hormone action. Topical application of zinc or oral supplementation has been reported to improve the rash, although circulating zinc levels are normal in patients with glucagonoma.

In addition to skin rash, the disease may affect the mucous membranes. Angular stomatitis, cheilitis, and atrophic glossitis with a beefy red tongue occur almost invariably. Blepharitis, vulvovaginitis, and urethritis accompanied by dysuria are frequent features. Mucosal lesions are associated with the cutaneous rash in about 70 % of patients. Glossitis or angular stomatitis was reported to occur in 34–68 % of patients. Nail and scalp involvement may result in onycholysis and alopecia. Some patients develop nail dystrophy with brittleness and crumbling.

Amino acid levels are frequently diminished in the serum of patients with glucagonoma. Glucagon, acting on the liver, increases both amino acid oxidation and gluconeogenesis from amino acid substrates [11]. The intensity of hypoaminoacidemia may vary with the intensity of the disease. Glucagon stimulates sustained gluconeogenesis, which depletes the glycogenic amino acids, particularly alanine, serine, and glycine, and hepatic proteolysis with conversion of amino acid nitrogen to urea nitrogen, which depletes all amino acids. It has been postulated that this results in an increase in protein degradation, depleting epidermal protein and ultimately resulting in skin necrosis. Amino acid deficiency is a frequent finding in the glucagonoma syndrome; in two large series, incidence was reported as 26 and 60 %. Plasma concentrations of amino acids are frequently less than 25 % of normal, with glycogenic amino acids most affected, whereas branch-chain amino acids are reportedly less affected. Hypoaminoacidemia may be clinically important because it may be the cause of NME; in one patient, amino acid infusion led to an improvement in the characteristic rash [15]. Intravenous amino acid infusion, although not correcting hypoaminoacidemia, has been reported to ameliorate the rash, but oral amino acid supplementation is ineffective. Similarly a high-protein diet, despite normalizing plasma amino acids and nitrogen balance, may have no effect on the rash. If glucagonoma-induced hypoaminoacidemia is corrected, the dermatitis may improve without changing plasma glucagon levels. The occurrence of the rash in patients without suppressed amino acid levels casts further doubt on a casual association. Resolution of rash, however, has been reported after simple hydration with glucose and saline; therefore, it is not established that either hypoaminoacidemia or zinc deficiency is causative in all patients [7, 9–11].

Glucose intolerance is usually mild and nonketotic since beta cell function is preserved and insulin secretion is normal. Diabetes mellitus occurs in 75–95 % of patients with glucagonoma [16]. Diabetes usually precedes skin rash (and diagnosis) by years, in one study with an average time of 5 years. The hyperglycemia due

to glucagonoma may be mild or moderate (median-glycated hemoglobin level of 9.8 % in one series [16]) and is easily controlled by diet, oral agents, or insulin. In another report, 42 % of patients required oral hyperglycemic agents and 24 % insulin. There is little correlation between the plasma glucagon levels and the degree of glucose intolerance. This probably reflects variability in beta (β) cell insulin secretion, which may be influenced by loss of β cells as a result of tumour growth. Other factors may include insulin gene expression in glucagonoma cells, secretion of biologically inactive forms of immunoreactive glucagon by the tumour, depletion of glycogen stores, and downregulation of glucagon receptors. Various treatments have resulted in changes in plasma glucagon concentrations that did not correlate with subsequent changes in blood glucose levels. In cases where infusion of a somatostatin analog decreased plasma glucagon levels in patients with glucagonoma, no effect on plasma glucose level was observed. Furthermore, tumour resection and normalization of blood glucagon may not result in normalization of the glucose tolerance in all of the patients. In some cases removal of glucagonoma improved glucose tolerance. It has been suggested that development of diabetes or glucose intolerance depends to a large degree on the patient's insulin reserve. If the latter is intact, enhanced insulin release may compensate for the hyperglucagonemia and increased hepatic glucose production. Otherwise, diabetes mellitus or glucose intolerance may develop.

Unrelenting weight loss affects the majority of cases (56–96 % of patients), usually despite a good appetite. Weight loss may be profound. A number of observations suggest that the weight loss is a unique aspect of the syndrome. It is seen even in patients with small tumour without metastatic spread. Weight loss is prominent in patients with small tumour as well as in those with metastatic tumour, suggesting that cachexia is a consequence of the catabolic actions of glucagon. This is supported by a study in which a patient's weight loss was reversed after being given long-acting somatostatin analog, octreotide, which reduced the plasma glucagon level to a near-normal range. The main cause is probably the organ protein catabolism, which occurs in response to the depletion of amino acids. If the weight loss exceeds 50 % of body weight, it can be fatal. It is not seen early in other pancreatic endocrine tumours, unless malabsorption is present. It is suggested that the anorectic effects of glucagonoma are due to the production of a novel substance by the tumour and not due to glucagon per se because other transplanted glucagonomas producing similar levels of plasma glucagon elevation do not cause anorexia.

Nonspecific abdominal pain has been reported in approximately 12 % of patients. The elevation of preproglucagon-derived peptides would be anticipated to affect gastrointestinal function. Enteroglucagon has been proposed as a humoral mediator of intestinal adaptation in response to injury. It appears to have a trophic action on the intestinal mucosa and elevated levels causing villous hypertrophy. Three patients have been described with an enteroglucagonoma, in whom the principal clinical finding was giant villous hypertrophy. Large-molecular-weight forms of glucagon inhibit gastrointestinal smooth muscle, resulting in constipation. Diarrhea is reported in 14–15 % of patients, occasionally with severe steatorrhea, and occurs far more common than constipation (15 % vs 4 % in one study). The etiology of the

diarrhea remains unclear. Jejunal biopsies have been reported to be normal or it may show hypertrophic folds. It remains possible that other hormones secreted by the tumour could be contributing to the development of diarrhea. Rarely, nausea and vomiting occurs, possibly as a result of local tumour effects.

A normochromic-normocytic anemia is common, being present in up to 90 % of patients in some series. It is probably due to the anemia of chronic disease, but a direct effect of glucagon on erythropoiesis has also been suggested [17]. The anemia is usually mild with a mean hemoglobin concentration of 9.4 g/dl, but occasionally the hemoglobin may fall as low as 4 g/dl. Serum iron, B_{12}, and serum folate concentrations usually are normal, and anemia does not respond to therapy with any of these agents. It does respond to resection of the tumour, however. Further evidence of anemia related to glucagon excess is that the prolonged therapy with a long-acting glucagon preparation decreased erythropoiesis in rats and mice.

Other abnormal laboratory findings in patients with glucagonoma are renal glycosuria and hypocholesterolemia. Renal glycosuria may occur early and may represent a direct renal effect of glucagon. Hypocholesterolemia was reported in 80 % of cases.

Neuropsychiatric symptoms may be underdiagnosed. Neurologic symptoms associated with glucagonoma include ataxia, dementia, and proximal muscle weakness. Depression is the most commonly reported abnormality; patients may suffer from severe depression or other psychiatric syndromes. The demonstration of glucagon gene expression in the central nervous system may provide an explanation for these manifestations. Paraneoplastic syndromes, notably optic atrophy, have also been attributed to this syndrome.

A late feature of the disease is widespread venous thrombosis and pulmonary embolism which is resistant to conventional anticoagulant therapy [18]. In one series venous thrombosis (VT) occurred in 24 % of patients and pulmonary emboli (PTE) in 12 %. Glucagon is not known to affect coagulation parameters, and the pathophysiological process of the thromboembolic events in glucagonoma has not been elucidated yet. Secretion of factor X, which is synthesized in α cells and is found in higher concentration in glucagonomas, may explain this complication. Venous thrombosis and PTE can frequently be a contributing factor to the patient's survival: therefore, it should be carefully sought. This association with thromboembolism appears to be unique among endocrine tumours. A single case of reversible dilated cardiomyopathy associated with a glucagonoma has been reported [19].

In MEN-1 syndrome associated glucagonoma cases, the characteristic syndrome associated with sporadic tumours is often absent, and in particular necrolytic migratory erythema rarely occurs [7, 9–11].

15.8 Diagnosis

Glucagonoma is often diagnosed relatively late in the course of the disease since many of the symptoms are nonspecific. NME is the most important clue which leads to suspicion of glucagonoma but it is not pathognomonic. With recognition of NME, further diagnostic workup should be done.

Glucagonoma is usually suspected in patients with chronic dermatitis or unexplained and therapy-resistant dermatitis, elevated sedimentation rates associated with glucose intolerance, thromboembolic phenomena, or the MEN-1 syndrome. Diagnosis rests on demonstrating a pathological elevation of the plasma glucagon concentration.

Once the syndrome has been recognized or suspected, the diagnosis is easily made by demonstrating increased plasma glucagon levels. Plasma glucagon level is usually elevated 10–20-fold, and up to 70 % of immunoreactive glucagon may be biologically inactive. In most laboratories the upper limit of normal for fasting glucagon concentration is 150–200 pg/ml. In one large review of glucagonomas, the plasma glucagon level was 200–500 pg/ml in only two patients, in four it was between 500 and 1,000 pg/ml, and in 52 it was >1,000 pg/ml. These results are in close agreement with another study in which the mean plasma glucagon concentrations was 2110 ± 334 pg/ml with a range of 550–6,600 pg/ml and with 0 % being <500 pg/ml, 30 % being 500–1,000 pg/ml, and the remainder >1,000 pg/ml in 73 cases. In some cases, glucagon levels remain in the "physiologically elevated" range even in patients with NME. Hence, a serum glucagon concentration below 500 pg/mL does not exclude a glucagonoma.

Hyperglucagonemia has been reported to occur in chronic renal insufficiency and hepatic insufficiency which reduce clearance of increased glucagon, diabetic ketoacidosis, prolonged starvation, acute pancreatitis, acromegaly, hypercortisolism, septicemia, severe burns, severe stress (trauma, exercise), familial hyperglucagonemia, and with some drugs (danazol and oral contraceptives). There has been one case of a familial hyperglucagonemia, with autosomal dominant inheritance, in which there were no sequelae attributable to the elevated glucagon levels. Plasma glucagon level in these conditions does not exceed 500 pg/ml. A plasma glucagon concentration of >1,000 pg/ml has therefore been suggested as diagnostic of glucagonoma. The one reported exception to this is in a patient with severe liver disease after portacaval anastomosis, in whom plasma level of glucagon was 1,000 pg/ml. Because a necrolytic migratory erythematosus-like rash has been reported in this patient with severe hepatic disease, diagnostic confusion could occasionally result. However, in one review of 13 cases, no overlap in plasma glucagon levels was found between patients with cirrhosis or any other of the aforementioned conditions and values seen in patients with glucagonoma. Glucagon levels are highest in patients with NME and DM.

Provocative testing with tolbutamid, secretin, a mixed or carbohydrate-rich meal, or arginine is of little additional value in the evaluation of patients with suspected glucagonoma. Other biochemical abnormalities associated with the syndrome are impaired glucose tolerance and hypoaminoacidemia.

Blood investigations reveal anemia, hypoaminoacidemia, hypoalbuminemia, and hypocholesterolemia as the most common disorders. Hypoaminoacidemia occurs in most patients, and as originally pointed out by Mallinson and colleagues, the levels may vary with the intensity of the disease [4].

Diabetes mellitus and glucose intolerance are among the most frequent findings in patients with glucagonoma syndrome. Because of the effect of glucagon on glucose

regulation, diabetes mellitus might be expected to be a constant feature of glucagonoma syndrome, but it is not uniformly present. Diabetes mellitus or even glucose intolerance is a much less constant feature of the syndrome than is hypoaminoacidemia.

Pancreatic polypeptide (PP) and chromogranine A are frequently elevated in glucagonoma syndrome. Most neuroendocrine tumours produce and secrete general tumour markers and circulating neuroendocrine tumour markers, such as CgA, PP, serum neuron-specific enolase, human chorionic gonadotropin, and subunit of glycoprotein hormones. Pancreatic polypeptide and CgA can be used for screening purposes in patients without distinct clinical hormone-related symptoms. Chromogranine A, although its precise function is not yet established, has been shown to be a very sensitive and specific serum marker for various types of neuroendocrine tumours. At the moment CgA is considered to be the best general neuroendocrine serum or plasma marker available for both diagnosis and therapeutic evaluation. Chromogranin A level increased in 50–100 % of patients with various endocrine tumours. Chromogranine A serum or plasma levels reflect tumour load, and it may be an independent marker of prognosis in patients with midgut carcinoids [20].

Radiological abnormalities of the small bowel, such as thickening of mucosal folds, villous hypertrophy, and delayed gastric emptying, may be demonstrated on abdominal computed tomography scanning or barium examination.

Histological specimens show the characteristic features of neuroendocrine tumours and immunocytochemistry can confirm the presence of immunoreactive glucagon. Most of the neuroendocrine tumours are immunostained positively for CgA, synaptophysin and neuron-specific enolase.

15.9 Tumour Localization and Diagnosis

Because most glucagonomas are malignant, it is important to attempt to localize the primary tumour and surgically resect it if it has not already metastasized or to establish the presence of metastases so that unnecessary surgery can be avoided. However, careful assessment of the extent of the tumour is indicated even in patients with extensive disease because aggressive surgical resection and tumour reduction may alleviate the debilitating effects of hyperglucagonemia. At the time of diagnosis, over 50 % of glucagonomas will be metastasized and most primary tumours will be greater than 3 cm, with tumours up to 35 cm in diameter reported. Thus, localization of these tumours rarely presents a problem, and abdominal ultrasonography or CT scanning is usually adequate for this purpose. The initial procedure of choice should be computed tomography, since CT will detect 95 % of primary pancreatic endocrine tumours more than 3 cm in diameter and 95 % of metastatic pancreatic endocrine tumours in the liver. CT is particularly useful for assessing local spread. Intravenous contrast enhances the detection of smaller lesions, especially when images are obtained during the arterial phase. In addition, arterial phase and portal venous phase sequences can be used to maximize the conspicuity of liver metastases compared to the surrounding normal liver parenchyma. In liver metastasis, magnetic resonance imaging may be considered more specific to CT.

For some patients especially those with smaller primary tumours, CT or USG may not localize the tumour, and then selective angiography or EUS is the procedure of choice, depending on the expertise of the institution. Endoscopic ultrasound can detect pancreatic tumours as small as 2–3 mm, provides accurate information on the local extent of disease and allows transmucosal needle biopsy of pancreatic lesions. The experience with selective venous sampling for localization of glucagonomas is much more limited than for insulinomas or gastrinomas. In the largest single-center experience reported to date, 82 patients underwent a total examination by EUS. One hundred tumours were visualized in 54 different patients and in 38 of cases, no tumour was found in the pancreas by EUS. On the basis of results of surgery, EUS correctly localized pancreatic tumour in 50 of 54 patients (93 %). Only one of them was glucagonoma. These results may support the use of EUS as a primary diagnostic modality in the evaluation and in the management of patients with pNET [21].

Peptide receptor scintigraphy has been developed successfully for visualization of somatostatin receptors on neuroendocrine tumours. After the intravenous administration of ^{111}In-diethylenetriamine pentaacetic acid octreotide (OctreoScan), the primary tumours and the previously unrecognized metastases of most carcinoids, islet cell tumours, paragangliomas, pheochromocytomas, medullary thyroid cancers, and small-cell lung carcinoma can be visualized. In one study, 38 patients with suspected pancreatic neuroendocrine tumours were evaluated by OctreoScan SPET (OCTSPET). SPET studies were acquired at 4 and 24 h after the injection of OctreoScan. The OCTSPET results were positive in 18 of 19 patients (10 gastrinomas, 5 insulinomas, 1 neuroendocrine tumour, 1 glucagonoma, and 1 carcinoid) and false negative in one insulinoma. These data indicate that OctreoScan represents an excellent tool for the diagnosis of pancreatic neuroendocrine tumours [22].

Angiography is highly specific since the vascularity of pNETs is high. Although angiography is considered to be the gold standard, it is invasive. Angiography is now performed almost exclusively for embolization and/or infusion of chemotherapy via the hepatic artery.

Tumour calcification and cystic degeneration are common findings of glucagonomas which reflect an indolent course of the disease. However, most of the cystic neoplasms of the pancreas are nonfunctioning glucagon-producing neuroendocrine tumours instead of glucagonoma.

Somatostatin receptor scintigraphy using the indium-111-labeled somatostatin analog, pentetreotide, is the best method for evaluating the extent of metastatic disease and to date, all glucagonomas have been somatostatin receptor positive. Such an evaluation is most crucial in patients being considered for hepatic transplantation but may also be of value in deciding on therapeutic modalities and assessing the response to treatment [7, 10, 11].

After the detection of primary tumour, needle biopsy is needed to confirm the diagnosis. EUS-guided biopsy or in the case of liver metastasis, CT-guided biopsy is the preferred method. Immunohistochemical staining is required to confirm the presence of glucagon in the tumour cells. The secretion of glucagon in tumour cells is heterogenous; therefore, occasionally the needle biopsy specimen may yield negative results for glucagon staining.

15.10 Staging

The prevalence of metastatic disease at the time of diagnosis varies from series to series, ranging from 50 to 100 % [18],. The liver is the most common site for metastasis, the regional lymph nodes, bone, adrenal gland, kidney, and lung following it. The American Joint Committee on Cancer (AJCC)/International Union Against Cancer (UICC) TNM staging is also proper for pancreatic NETs (Table 15.1) [23]. Survival rates according to stages are summarized in Table 15.2 [24].

Table 15.1 NM staging for exocrine and endocrine tumours of the pancreas

Primary tumour (T stage)		Lymph nodes, regional (N stage)		Distant metastasis (M stage)	
Tx	Cannot be assessed	Nx	Cannot be assessed	M0	No distant metastasis
T0	No evidence of primary tumour	N0	No metastasis	M1	Distant metastasis
Tis	Carcinoma in situ: PanInIII	N1	Lymph node metastasis		
T1	Limited to the pancreas, <2 cm				
T2	>2 cm but limited to pancreas				
T3	Tumour extended from the pancreas but no involvement of celiac axis or SMA				
T4	Unresectable, celiac axis, or SMA involvement				

SMA superior mesenteric artery

Table 15.2 Five- and ten-year survival rates for resected pancreatic neuroendocrine tumour patients

Stage		Observed survival		Median survival (months)
		5 years (%)	10 years (%)	
T1N0M0	Stage 1A	61.0	46.0	112
T2N0M0	Stage 1B			
T3N0M0	Stage IIA	52.0	28.8	63
T1N1M0 T2N1M0 T3N1M0	Stage IIB			
T4 anyN M0	Stage III	41.4	18.5	46
Any T and any N M1	Stage IV	15.5	5.1	14

15.11 Treatment

Surgery is the only curative therapeutic option. The cure potential is as low as 5 %. The exact percentage of cases that can be cured by surgical resection is unknown. Glucagonomas are usually diagnosed at later stages; therefore, the probability of surgical resection is probably less than 20 %. In one large review of 92 cases with glucagonoma, only 16 of the malignant cases were treated by surgical resection alone. Postoperatively only seven patients had normal plasma glucagon levels. Recurrence with elevated plasma glucagon level was reported in a number of patients after R0 resection. Even if R0 resection is amenable, there will be an eventual recurrence. However, despite the recurrence of disease, an extended disease-free interval may be achieved. In another study, 125 patients with neuroendocrine tumour who underwent surgery were investigated. Only two (3 %) patients had glucagonoma. The most common operative procedures were 50 pancreaticoduodenectomies (40 %), 39 distal pancreatectomies (31 %), and 21 tumour enucleations (17 %). Nine synchronous hepatic resections were performed for metastases. Of the evaluable patients, 46 (43 %) had postoperative complications, the most common of which were pancreatic fistula (16 %), wound infection (15 %) and delayed gastric emptying (8 %). There were three in-hospital deaths. The most favorable outcomes for neuroendocrine tumours were observed in patients with benign functional tumours and in those with completely resected malignant pancreatic NETs [25].

Cure can be achieved for malignant disease if tumour metastases are confined to the liver and the patient is a candidate for hepatic transplantation. Cytoreductive surgery may be appropriate as palliative therapy to reduce hyperglucagonemia if there are well-circumscribed metastases amenable to enucleation. However, this can be more complicated by venous thrombosis, hypercatabolic state and anemia. Total parenteral nutrition and blood transfusions are often required preoperatively. As a result of these factors, medical options are usually preferred for palliative therapy for metastatic disease. Control of liver metastases by metastasectomy, cryoablation, RFA or chemoembolization may be feasible. Hepatic artery embolization involves occlusion of hepatic artery collaterals to glucagonoma metastases in the liver, the portal vein maintaining supply to the normal liver. There is usually dramatic symptomatic relief following embolization, and the procedure can be repeated at 6–9 month intervals. The response rates associated with embolization or chemoembolization, as measured either by decrease in hormonal secretion or by radiographic regression, are generally greater than 50 %. However, the necrotic tumour load and the remaining low amount of healthy liver tissue may be poorly tolerated in frail patients.

Another approach for liver predominant metastatic disease is radiofrequency ablation and cryoablation. They can be used alone or in conjunction with surgical removal. Long-term efficacy of both these procedures remains uncertain.

Recent studies suggest that conventional contraindications to surgical resection, such as superior mesenteric vein invasion and nodal or distant metastases, should be redefined in patients with advanced neuroendocrine tumours. These patients will

benefit from extensive surgical debulking in combination with adjuvant medical treatments, such as somatostatin analogs. This combination may result in enhanced survival rates compared with either procedure alone.

The morbidity associated with glucagonomas results principally from the hormonal syndrome. The effects of tumour bulk rarely become apparent until the patient is in a terminal condition. Initial treatment should be directed to controlling symptoms, restoring nutritional status, and controlling the hyperglycemia. Thus, palliative medical therapy aims to reduce hormone levels either by reducing tumour bulk or by inhibiting the release of hormone. Cytotoxic chemotherapy or hepatic embolization may be used to reduce tumour bulk, while the somatostatin analog, octreotide, both inhibits hormone levels and antagonizes hormone action.

The glucagonoma patients suffer from a prolonged catabolic state due to the effects of glucagon in the liver. Hence, nutritional support is an integral component of treatment. Total parenteral nutrition should be considered preoperatively in potentially operable patients to decrease the perioperative morbidity.

In patients with widely metastatic disease in whom surgical debulking is not possible, various chemotherapeutic agents are frequently used. The conventional chemotherapy for glucagonomas and pancreatic endocrine tumours is 5-flouro-uracil and streptozotocin. For patients with a glucagonoma, the response rate, measured by 50 % reduction in plasma glucagon levels, is about 70–80 %, with remission often lasting a year, although no effect on overall survival has been observed. An alternative chemotherapeutic agent is dacarbazine which has been reported to be particularly successful in patients with glucagonoma, with a biochemical response sustained for over a year in all patients. The modest efficacy of cytotoxic treatments has prompted the development of new treatment strategies. Recently, orally active alkylating agent temozolomide has been found to be effective on PNETs. Progress in the understanding of pathophysiology of pancreatic NETs has revealed the importance of growth factors and their receptors (vascular endothelial growth factor receptor, VEGFR) and the involvement of the mammalian target of rapamycin (mTOR) in pancreatic neuroendocrine tumourigenesis [26–28]. Many of the growth factor receptors function as tyrosine kinases. Two molecularly targeted agents, an oral tyrosine kinase inhibitor (TKI) sunitinib and an inhibitor of mammalian target of rapamycin (mTOR) everolimus, have been approved in the treatment of metastatic pNETs.

Sunitinib is a small-molecule TKI with multimodal inhibition on TKs. In a phase II trial, sunitinib was given to 109 advanced stage PNETs [29]. Glucagonoma was presented by 4 cases in the study group. 18 % of patients had a partial response and 68 % had a stable disease. Median time to tumour progression was 7.7 months. Continuous 37.5 mg daily administration of sunitinib was tested in a phase III trial [30]. Accrual was stopped prematurely due to efficacy of sunitinib in the treatment arm. Median PFS was significantly longer with sunitinib (11.4 vs 5.5 months). Although a small number of patients had glucagonoma, sunitinib treatment may be considered for advanced stage diseases in which symptomatology is not much prominent and visceral crises does not exist. Side effects may include hypertension, proteinuria, other forms of renal toxicity, arterial thromboembolism, left

ventricular dysfunction, clinical heart failure, thyroid dysfunction, bleeding, myelosuppression, hand-foot skin reaction, delayed wound healing, hepatotoxicity, and muscle wasting.

Everolimus activity was explored in many phase II and III trials in pNET [31]. In RADIANT-3 trial everolimus monotherapy was tested [32]. Everolimus was associated with a significant prolongation in median PFS (11.0 vs 4.6 months, hazard ratio [HR] for progression 0.35, 95 % CI 0.27–0.45). There were confirmed objective partial responses in 5 % versus 2 % of the placebo group. The most common grade 3 or 4 drug-related adverse events were stomatitis (7 %), anemia (6 %) and hyperglycemia (5 %). The rates of grade 3 or 4 hyperglycemia were similar in those with glucagonoma versus those without glucagonoma (9.1 vs 7.8 %). However, the safety of everolimus should be tested in glucagonoma patients further.

Pancreatic neuroendocrine tumours are generally considered to be radioresistant. However, some anecdotal reports have showed that in some patients local control and symptom control can be achieved by external beam radiotherapy [33–36].

Somatostatin and its long-acting analogs (octreotide and lanreotide) have been introduced for the treatment of endocrine tumours of the gastrointestinal tract [37]. Somatostatin analogs have been shown to effectively control symptoms resulting from excessive hormone release in patients with carcinoid, Verner-Morrison syndrome and glucagonoma syndromes. This beneficial effect is due to the presence of somatostatin receptors in high density in the majority of these tumours. Lanreotide is as effective as octreotide and also available in depot form. Long-term studies have shown that somatostatin analogs are safe. The most serious adverse effect is the development of gallstones. The antiproliferative potency of somatostatin and its analogs in vitro in experimental tumour models prompted a number of studies in patients with metastatic endocrine tumours that are generally unresponsive to conventional chemotherapeutic protocols. PROMID and CLARINET studies now clearly showed the antiproliferative potency of somatostatin analogs in patients with neuroendocrine tumours. Therefore, octreotide or lanreotide now the first-line therapy for the glucagonoma syndrome. Octreotide dose ranges from 150 to 1,500 μg daily. Therapy is generally initiated with short-acting octreotide 50 mcg subcutaneously three times a day [16]. The dose is increased gradually as needed to control symptoms. The patient is then rapidly transitioned to a long-acting formulation. The duration of response ranges from 2 months to 3.5 years. It is particularly effective against the NME, with resolution usually occurring within a week, independently of changes in plasma amino acids or glucagon. According to various reviews, the rash improved with octreotide treatment in 54–90 % of patients; complete remission of rash occurs in up to 30 %. The effect is prolonged at least 6 months and frequency of recurrence is further reduced thereafter. Octreotide is less effective at reversing weight loss and has an inconsistent effect on diabetic control, perhaps reflecting inhibition of secretion of other hormones, such as insulin. It generally improves abdominal pain and diarrhea. Plasma glucagon levels decrease in 80–90 % of patients, but the normal range can be reached in 10–20 % of patients with octreotide treatment. It is not known whether octreotide has any effect on the tendency to develop VT. The

combination of chemotherapy or hepatic embolization with octreotide may delay the development of octreotide resistance, which usually develops after a mean of 2 years of therapy.

Octreotide long-acting repeatable (LAR) is a new somatostatin analog whose activity lasts 28 days. It appears to have good therapeutic efficacy, tolerability, and safety in the treatment of neuroendocrine tumours. Its effects are similar to those of octreotide and lanreotide. However, because it only needs to be administered once every 28 days, it is preferable in clinical practice [38].

Interferon-alpha (IFN-α) is another effective agent that can be used in treatment. IFN induces tumour stabilization in 20–40 % and improves symptom control in 40–50 % and objective tumour regression in 15 %. However, its efficacy is concealed by high toxicity. Adverse effects like myelosuppression, mental changes like depression, and alterations of thyroid functions result in detrimental effects in quality of life of patients.

Combination treatment with SA and IFN may be an option for SA-resistant patients; however, the superiority of combination is not proven yet.

Simple measures to ameliorate the NME such as topical and oral zinc and a high-protein diet, although unproven, are worth to try. Acetylsalicylic acid may be useful in preventing thromboembolic disease, but conventional anticoagulants are ineffective. If diabetic control cannot be achieved with diet alone, insulin should be used.

Radioligand therapy based on ^{111}Indium, ^{90}Yttrium, and ^{177}Lutetium coupled to somatostatin analogs via bifunctional chelators is currently under investigation with promising data concerning long-lasting control of symptoms and tumour growth from phase I studies [39].

15.12 Prognosis and Follow-Up

The prognosis for patients with glucagonoma is unpredictable. Glucagonomas are slow growing tumours but due to this indolent behavior, most patients present late in the course of the disease. The 10-year survival rate in patients with DDS was 51.6 % in those with metastases and 64.3 % in those without metastases ($P < 0.001$) [14]. Once metastasis occurs, cure can be rarely achieved. However, with more advanced knowledge about the disease, better supportive care and better management of complications and with the implementation of new therapeutic modalities, prolonged survival is expected. The median survival from diagnosis is about 3 years; according to recent reports, more patients are living longer, an average of 4.9 years after the diagnosis of metastasis.

A postsurgical prognostic system has been proposed upon a series of 3,851 patients (75 of them were glucagonoma) [24]. Age at the diagnosis, tumour grade and presence of metastasis are the most important predictive factors. Scoring allows patients to be categorized into 3 groups: prognostic score 1 (raw score of 0), prognostic score 2 (raw score 1–2), and prognostic score 3 (raw score ≥3). The expected 5-year survival rates are 77–51 % and 36 %, respectively.

Postsurgically (R0 resection), patients can be followed 3–6 months with history and physical examination, serum glucagon, and CT/MRI. Exacerbations of the rash are a surrogate marker in the long term. Those patients in remission should be seen at least yearly, when a gut hormone screen should be performed, since up to 10 % of tumours will be associated with secondary hormone syndromes. In the long term, patients should be followed with history and physical examination with tumour markers every 6–12 months for years 1–3, and as clinically indicated thereafter. Imaging studies are recommended as clinically indicated.

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Somatostatinoma

16

James M.L. Williamson

16.1 Introduction

Somatostatinomas are rare neuroendocrine tumours (NETs) that arise in the pancreas or duodenum. They are the fifth most common pancreatoduodenal NET (after insulinomas, non-functioning tumours, gastrinomas and VIPomas), with an incidence of 1 in 40 million [1–3]. They tend to be non-functioning, although they are rarely active and can produce a spectrum of symptoms called 'somatostatinoma syndrome' [4–6]. A degree of discord exists regarding the functional component of a somatostatinoma; some clinicians argue that a 'syndrome' of clinical symptoms must be present for the diagnosis of somatostatinoma and others that the diagnosis is purely immunohistochemical [7]. As most of these tumours are biochemically inert, they are either detected incidentally or by causing mass-effect pressure symptoms, and most will have metastasised by the time of diagnosis [6, 8, 9].

There is little to distinguish somatostatinomas from other pancreatoduodenal NETs on radiological imaging or preoperative histology, but the presence of strong and diffuse positive somatostatin staining on immunohistochemistry is diagnostic [4]. Thus, many somatostatinomas are only diagnosed postoperatively when the histopathologist has had access to the resected specimen [6]. Like most NETs, operative resection even in the presence of metastases is recommended, with somatostatin analogues and chemotherapy reserved for patients not amenable to operation [10]. A variety of techniques exist for the management of metastases, with operative resection being advocated when suitable [7, 10–12]. Outcomes tend to much be more favourable than for an associated adenocarcinoma of the pancreas or duodenum, largely due to the indolent nature of these tumours.

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16.2 Incidence

Somatostatinoma was first described in 1977 [1], and since then, approximately eight cases per year have been reported [2]. The suggested incidence of 1 in 40 million may increase with improved understanding and diagnosis of the tumour [1, 2]. They are the fifth most common NET in the pancreas [4], while in the duodenum, they account for 15 % of all NETs [13]. Patients typically present between the ages of 40–60 years of age, and there is a possible slight female predominance [6, 14, 15]. There are no known environmental risk factors [3]. Somatostatinomas can either be functioning or non-functioning: cases in the literature have not been associated with somatostatinoma syndrome.

Somatostatinomas tend to be solitary and almost exclusively confined to the pancreas or duodenum, presumably reflecting the high concentration of delta cells in these organs [3, 6, 14]. Between 50 and 60 % of somatostatinomas originate in the pancreas, where there is a preference for the head of the gland (50 %), followed by the tail (25 %), and then diffuse infiltration of the gland (25 %) [6, 10, 13]. Of the remaining extrapancreatic lesions, approximately 50 % originate in the duodenum and 50 % precisely at the ampulla; less than 1 % arise from the jejunum, and isolated cases in the colon and rectum have been reported [6, 16, 17].

Somatostatinomas are associated with malignancy rates of up to 78 %, while 70–92 % have metastatic disease at presentation [6, 8, 9]. Rates of malignancy and metastasis are highest for pancreatic tumours [18, 19]. The liver is most commonly involved, followed by regional lymph nodes and then bony deposits [6]. The presence of metastatic disease is thought to be as a consequence of late diagnosis and does not appear to be dependent upon the tissue of origin [6].

16.3 Associations

Most somatostatinomas are sporadic, but there are well-known associations with MEN (multiple endocrine neoplasia) type 1 and neurofibromatosis type 1 (NF1), also known as von Recklinghausen disease. Less than 1 % of somatostatinomas are associated with MEN, however, this percentage being lower than for other pancreatoduodenal NETs [20]. Duodenal somatostatinomas are associated with NF1 in up to 50 % of patients, but there is less of a clear-cut association for pancreatic tumours [9, 14]. NF1 patients have an inherited mutation of the NF1 gene on chromosome 17q11, which codes for the protein neurofibromin. Somatostatinomas diagnosed in the presence of NF1 are thought to have a lower risk of metastases at presentation. There is a well-recognised association between NF1, duodenal somatostatinomas and pheochromocytomas, so that the presence of an adrenal lesion should be sought in this group of patients [3, 15, 21].

There have been occasional case reports of somatostatinomas in combination with conditions including von Hippel-Lindau disease [22, 23], tuberous sclerosis [24] and gastrointestinal stromal tumours [25, 26].

16.4 Biochemistry

16.4.1 Somatostatin

Somatostatin is also known as growth hormone-inhibiting hormone (GHIH), somatotropin release-inhibiting factor (SRIF) or somatotropin release-inhibiting hormone. It is a small cyclic peptide derived from proteolytic processing of two larger molecules (prepro-somatostatin and pro-somatostatin) [14]. Somatostatin is secreted from exocrine cells in the gastrointestinal (GI) tract and from delta cells of the pancreas under physiological conditions. It has two active forms, somatostatinoma-14 and somatostatin-28 (consisting of 14 and 28 amino acids, respectively) [27].

Somatostatin has a general inhibitory effect up on the gastrointestinal tract. It affects gastrointestinal motility, gastric acid production, pancreatic enzyme secretion, bile secretion and colonic fluid secretion [27]. It also inhibits the secretion of pancreatic and intestinal hormones such as insulin, glucagon, secretin and vasoactive intestinal polypeptide. Some of these actions are thought to occur via paracrine modulation and a reduction in splanchnic perfusion [3]. Somatostatin may also control cell proliferation both in normal tissues and NETs [27].

Since these effects have therapeutic benefits, somatostatin analogues, such as octreotide, octreotate, edotreotide and lanreotide, can be used in a variety of clinical contexts, including the palliation of vomiting, symptomatic control in neuroendocrine and thyroid tumours and prevention of complications following pancreatic surgery.

16.4.2 Somatostatin Receptors

The physiological effects of somatostatin are mediated by a family of seven G-protein-coupled transmembrane receptors; five subtypes exist ($SST_{1–5}$), each being coded by its relevant gene (SSTR1–5) [27, 28]. Most cells within the body express somatostatin receptors in various concentrations [29]. Of note, SSTR1 is expressed in its highest concentrations in the jejunum (and stomach), and SSTR3 is expressed in its highest concentrations in pancreatic islet cells [28]. The expression of somatostatin receptors by a tumour does not necessarily indicate that it is functional, but it can influence patient management [8, 9]. Approximately 80 % of all NETs express somatostatin receptors [10].

Tumours that express SST2 and SSTR5 are associated with a better outcome than those that do not [30]. In addition, tumour expression of receptors can be useful for both diagnostic and treatment purposes (see below): somatostatin receptor scintigraphy (SRS) relies on this expression for the accurate location of both primary tumours and metastatic foci [6, 29, 31]. Somatostatin analogues (e.g. octreotide, octreotate, edotreotide and lanreotide) can be used to control symptoms and inhibit cell growth [5, 7, 10, 32], while radionuclide therapy (via radiolabelled somatostatin analogues) can be used to deliver targeted radiotherapy to the tumour [4, 31, 33].

16.5 Pathology

Immunohistochemistry is the diagnostic investigation of choice for somatostatinomas as it reveals characteristic immunoreactivity for somatostatin [3, 13]. Management and survival of somatostatinomas is based on pathological findings, the Ki-67 index and the TNM (tumour, node, metastases) staging. All pathology reports from NETs should include a minimum data set of tumour site, immunohistochemical staining, grade (mitotic rate and Ki-67 index), presence of non-ischaemic tumour necrosis and presence of other pathological components (e.g. non-neuroendocrine components) [34].

16.5.1 Macroscopic

Macroscopic examination of a somatostatinoma reveals a round lesion which is well demarcated [13]. Lesions are typically solitary and measure 1–5 cm in diameter [3]. There is nothing to distinguish these tumours from other pancreatoduodenal NETs on macroscopic appearance.

16.5.2 Microscopic

Most somatostatinomas show typical features of a NET on histological examination: they tend to be well differentiated and show a glandular pattern (Fig. 16.1)

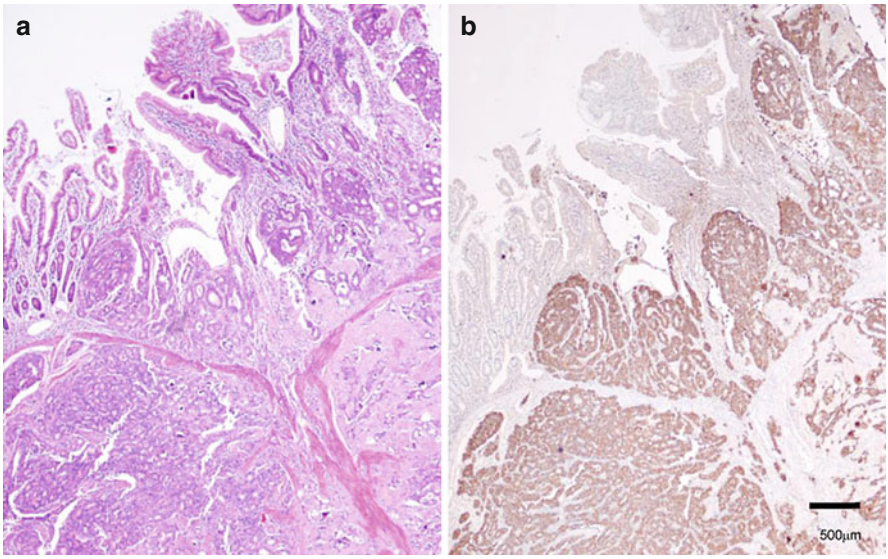

Fig. 16.1 (**a**, **b**) Microscopic appearances of ampullary somatostatinoma. Haematoxylin and eosin staining (*left*) and strongly positive immunostaining with somatostatin (*right*) (ANNALS IMAGE)

[3, 6, 13]. Some somatostatinomas show a mixed picture, with separate zones of well-differentiated and anaplastic cells. The differentiated areas have cells arranged in lobular or acinar patterns, which are separated by fibrovascular stroma. The less well-differentiated areas have cells interrupted by fibrous septa [6].

Psammoma bodies (round collections of calcium that arise after infarction and calcification) may be present in somatostatinomas. They are circular in appearance and are laminar, acellular and basophilic on histological examination. Psammomas are more commonly found in duodenal lesions and may be associated with neurofibromatosis [13].

16.5.3 Differentiation

Tumour differentiation – the extent of resemblance to normal cellular architecture – plays a role in assessing how aggressive a NET is [35]. Most somatostatinomas are well differentiated and have a uniform cellular pattern consistent with the organ of origin. Cells produce abundant neurosecretory granules and may also be arranged in nesting, trabecular or gyriform patterns [13, 35]. Poorly differentiated somatostatinomas less closely resemble non-neoplastic cells and have a more sheet-like or diffuse structural arrangement, with irregular nuclei and less cytoplasmic granularity.

16.5.4 Immunohistochemistry

Most NETs reveal some positivity for somatostatin immunohistochemistry due to the presence of somatostatin receptors. Somatostatinomas show diffuse positive immunoreactivity for somatostatin, and this expression is characteristic (Fig. 16.1) [3, 6, 13]. The immunoexpression is related to the differentiation of the tumour, with well-differentiated somatostatinomas revealing strong immunoreactivity for somatostatin staining and poorly differentiated tumours showing less immunoreactivity [35]. The absence of staining for other NET hormones (vasoactive intestinal polypeptide (VIP), gastrin, insulin and glucagon) also confirms the diagnosis. In addition, there may be immunoexpression of general neuroendocrine markers such as chromogranin A and synaptophysin [35].

16.5.5 Grading

Although no grading system can fully predict the behaviour of somatostatinoma, an estimate of the biological aggressiveness of NET can provide significant information on prognosis [35]. Biological activity can be assessed by cellular mitotic activity and the Ki-67 index (to estimate the growth fraction of a cellular population). Both of these measures assess tumour proliferative rate; mitotic activity requires counting the number of mitoses seen over a microscopic field,

Table 16.1 Grading system for foregut neuroendocrine tumours [35, 36]

Grade	Mitotic count (10 HPF)	Ki-67 index (%)
Low grade G1	<2	≤2
Intermediate grade G2	2–20	3–20
High grade G3	>30	>20

10 HPF high-power field = 2 mm^2, at least 40 fields (at 40× magnification) evaluated in areas of highest mitotic density, *Ki-67 index* labelling with MIB1 antibody; % of 2,000 tumour cells in areas of highest nuclear labelling

whereas the Ki-67 index provides a percentage of cells reacting to labelling with MIB1 antibody [35, 36]. These measures are broadly equivalent in assessing grading but can sometimes give conflicting information (in which case the more aggressive score is adopted) [35]. If limited amounts of tissue are available (e.g. following biopsy), a mitotic index may not be possible to perform as it requires counting 40–50 high-power microscopic fields (more than most biopsy samples contain). In these cases, Ki67 staining provides a more accurate assessment of proliferative rate [35].

Histological grading for pancreatoduodenal NETs is either low (G1), intermediate (G2) or high (G3) (Table 16.1). Low-grade NETs are relatively indolent, while intermediate grade tumours have a less predictable, moderately aggressive course [35]. Somatostatinomas that are either G1 or G2 tend to be well-differentiated NETs, while G3 tumours are poorly differentiated neuroendocrine tumours [35, 36].

16.6 Staging

A number of different staging systems exist to classify the extent of tumour spread for NETs, and while the criteria for assessment vary between each method, the underlying basic data are similar [35]. The TNM staging system is becoming the most widespread method for assessment; it reveals the extent of invasion into the organ of origin and regional or distant spread (Table 16.2) [35, 36].

16.7 Presentation

The functionality of the somatostatinoma greatly influences presentation. Non-functional tumours, which are not associated with somatostatinoma syndrome, tend to present with local mass effects, though many are detected incidentally. Symptoms are non-specific but include upper abdominal pain, abdominal swelling and mass, jaundice, weight loss, nausea and vomiting [3]. Some patients will present with tumour burden from metastatic disease. Often non-functioning tumours are detected incidentally during investigation of non-specific gastrointestinal symptoms in the same manner as other gastroduodenal NETs.

Table 16.2 TNM classification for pancreatoduodenal neuroendocrine tumours

TNM grade	Duodenum/ampulla/proximal jejunum	Pancreas
Tx	Primary tumour cannot be assessed	Primary tumour cannot be assessed
T0	No evidence of primary tumour	In situ tumour/dysplasia
T1	Tumour invades lamina propria or submucosa and size ≤1 cm	Tumour invades lamina propria or submucosa for ≤1 cm
T2	Tumour invades muscularis propria or size ≥1 cm	Tumour invades muscularis propria or subserosa for ≥1 cm
T3	Tumour invades the pancreas of the retroperitoneum	Tumour penetrates serosa
T4	Tumour invades peritoneum or other organs	Tumour invades adjacent structures
Nx	Regional lymph nodes cannot be assessed	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis	No regional lymph node metastasis
N1	Regional lymph node metastasis	Regional lymph node metastasis present
Mx	Distant metastases cannot be assessed	Distant metastases cannot be assessed
M0	No distant metastases	No distant metastases
M1	Distant metastases	Distant metastases

Adapted from Rindi et al. [36]
T primary tumour (add (m) for multiple tumours), *N* regional lymph nodes, *M* distant metastases
M1 specific sites as defined according to Sorbin and Wittekind [37]

16.7.1 Somatostatinoma Syndrome

Functioning tumours are rare and cause somatostatinoma syndrome as a result of overexpression of somatostatin. This syndrome comprises diarrhoea (secondary to decreased pancreatic enzyme and bicarbonate secretion), steatorrhoea and diabetes (resulting from insulin inhibition), gallstones (from cholecystokinin inhibition) and hypochlorhydria [4–6]. Some authors argue that this syndrome must be present for a secure diagnosis of somatostatinoma [7]. Functioning tumours are likely to present earlier than non-functioning tumours, given their symptomatology and consequent investigation.

16.8 Investigation

Although radiology provides the mainstay of investigation, it will only reveal the presence of a pancreatoduodenal NET rather than a definitive diagnosis of a somatostatinoma [3]. Nuclear medicine and endoscopic assessment play an increasingly important role in the management of this group of tumours. Biochemical assessment may also reveal the presence of a NET, and it can be diagnostic for somatostatinoma. Definitive diagnosis tends to come from tissue diagnosis and immunohistochemistry (either biopsy or pathological specimen).

16.8.1 Biochemical and Haematological Assessment

Patients with pancreatoduodenal NETs should be investigated with both standard and specific blood tests. Routine full blood count, liver function tests, urea and electrolytes and clotting screen should be performed to check for any derangement in organ function: significant hepatic metastases may result in derangement in liver function. Blood glucose should also be measured for signs of diabetes, either due to somatostatin syndrome or to pancreatic dysfunction secondary to tumour invasion. These tests should also be used as a workup for further management, both in terms of radiological investigation and suitability for operative intervention.

Chromogranin A and pancreatic polypeptide are non-specific markers for pancreatoduodenal NETs; elevated levels are found in 50–80 % of tumours, including somatostatinomas [20]. Although elevated plasma somatostatin levels (SLI) are strongly indicative of somatostatinoma, they are rarely found [5, 7]. Pancreatic somatostatinomas tend to have higher SLI concentrations (up to 50 times normal) compared to intestinal somatostatinomas in which the concentration is often normal [6]. Patients with high plasma levels of somatostatin are thought to present earlier than those with normal levels [4].

16.8.2 Radiological Imaging

Accurate imaging of the primary tumour and the extent of disease is vital in all stages of management of somatostatinomas. Imaging should provide the location and extent of the primary tumour and assist in determining whether operative intervention should be performed, either curative resection or debulking. In addition, local invasion and the presence of metastases should be sought on investigation. Functional somatostatinomas may be harder to identify than tumours without somatostatinoma syndrome, as they are likely to be smaller at initial presentation.

Conventional imaging provides the mainstay of initial investigation. Computed tomography (CT), ultrasound scanning and magnetic resonance imaging (MRI) can all be employed to assist diagnosis, with selective use of small bowel series (barium or Gastrografin) (Fig. 16.2) and angiography (Fig. 16.3) [3]. Dual-phase, thin-slice CT (Fig. 16.4) is the usual first-line investigation; detection rates are proportional to the size of the lesion. Somatostatinomas are isodense (and thus not visible on unenhanced CT), but intravenous contrast will reveal a characteristic hypervascular lesion [38]. Typically lesions are 5 cm in diameter at presentation [34]. More than 70 % of lesions greater than 3 cm in size can be identified, but only 50 % of lesions measuring less than 1 cm are detected [10, 39]. Thus, small somatostatinomas and hepatic metastases may be missed. Dual-phase CT and MRI have equivalent sensitivities for the detection of NETs; CT is thought to be better for detection of peritoneal and mesenteric disease than MRI, which is more sensitive for detecting liver and bony metastases [6, 40].

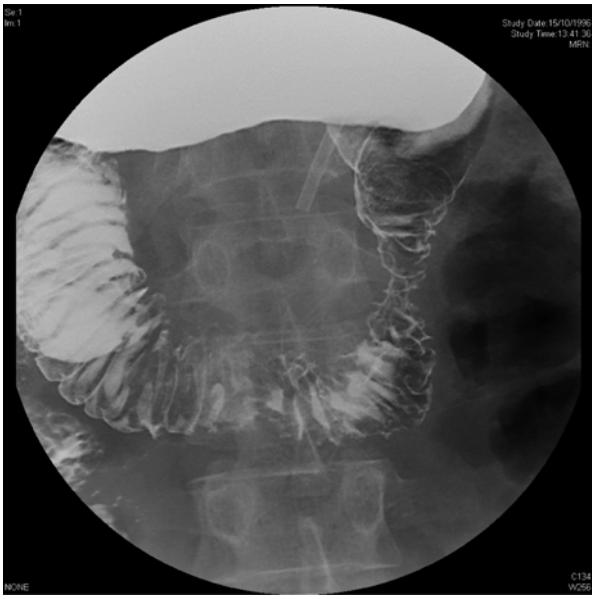

Fig. 16.2 Small bowel Gastrografin study showing constricting lesion in the second part of the duodenum (patient in prone position). Lesion subsequently confirmed as somatostatinoma on postoperative immunohistochemistry

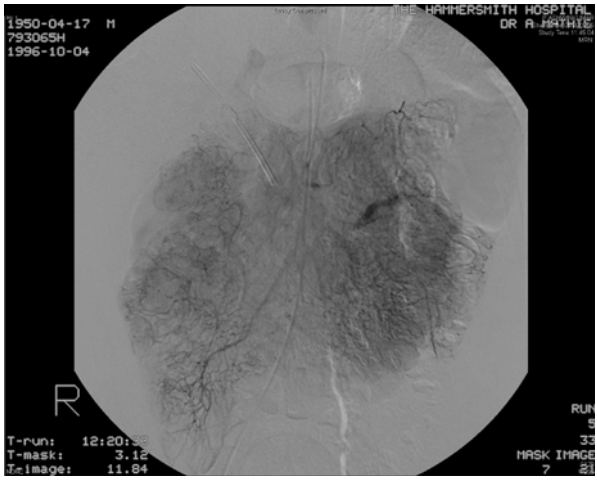

Fig. 16.3 Visceral angiography of patient with duodenal somatostatinoma. Increased tumour 'blush' noted at the right-hand side of the image, but no gross vascular invasion present (patient subsequently underwent successful pylorus-preserving proximal pancreatoduodenectomy)

Transabdominal ultrasound is not used routinely because it has a low sensitivity (9–64 %) for pancreatic lesions [10, 39]. Angiography may reveal a characteristic tumour blush and can be combined with selective visceral cannulation and assessment of hormonal gradients. These techniques have now been largely superseded by other imaging modalities, notably CT.

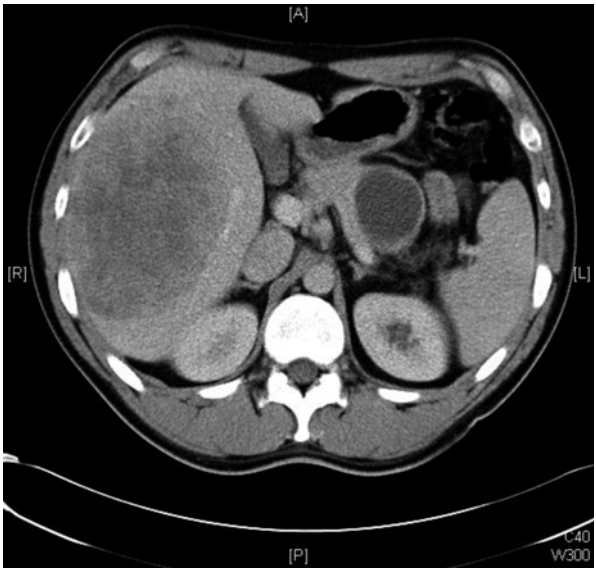

Fig. 16.4 Computed tomography showing the presence of a large pancreatic primary somatostatin and hepatic metastases

16.8.3 Nuclear Medicine Imaging

16.8.3.1 Somatostatin Receptor Scintigraphy

The overexpression of somatostatin receptors (particularly subtypes SSTR2 and SSTR5) by more than 80 % of pancreatoduodenal NETs has allowed the development of somatostatin receptor scintigraphy (SRS) [31, 41, 42]. A number of different radiolabelled somatostatin analogues will bind to the receptors with high affinity. One of the agents most widely used worldwide for SRS is ^{111}IN-DTPA-octreotide (Octreoscan), which has an overall sensitivity of 80–90 % [6, 10, 20, 31, 43]. SRS allows whole body scanning, which is invaluable in detecting metastases (especially at unexpected locations), but it does not provide information on tumour size or resectability [4, 6]. After the radiolabelled agent is injected into the patient, scans are generally performed at either 4–6 h or at 24 h. With earlier scans, lesions may be obscured by a relatively high background activity. Delayed scans provide better contrast (due to lower background activity) but can result in false-positive results secondary to physiological bowel activity [6].

16.8.3.2 SPECT (Somatostatin Receptor Scintigraphy Combined with Computed Tomography)

SPECT is more sensitive than conventional imaging for detection of both the primary somatostatinoma and its metastases [31, 44]. Seventy per cent of primary lesions and more than 90 % of distant disease can be detected with SPECT [31, 44]. It has the additional advantage over conventional imaging that it can provide whole body scanning at one time, and it alters patient management in up to 47 % of patients

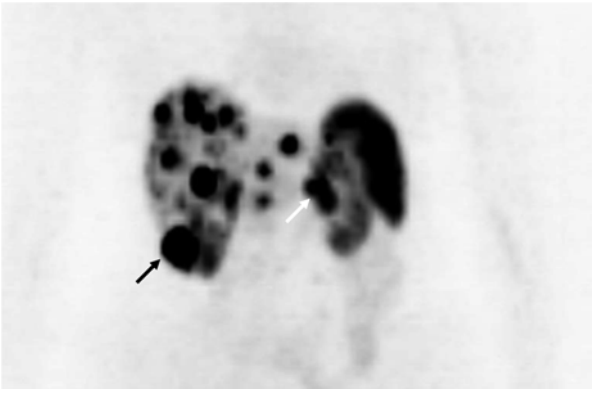

Fig. 16.5 PET scan showing extensive hepatic metastases as well as uptake in the inferior pole of the right kidney (*black arrow*) and the pancreatic primary tumour (*white arrow*)

[7, 31]. False-positive localisations can occur in up to 12 % of patients, but this figure can be reduced to 3 % when findings are corroborated with clinical assessment [7, 10, 31].

16.8.3.3 Positron Emission Tomography

Positron emission tomography scanning (Fig. 16.5) has greater sensitivity than either SRS or conventional cross-sectional imaging [10]. As a result, this new technique is becoming increasingly employed for the detection of all pancreatoduodenal NETs, including somatostatinomas [3]. Two main agents are used: ^{11}C-5-HTP and 68gallium-labelled somatostatin analogues.

16.8.4 Endoscopic Examination

Endoscopy allows direct visualisation of the upper gastrointestinal tract and can help diagnose gastroduodenal lesions. It should be combined with concurrent endoscopic ultrasound, which can detect lesions as small as 0.5 cm [45]. Endoscopic ultrasound is more effective at localising tumours in the pancreas than the duodenum, although the tail of the gland can be inaccessible [7, 46]. Fine needle aspirates can be taken, which can be useful in providing cytological assessment of suspect lesions.

16.8.5 Intraoperative Techniques

Both intraoperative ultrasonography and endoscopic transillumination are recommended to assist in detection of small lesions not appreciated on conventional imaging [7, 10]. In addition they can be used to help plan the route of resection and enucleation of lesions [4]. However, these techniques may fall out of routine use with the increasing availability and sensitivity of nuclear imaging.

16.9 Management

The overall 5-year survival rate in pancreatic and periampullary somatostatinoma is 60–100 % with localised disease or 15–60 % in metastatic disease [15, 47]. Large size (>3 cm), poor differentiation and lymph node involvement are poor prognostic markers. Tumours that are hormonally inactive (non-functioning), which predominate, have a worse prognosis than functioning somatostatinomas.

Operative resection, when feasible, is recommended for all pancreatoduodenal NETs, including somatostatinomas. Curative resection is the aim of intervention, but in the presence of liver metastases, either complete resection or debulking of primary tumour is recommended. Medical management, in the form of chemotherapy, somatostatinoma analogues or radionucleotide therapy, plays an important role in controlling both symptoms and tumour growth, but it is not considered a first-line intervention.

16.9.1 Medical

16.9.1.1 Somatostatin Analogues

Somatostatin analogues, such as octreotide, octreotate, edotreotide and lanreotide, help control the symptoms of pancreatoduodenal NETs [5, 7]. These analogues act on somatostatin receptors and stimulate the inhibitory effects of somatostatin. Long-acting depot forms of these analogues allow monthly injection therapy, which increases patient convenience and therefore compliance [10]. Long-term treatment may be associated with symptomatic breakthrough, in which case a stronger or more frequent doses may be required; in some cases a treatment 'holiday' is required before recommencing the analogue [10]. These analogues have mild side effects in up to half of all patients, including flatulence, diarrhoea/steatorrhoea, nausea, gallstones and glucose intolerance [5, 7]. The addition of α-interferon therapy may help control symptoms if first-line intervention is inadequate [20].

In addition to symptomatic relief, somatostatin analogues are thought to have tumourostatic effect in 40–80 % of patients, but have not been shown to cause tumour regression [7, 10, 31]. This stabilisation of disease has increased progression-free survival for midgut neuroendocrine tumours [4, 48].

16.9.2 Chemotherapy and Radiotherapy

Generally pancreatoduodenal NETs do not respond well to oncological agents because they are slow growing and thus have an inherent resistance to therapies targeted at rapidly dividing cells. Nevertheless, chemotherapy and radiotherapy are frequently employed for disease that is not amenable to operative resection. A number of different chemotherapeutic regimes have been employed, with an overall objective response rate of 10–45 % [4]. Chemotherapeutic agents include streptozotocin, doxorubicin, 5-FU, temozolomide, dacarbazine and chlorozotocin;

streptozotocin is thought to be the best therapy with response rates as high as 70 % [3, 4]. However, these regimes result in appreciable toxicity. Complete tumour response to any therapy is rare, and all drugs are associated with side effects that may make the regime intolerable. Trans-arterial chemoembolisation (TACE) can also be successfully employed for the treatment of hepatic metastases (see below).

Systemic radiotherapy is not usually suitable for patients with somatostatinomas (or any pancreatoduodenal NETs) due to the location of the primary tumour and associated lymphatic spread. Its use is not supported in the literature except for providing symptom relief for bony metastases [4].

16.9.3 Peptide Receptor Radionuclide Therapy [PRRT]

Radionuclide therapy is a novel technique which is being increasingly utilised for the treatment of inoperable or metastatic pancreatoduodenal NETs [33, 49]. This therapy delivers targeted local radionucleotides that release γ-radiation or β-radiation to the tissues, thus decreasing overall radiation exposure to the patient [31, 33]. The exact dosage of radiation needs to be calculated to ensure that sensitive organs, for example, the kidneys and bone marrow, do not suffer irreversible damage [33].

The overexpression of somatostatin receptors by most NETs, particularly somatostatinomas, makes targeted peptide receptor radionuclide therapy (PRRT) particularly appealing. Radiolabelled somatostatin analogues with attached radionuclides of ^{177}lutrium, ^{90}yttrium or ^{111}indium can be used to target pancreatoduodenal NETS. No randomised controlled studies exist, but several studies have reported favourable response rates [4, 31, 49]. Of all the agents commercially available, [^{177}Lu-DOTA^{0},Tyr3]octreotate (DOTATATE) has showed a complete response rate in 2 % of patients, a partial response in 32 % and stabilisation of disease in 34 % [49]. Overall, median survival rates with DOTATATE of more than 40 months have been reported.

16.9.4 Surgery

Operative resection of the primary tumour, even in the presence of metastases, is recommended for all pancreatoduodenal NETs unless the patient has [10]:

1. Another medical condition limiting life expectancy or increasing surgical risk
2. Diffuse metastatic liver disease
3. One of the inherited PNET syndromes

This strategy is governed, in part, by better outcomes of operative resection and the traditionally poor outcomes associated with nonoperative interventions. When curative resection cannot be performed, surgical debulking of the primary tumour is advocated; hepatic resection of metastatic disease is also advocated when possible, although there are a number of alternatives to hepatectomy (see below). Any

resection should be combined with cholecystectomy, because of the likelihood of gallstone formation with increasing somatostatin levels (with associated cholecystokinin inhibition) postoperatively [4].

16.9.4.1 Curative Resection

Any patient without metastatic disease should be considered for curative resection of the tumour. Unfortunately with up to 90 % of patients presenting with metastatic disease, curative resections are not that common particularly when sensitive whole body scanning, in the form of SRS or PET, is now commonplace [6, 8, 9]. When possible, somatostatinomas should be locally excised or enucleated, with major resections reserved for more extensive disease in patients who are fit enough to tolerate the operation [7, 10, 20]. As somatostatinomas are located in the periampullary duodenum or pancreatic head, a pylorus-preserving pancreatoduodenectomy or Whipple's procedure is the most common resection, although total pancreatoduodenectomy may occasionally be required for multiple tumours or bulky tumours in the pancreatic neck [3].

Well-defined and localised intrapancreatic lesions can be resected laparoscopically, but most somatostatinomas are approached by laparotomy. An extensive abdominal exploration has traditionally been recommended to search for lymph node metastases, although this may not be required given the sensitivity of SRS and PET [7, 10, 20].

16.9.4.2 Palliative Resection

By definition, tumours that have metastasised cannot undergo curative resection, but resection of the primary tumour even in the presence of hepatic metastases is advocated [3, 10]. There are many options for the management of hepatic metastases (see below) that can improve patient survival and alter the natural history of the disease process [50]. A recent systematic review has shown a trend towards improved survival post resection of primary lesion in the presence of metastases, although it is unknown whether this is a true reflection or simply that patients in the operative group are healthier than those not deemed for surgery, i.e. a positive selection bias for treatment in patients with better overall performance status [12]. Regardless of the underlying mechanism, a 30 % improvement in 5-year survival in patients undergoing resection has been reported [12].

Thus all patients should be considered for operative intervention if complete (or near-complete) resection of hepatic metastases can be achieved [50]. Resection of the primary tumour should be undertaken in the same manner as outlined above, and it can be combined with concurrent resection of metastatic disease.

16.9.4.3 Debulking of Primary Tumour

Operative tumour debulking, to remove at least 90 % of primary tumour volume, is only possible in 5–15 % of patients [7, 10, 51, 52]. Although there is little evidence to suggest that cytoreductive surgery improves symptoms and survival, it is generally advocated especially since other interventions have low efficacy [4, 7, 51–53].

Some studies have shown that debulking can improve symptoms associated with 'tumour mass', i.e. pain and vomiting, with up to half of patients reporting benefit for a mean duration of 39 months, compared to best combination chemotherapy offering a median survival of 26 months [53, 54].

16.9.5 Management of Liver Metastases

Given that most non-functioning somatostatinomas are indolent and present with metastatic deposits, aggressive management of these liver lesions is advocated; hepatectomy should be considered in all cases and can be performed in isolation or in combination with other hepatic interventions (see below). Patients who are deemed unresectable, either due to co-morbidities or diffuse hepatic involvement, may be managed with targeted tissue destruction.

16.9.5.1 Resection of Liver Metastases

Aggressive resection of liver metastases is associated with better long-term outcomes compared with nonoperative intervention, such as hepatic artery embolisation (HAE), radio-frequency ablation or radioactive octreotide [3]. Five-year survival rates of 76 % have been reported following hepatectomy, compared with 50 % for HAE and 26 % for medical therapy [53]. Complete resection of hepatic metastases improves survival threefold compared with incomplete resection [55]. For young, otherwise healthy patients, liver transplantation for widespread hepatic metastatic infiltration should be considered, especially if the patient's symptoms cannot be managed by other therapies [10].

16.9.5.2 Nonoperative Management of Liver Metastases

Several hepatic interventions exist for the management of metastatic disease, but no randomised studies exist comparing the efficacy of any procedure either against each other or against resection [4, 11]. Nonoperative intervention is recommended for palliation in patients who are unsuitable for surgical resection provided they have an otherwise preserved performance status, with disease confined to the liver and a patent portal vein [10, 11]. These techniques are considered particularly relevant for patients with hormone excess who cannot be controlled by any other means [11]. Nonoperative strategies include gel foam embolisation, transhepatic arterial chemoembolisation (TACE), hepatic artery embolisation (HAE) with radioactive microspheres, percutaneous alcohol ablation, radio-frequency ablation and cryoablation.

Clinical response rates following TACE are generally greater than 50 % and are measured by radiographic regression and/or a decrease in serum hormones [11]. No data exist showing superiority of any one of the TACE techniques (bland embolisation, chemoembolisation, embolisation with chemotherapy beads and embolisation using radioisotopes) compared with each other. RFA, Microwave Ablation (MWA) and cryoablation are recommended only in selected patients who have low-volume

disease [10, 11]. All these techniques can be performed either intraoperatively (either combined with resection of primary metastatic disease or during separate laparoscopy) or via a percutaneous approach [10].

16.10 Surveillance

Like most pancreatoduodenal NETs, somatostatinoma has an indolent course compared with pancreatic adenocarcinoma. These tumours therefore generally have a longer overall survival even if untreated [4]. The natural history of the disease is not fully understood, and as such, there are no guidelines for prognosis or surveillance of disease. Generally patient surveillance is advocated, especially when operative resection has occurred. Patients should have their chromogranin A, pancreatic polypeptide and plasma somatostatin levels monitored in addition to periodic cross-sectional imaging of the abdomen [10]. Some clinicians advocate yearly postoperative SRS studies to detect signs of disease recurrence [10].

16.10.1 Tumour Recurrence or Progression

No established guidelines exist for the management of patients with progressive or recurrent disease. Options include reoperation (including debulking for symptom relief from space-occupying lesions or hormonal effects) and systemic chemotherapy. Therapy should be based on the individual's characteristics, site of recurrence and prior therapy [4].

Conclusion

Somatostatinomas are rare neuroendocrine tumours that arise in the pancreas or peripancreatic duodenum. They are a fascinating group of tumours that are poorly understood and relatively unpredictable. These tumours are relatively indolent and present late, often with metastatic disease. Although there is some dispute as to whether functional activity (somatostatinoma syndrome) is required for diagnosis, most tumours are diagnosed histologically on diffuse positive immunoreactivity to somatostatin. An increased awareness of this group of tumours and improved imaging (particularly in the form of SRS and PET scanning) is thought to be related to an increasing incidence of disease.

Operative resection provides the mainstay of treatment for both primary lesions and metastatic disease, with somatostatin analogues, chemotherapy and radionucleotide therapy considered second-line intervention. No randomised control trials for the management of somatostatinomas exist to guide treatment, owing to their rarity and the difficulty with correct preoperative diagnosis. Survival is generally good and certainly much better than for an equivalent pancreatoduodenal adenocarcinoma.

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Less Common Types of Pancreatic Neuroendocrine Tumors

17

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17.1 Introduction

Less common types of pancreatic neuroendocrine tumors (pNETs) can trigger clinical syndromes, such as ACTHoma, CRHoma, serotoninoma, calcitoninoma, GHRHoma, GRFoma, parathyroid hormone-related peptide tumor, and ghrelinoma (Table 17.1). In the majority of cases, it is not clear whether they involve specific syndromes due to their low incidence rate [1–4].

Less common pNETs make up less than 10 % of all pNETs [1]. They can occur in areas other than the pancreas, and when they occur in the pancreas, they are often metastatic to the liver (40–90 %) [1].

Mostly, less common pNETs are diagnosed as WHO Group 2. In general, patients within the age range of 50–55 years are likely to be affected, with no significant gender differences. Patients diagnosed with malignant tumors show mixed syndromes, which can change over time [1].

Most often, pNETs can occur as part of four inherited disorders, including multiple endocrine neoplasia type 1 (MEN1), von Hippel–Lindau disease (VHL), neurofibromatosis 1 (NF-1) (von Recklinghausen disease), and the tuberous sclerosis complex (TSC). The order of their frequencies is MEN1 > VHL > NF-1 > TSC [5].

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Table 17.1 Less common types of pNETs

Name	Biologically active peptide(s) secreted	Incidence	Tumor location	Malignant %	Associated with MEN-1, %	Main symptoms/signs
ACTHoma	ACTH	Rare	Pancreas	>95	Rare	Cushing's syndrome (100 %)
CRHoma,	Corticotropin-releasing hormone	Rare	Pancreas, lung	Unknown	Unknown	Cushing's syndrome, rare
Serotoninoma	Serotonin, tachykinins	Rare	Pancreas	60–88	Rare	Diarrhea, flushing
Calcitoninoma	Calcitonin	Rare	Pancreas	>80	16	Diarrhea (50 %)
GHRHoma	Growth hormone-releasing hormone	Unknown	Pancreas, lung, jejunum, others	>60	16	Acromegaly (100 %)
GRFoma	Growth hormone-releasing factor	Rare	Lung, pancreas, others	30	<1 %	Acromegaly
PET causing hypercalcemia (PTHrp-oma)	PTHrp; others unknown	Rare	Pancreas	>90	Rare	Symptoms due to hypercalcemia
Ghrelinoma	Ghrelin	Rare	Pancreas, stomach, intestine	Unknown	Unknown	Starvation, anorexia nervosa, bulimia, cachexia
PET secreting renin	Renin	Rare	Pancreas	Unknown	No	Hypertension
PET secreting luteinizing hormone	Luteinizing hormone	Rare	Pancreas	Unknown	No	No anovulation, virilization (female); reduced libido (male)
PET secreting erythropoietin	Erythropoietin	Rare	Pancreas	100	No	Polycythemia
PET secreting IF-II	Insulin-like growth factor II	Rare	Pancreas	Unknown	No	Hypoglycemia

17.2 Clinical Features and Prognosis

The majority of less common pNETs have already metastasized to other areas at the time of diagnosis, and a 5-year survival is weakly associated with the hormone excess state compared to the growth rate of the tumor. The 5-year survival rate is generally 29–45 % [1, 6, 7].

The survival/prognostic data of pNETs demonstrate that tumor Ki67 ≥ 2 % and the presence of liver metastasis were associated with a poor prognosis [7].

Less common pNETs typically give rise to symptoms specific to hormone excess states (Table 17.1), which mostly occur during the later phases of the disease [6].

17.3 Diagnosis

The minimum diagnostic work-up for less common pNETs includes specific biochemical analyses associated with specific hormonal activities, clinical symptoms of the disease, and evidence of a hormone excess state. Commonly used markers such as serum chromogranin A are useful for identifying a neuroendocrine tumor and monitoring during the disease's course [1, 5–7]. All biochemical tests should be performed at the initial diagnosis.

In terms of diagnostic imaging, it is recommendable to perform mdCT scan (or MRI) and SRS-SPECT [1, 6, 7]. Other useful tests include PET/CT, gallium-68-labeled somatostatin analogue PET, 18 F-DOPA-PET, or 11 C-5-HTP-PET [1, 6, 7].

Detailed pathological examinations such macroscopic, microscopic, and immunohistochemical findings are essential for the diagnosis of pNETs, and the current WHO TNM classification is applied [1].

Histological examination of HE-stained sections as well as immunostaining for chromogranin A and synaptophysin should be performed in all cases. It is also essential to determine both the mitotic index and Ki67 index [1, 6, 7].

17.4 Surgery

Indications for surgery are determined by factors such as clinical symptom control, tumor size/location/extent, malignancy, and metastatic spread [1, 6, 7]. It is recommended to consider curative surgery even in metastatic states. In the case of localized metastatic disease to the liver, cytoreductive surgery is feasible, if the tumor site is potentially resectable and the patient's conditions allow surgery. The selection of the type of surgery is generally based on the location of the primary tumor. Because less common pNETs are predominantly malignant, adequate lymph node clearance should always be ensured [1, 6, 7].

17.5 Medical Treatment

Somatostatin analogues are effective in treatment in the control of symptoms of less common functional pNETs. If somatostatin analogues are ineffective or lose efficacy in controlling the hormone excess state, interferon-α can be effective in standalone use or in combination with somatostatin analogues.

In the case of pNETs, G2 foregut NET of extrapancreatic site, and neuroendocrine carcinoma (G3) of any site, chemotherapy is recommended. Systemic cytotoxics are useful in patients with inoperable progressive liver metastasis from well-differentiated pNETs, and combinations of streptozotocin and 5-FU or doxorubicin are generally administered [1, 6, 7].

Other interventions, such as transarterial embolization and/or chemoembolization and liver-directed therapy with radiolabeled particles, may be performed when the liver predominantly shows metastases and the symptom control of functional pNETs becomes difficult [1, 6, 7].

Everolimus and sunitinib can be administered as molecular-targeted therapies, which are relatively new remedies for advanced pNETs. Everolimus is primarily administered to pNET patients not responding to chemotherapy, but its use can also be considered for some patients as a first-line therapy. Sunitinib is primarily used as a second- or third-line therapy, but it is also applicable to some patients as a first-line therapy [1].

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Nonfunctioning Pancreatic Neuroendocrine Tumors

18

Anna Koumarianou and Nicola Fazio

18.1 Epidemiology and Genetics

Pancreatic neuroendocrine tumors (PNETs), in general, include a heterogeneous group of malignant diseases exhibiting unique clinical and biological features [87, 120]. They can range from indolent to highly aggressive and when unresectable have no curative therapy. The term nonfunctioning (NF) describes the lack of a specific clinical syndrome related with hormone oversecretion. This does not necessarily mean that no peptide or amine production takes place. A clinically undefined syndrome in PNETs may well be associated with positive immunohistochemistry (IHC) for hormonal production, indicating that the quantity secreted is small or biologically inactive (e.g., pancreatic polypeptide) or simply accumulated in the cytoplasm rather than released in the intercellular compartment [63].

A recent SEER database analysis reported that NF-PNETs have an annual incidence of three per million, representing 85 % of all PNETs and 2 % of all pancreatic malignancies [38]. In the same study, 2,158 NF-PNET patients were found to have a median age of 59 years, a presence of metastatic disease in 60 % of cases, and an overall survival (OS) at 5, 10, and 20 years of 33, 17, and 10 %, respectively. Nonfunctioning PNETs can present with symptoms due to mass effect, including obstruction, bleeding, abdominal pain, vomiting, and jaundice, or

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systemic ones as anorexia and weight loss [7, 38]. More than 50 % of NF-PNET patients who initially present with localized disease will develop liver metastasis after resection of their primary tumor [99]. The survival of patients with NF-PNETs was found to be similar to that of the functioning counterpart, an interesting finding given that the lack of clinical syndrome was thought to be associated with a more aggressive biology [19].

The molecular basis of NF-PNET tumorigenesis is not well understood, and although 90 % of cases are sporadic, they may be included in the context of important genetic syndromes such as multiple endocrine neoplasia type 1 (MEN1), von Hippel–Lindau (VHL) disease, and tuberous sclerosis complex (TSC) syndrome [12].

MEN1 syndrome is associated with many neoplasms, most commonly pituitary adenoma, parathyroid adenoma, and pancreatic islet cell tumor. Menin is a predominantly nuclear protein that has a role in transcriptional regulation, genome stability, cell division, and proliferation. Until recently, 1,336 mutations have been reported in the menin chromosomal region 11q13 [62]. Menin has been recently found to epigenetically suppress Hedgehog signaling, a proproliferative and oncogenic pathway, and to downregulate AKT and its kinase activity that in turn induces proliferation and antiapoptotic signaling [40, 111].

In the context of MEN1 syndrome, NF-PNETs occur typically within a field of numerous pancreatic microadenomas, and a prospective study indicated their frequency to be approximately 55 % [104]. PNETs arising in the context of MEN1 syndrome are usually nonfunctioning [72]. With respect to the time of clinical onset, a study in children with MEN1 syndrome has shown that they may have asymptomatic NF-PNETs well before the age of 20 years [74].

VHL disease is a very rare autosomal dominant inherited syndrome characterized by inactivating mutations of the VHL gene that resides on chromosome 3p25-26. The VHL gene encodes, by alternative splicing, two proteins (pVHL) with a main role of inactivation of hypoxia-inducible factor 1a (HIF1a) [46]. Loss of heterozygosity of VHL leads to the upregulation of HIF1a and other angiogenic factors such as VEGF resulting in the proliferation of vascular tissue and the development of retinal and cerebellar hemangioblastomas, renal cell carcinoma, pheochromocytoma, and NF-PNETs [16].

Tuberous sclerosis complex is a very rare hereditary autosomal dominant syndrome that involves two genes located on 9q34 (TSC1) and 16p13.3 (TSC2) encoding for hamartin and tuberin, respectively. In the normal cell, hamartin and tuberin form a heterodimer complex that inhibits the mammalian target of rapamycin (mTOR) pathway and thus cell growth and proliferation [103]. Patients with deficiency in these proteins develop widespread hamartomas in several organs, including the brain, heart, skin, eyes, kidney, lung, and liver, but also neurological disorders and NF-PNETs [18, 47]. Most features of tuberous sclerosis become evident in childhood at 3 years of age, limiting their usefulness for early diagnosis.

18.2 Molecular Biology of Sporadic NF-PNETs

The genetic background of sporadic PNETs is complex. Many studies support that PNETs have different deregulated cellular mechanisms when compared to other NETs or are related with different grades of disease and presence or absence of a syndrome [66, 80].

Functioning PNETs compared with normal pancreas and NF-PNETs showed several differences at gene expression level including decreased protein expression of TSC2 and PTEN and increased expression of somatostatin receptor type (SSTR) 2 [66]. PTEN was one of the first reported proteomic alteration observed in PNETs, and it was found to be mutated or abnormally localized in the cytoplasm instead of the nucleus [90]. Mutations in chromosome 11 involving menin are also observed in 8–26 % of NF-PNETs [72].

Except the well-studied genetic mutations, several newer studies support the role of epigenetics in the pathogenesis of neoplastic diseases. Epigenetics are potentially modifiable/reversible factors by environmental conditions or even heritable changes in gene expression caused by several other factors other than mutations of DNA sequence such as DNA methylation, histone alterations, and miRNAs. Some of these recently recognized changes have been observed in sporadic NF-PNETs and mainly include the inactivated pathway of menin that has recently been linked to H3 histone variation [43, 60]. Histone variants cause posttranslational modifications (histone code) and are one of the reasons of silencing genes that are not found mutated.

Another important pathway in the pathogenesis of the tumors is telomere preservation that is under the control of several enzymatic processes and is gradually decreasing in size in normal cells and is responsible for the cellular senescence. This pathway is commonly found deranged in tumor cells. Telomere maintenance is indispensable for cellular immortalization, and tumors achieve that either by activation of telomerase or by activation of the alternative lengthening of telomeres (ALT). In a recent study of 149 genes, 16 tumors were found mutated in PNETs, a much lower incidence than in adenocarcinomas. Three main groups were identified: (a) MEN1 in 44 %, (b) death domain-associated protein (DAXX)/alpha thalassemia/mental retardation syndrome X-linked (ATRX) in 45 %, and (c) TSC2, PTEN, and PIK3CA genes involved in the mTOR pathway in 14 %. The DAXX and ATRX are both required for histone 3.3 incorporation in telomeres. When ATRX and DAXX are mutated, the ALT pathway becomes activated [47]. In 19/19 patients with mutated ATRX or DAXX, ALT was positive as determined by fluorescence in situ hybridization. Six out of twenty ALT-positive patients without ATRX or DAXX mutations had lost nuclear expression of ATRX or DAXX nuclear protein by IHC, indicating an error in another point of the telomere pathway. Nevertheless, ALT- and MEN1-positive patients had a superior survival, and mutations of the mTOR pathway may be used as predictive factors of response to treatment with everolimus [47].

A different study in poorly differentiated PNETs showed overexpression of PTEN, mothers against DPP homologues (SMAD)4, and B-cell CLL-lymphoma (Bcl)-2 [113].

18.3 Classification and Staging

The World Health Organization (WHO) 2010 classification distinguishes PNETs on the basis of the Ki-67 proliferation index [9] in grade (G) 1 (<3 % Ki-67), G2 (3–20 % Ki-67), and G3 (>20 % Ki-67). The first two categories are called *tumors* and the third one *carcinomas*.

Staging involves a site-specific tumor–node–metastasis (TNM) classification according to the recommendations of the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC) (Table 18.1). The WHO classification and staging aims to standardize pathology reporting and staging procedures and ultimately to enable meaningful patient stratification into prognostic and treatment groups. However, a recent

Table 18.1 TNM staging definitions in the European Neuroendocrine Tumor Society (ENETS) and the Union for International Cancer Control/American Joint Committee on Cancer/World Health Organization (UICC/AJCC/WHO) 2010 TNM staging systems

ENETS TNM	UICC/AJCC/WHO 2010 TNM
T classification	
Limited to the pancreas, <2 cm	Limited to the pancreas, ≤2 cm in greatest dimension
Limited to the pancreas, 2–4 cm	Limited to the pancreas, >2 cm in greatest dimension
Limited to the pancreas, >4 cm or invading duodenum or bile duct	Beyond the pancreas but without involvement of the superior mesenteric artery
Tumor invading adjacent organs (stomach, spleen, colon, adrenal gland) or the wall of large vessels (celiac axis or the superior mesenteric artery)	Involvement of celiac axis or the superior mesenteric artery (unresectable tumor)
N classification	
No regional lymph node metastasis	No regional lymph node metastasis
Regional lymph node metastasis	Regional lymph node metastasis
M classification	
No distant metastasis	No distant metastasis
Distant metastasis	Distant metastasis
Stage classification	
I: T1, N0, M0	IA: T1, N0, M0
	IB: T2, N0, M0
IIa: T2, N0, M0	IIA: T3, N0, M0
IIb: T3, N0, M0	IIB: T1–T3, N1, M0
IIIa: T4, N0, M0	III: T4, N0–N1, M0
IIIb: T1–T4, N1, M0	
IV: T1–T4, N0–N1, M1	IV: T1–T4, N0–N1, M1

Data from Rindi et al. [92, 93], Bosman et al. [9], Edge et al. [25]

Abbreviations: *NA* not applicable

large retrospective analysis of 1,072 patients with a resected PNET concluded that ENETS TNM is superior in performance to the UICC/AJCC/WHO 2010 TNM and is more accurate [92].

18.4 Prognostic and Predictive Factors

Over the past few years, a few studies have identified certain clinical parameters that may contribute to the formation of an accurate prognostic assessment, which is of paramount importance for therapeutic decision-making. The most prognostically important pathological factors are the presence of angioinvasion as well as the proliferation index [59, 89]. With respect to the latter, the Ki-67 labeling index emerges as an independent predictor of survival using a cutoff value of 5 % in both functioning and NF-PNETs [96]. Together with G3 histology and the proliferative index Ki-67, with an increasing risk of progression of 2 % for each increasing Ki-67 unit, are the major factors to predict tumor progression [86]. One Italian study evaluating prognostic factors in NF-PNETs including 180 patients supports that the evaluation of nodal metastasis and weight loss should be included along with liver metastasis, Ki-67 index, and grade of differentiation [7]. One of the largest studies in the literature evaluating 2,158 NF-PNETs showed that systemic metastases, local/vascular/lymphatic invasion, and grade are more powerful indicators of outcome with tumor grade influencing survival more than distant metastases [38].

It is well established that radical surgery is the only chance for cure in NF-PNETs, with a 5-year survival rate of 93 % [7]. Nevertheless, the surgical choice is the direct result of the disease stage at diagnosis, a well-established prognostic factor in all tumors.

With respect to predictive factors and response to chemotherapy, this was found to be related to Ki-67 LI >10 %, the performance status, and age <60 years [108]. Additionally, a recent study showed that apart from a high Ki-67 LI >5 %, the lymph node ratio in resected PNETs is a powerful predictor of recurrence and supports its investigation in relation to adjuvant treatment in the context of clinical trials [8].

18.5 Therapies

18.5.1 Surgical Therapy

Surgical resection remains the cornerstone therapy and the only approach that may cure patients with G1 and G2 PNETs. With respect to G3 tumors, surgery is rarely curative but offers a significant long-term palliation [34].

Over the last decade, novel and less invasive pancreatic surgical approaches have been developed and depending on clinical scenarios are applied in patients with PNETs [41]. These involve mainly partial pancreatectomies or tumor enucleation that may also be performed laparoscopically [34]. Generally, typical and atypical pancreatic resections are intended for different sites and sizes of PNETs, and the

decision should be ideally based on previous histological confirmation by endoscopic ultrasound. Tumor enucleation has been proposed in well-demarcated, less than 2 cm, and benign or uncertain-behavior lesions [32]. Centrally located lesions of the pancreatic head require pancreaticoduodenectomy, while lesions of the body and tail may be handled with a left pancreatectomy with or without spleen preservation. Middle pancreatectomy is usually performed for small tumors of the pancreatic body, while enucleation is considered when the main pancreatic duct can be preserved.

The role of surgical resection of the primary PNET in the presence of inoperable metastases is controversial. In 2,158 NF-PNET patients from the SEER database, it was shown that removal of the primary tumor significantly prolonged survival in the entire cohort (median 1.2 vs. 8.4 years; $p < 0.001$) even among those with metastatic disease (median 1.0 vs. 4.8 years; $p < 0.001$) [38]. On the other hand, a systematic review of small studies specifically investigating the role of surgery in this scenario was inconclusive supporting the need of a prospective randomized trial [11]. Furthermore, no survival difference was seen between enucleation and more aggressive resection of the primary tumor (median 10.2 vs. 9.2 years, $p = 0.456$) [38].

Commonly, typical pancreatectomies are associated with a high rate of perioperative complications as well as exocrine and endocrine insufficiency [98]. Atypical resections, on the other hand, are associated with a decreased long-term endocrine/exocrine impairment but with a high incidence of pancreatic fistulas, a disturbing but ultimately a manageable complication [2, 31].

Whichever the type of resection, for a more than 2 cm suspicious lesion, it is imperative to perform a wide excision with clear margins and to remove draining lymph nodes for proper TNM assessment particularly in cases with G2 and G3 PNETs. Controversial is the management of tumors 1–2 cm and those found incidentally as several studies have shown that these may be malignant and have lymph nodal metastases [35, 42, 88]. It is recommended depending on each case either to closely monitor or to remove with draining lymph nodes.

18.5.1.1 Surgical Therapy of Patients with NF-PNETs in the Context of MEN1

MEN1 syndrome represents a difficult challenge for surgeons mainly because it is associated with heterochronous and multifocal NF-PNETs. Prophylactic pancreatectomies could remove all premalignant lesions prior to malignant conversion but are not an accepted approach. Therefore, the careful monitoring of these patients for the early diagnosis and surgical excision of any arising tumors with malignant potential is of utmost importance [55]. Based on previous evidence, the European Neuroendocrine Tumor Society (ENETS) recommends operating MEN1-related NF-PNETs with a diameter of more than 2 cm, with a yearly size increment in diameter of more than 0.5 cm, or with metastases [30, 105]. The management of NF-PNETs of less than 2 cm is debated as they may also have an aggressive biology, but they usually are microadenomas and have a more indolent behavior, and their management is close monitoring.

Overall, the refinement and personalization of surgical procedures in NF-PNETs have resulted in improved long-term survival in patients with locally advanced and metastatic disease, as well as shorter hospital stays and comparable long-term outcomes in patients with limited disease treated minimally invasively. There are still controversies related to issues of surgical treatment of PNETs, such as to what extent enucleation, lymph node sampling and vascular reconstruction are beneficial for the oncologic outcome particularly in MEN1 patients and in tumors 1–2 cm in diameter. It is expected that the recently developed endoscopic ultrasound-guided biopsies will shed light to all these gray zones.

18.5.2 Medical Therapies

Recent advances in therapeutics have introduced novel exciting agents in the treatment of PNETs that are expected to improve the survival of patients. Some interesting combinations including triplets of chemotherapy, radiopeptides, or targeted agents have recently been exploited in this rapidly evolving field (Table 18.2).

Table 18.2 List of mainstream clinical studies in pancreatic neuroendocrine tumors

Drugs	N pts	Ki-67	SSTR+	PR %	PFS[a]/TTP[b] months	Study type	Author
STZ/ ADM/5-FU	84	NR	NR	34	9[a]	Retrospective	Kouvaraki et al. [54]
TMZ	36	NR	NR	14	7[b]	Retrospective	Ekeblad et al. [26]
STZ/ CDDP/5-FU	49	25	39	38	9[b]	Retrospective	Turner et al. [106]
TMZ/Xeloda	30	NR	NR	70	18[a]	Retrospective	Strosberg et al. [101]
TMZ/BEV	15	<20 %	NR	33	14.3[a]	Phase II	Chan et al. [15]
PRRT (Lu)	91	NR	91	43	40[a]	Retrospective	Kwekkeboom et al. [57]
PRRT (Lu)	52	NR	52	29	29[a]	Phase II	Sansovini et al. [95]
Sunitinib	86	36	NR	7	11.4[a]	Phase III	Raymond et al. [91]
Placebo	85	36		0	5.5[a]		
Everolimus	207	NR	NR	5	11[a]	Phase III	Yao et al. [117]
Placebo	203				4.6[a]		

STZ streptozotocin, *ADM* Adriamycin, *TMZ* temozolomide, *XELOX* Xeloda/oxaliplatin, *CDDP* cisplatin, *5-FU* 5-fluorouracil, *BEV* bevacizumab, *PRRT* peptide receptor radionuclide therapy, *Lu* lutetium, *SSTR* somatostatin receptors, *PR* partial response, *TTP* time to progression, *PFS* progression-free survival, *NR* not reported

[a]Progressive free survival (PFS)

[b]Time to disease progression (TPP)

18.5.2.1 Somatostatin Analogs

The clinical application of long-acting somatostatin analogs (SSAs) has changed dramatically the quality of life and prognosis of patients with NETs [79]. Their benefit was initially thought to be related to their antisecretory effect as they were shown to relieve patients from troubling clinical syndromes related to the peptide secretion. Altogether, SSAs have been shown to be well tolerated and safe in all available forms as patients may experience only mild and infrequent side effects such as abdominal pain, steatorrhea, and cholelithiasis [81]. This last effect is due to the inhibitory effects of octreotide on gallbladder contractility, and although it requires follow-up attention, is rarely of clinical significance.

From the advent of the first SSA, an improved survival has been shown in NET patients, and although a clear gain could be seen by the fact that patients' quality of life was improved together with their symptoms, it was not clear whether there was also an antiproliferative effect. Several groups have reported a possible antiproliferative action from SSA, by induction of apoptosis [1, 23, 44], although significant tumor shrinkage has been reported to occur in <5 % of cases [79]. Initial in vitro studies suggested a number of direct and indirect mechanisms of such an effect. Direct mechanisms involve the activation of somatostatin receptors (SSRs) leading to modulation of intracellular signaling transduction such as the activation of MAP kinase pathway [118]. Indirect antiproliferative effects are exerted through activation of SSRs on endothelial cells and monocytes leading to blocking of tumor angiogenesis [20, 49]. A proof of direct antiproliferative effect came from the PROMID trial, a phase III study that randomized patients to receive octreotide long-acting repeatable (LAR) or placebo [94]. This placebo-controlled, double-blind study involved patients with well-differentiated metastatic NETs originating in the distal intestine and proximal colon and showed a clear benefit in all patients receiving octreotide including those with NF tumors. Nevertheless, in this trial, disease stabilization was the main clinical response observed as hardly any tumor shrinkage was achieved and there were no patients with NF-PNETs included in this study. Furthermore, no clear prospective evidence supporting the antitumoral effect of SSAs in NF-PNETs exists. However, based on these encouraging results, octreotide LAR was recommended [84] and approved in several countries in progressing well-differentiated, metastatic nonfunctioning NETs, including PNETs. Supporting are the results of a randomized phase III double-blind trial testing lanreotide versus placebo in NF well-differentiated enteropancreatic NETs (the CLARINET study; clinical trial link: http://clinicaltrials.gov/show/NCT00353496). In a previous study including 21 well-differentiated advanced NF-PNETs, treatment with octreotide LAR was associated with disease stabilization and good quality of life in 38 % of patients [10]. A Ki-67>or =5 % and/or weight loss was associated with more aggressive disease and would justify more aggressive therapy [10].

18.5.2.2 Interferon

Early studies had shown that interferon-alpha (IFN-α) has antiproliferative, through Jak1/Tyk2 kinase and STAT1/STAT2 activation, antisecretory, antiangiogenic, and antiapoptotic effects [22, 76, 109]. A possible positive immunomodulatory effect on

T-cell and natural killer activity was also suggested [39, 77], but it has not been confirmed since by later studies. Interferon-α may be used as monotherapy or in combination with SSAs, as antisecretory and antiproliferative treatment, with variable response rates (RRs). There has been biochemical response in 40–60 % of patients, symptomatic improvement in 40–70 % of patients, and significant tumor shrinkage in a median of 10–15 % of patients [37, 78, 82]. Side effects of IFN-α are significant and include flu-like syndrome, chronic fatigue, depression, weight loss, polyneuropathy, myositis, thrombocytopenia, anemia, leukopenia, and autoantibodies in 15 % of patients (autoimmune hepatitis/thyroiditis, psoriasis, systemic lupus erythematosus syndrome with antinuclear factors, and parietal cell antibodies with pernicious anemia) [78, 84].

One of the first reports to compare results from different studies on octreotide and on IFN-α monotherapy in NETs suggested that better biochemical responses and disease stabilization were obtained with octreotide and not with IFN-α [17]. An initial single-arm study suggested that for patients with metastasized endocrine gastroenteropancreatic tumors, the combination of octreotide and IFN-α is superior to either as monotherapy [75]. Another early study showed that the addition of IFN-α to octreotide has antiproliferative efficacy in a subgroup of patients with advanced metastatic disease unresponsive to octreotide monotherapy [37]. Similarly, in another study, patients having progressed on either octreotide or IFN-α benefited from the subsequent combination of both drugs [36]. There are very few studies comparing SSA monotherapy to IFN/SSA combinations, and the results are conflicting. There are no studies employing IFN monotherapy or in combination with SSA in NF-PNETs. A prospective randomized study showed a significantly reduced risk of tumor progression during follow-up in patients with midgut carcinoid tumors metastatic to the liver receiving octreotide and IFN-α as compared to single-agent octreotide [50]. A randomized phase III study comprised three arms to compare lanreotide, IFN and lanreotide plus IFN [28]. All arms had equivalent results with respect to symptom control. No arm showed statistically significant survival improvement, but there was a trend of better survival in the combination arm. In the same study, the most toxic arm was the combination one. Another recent randomized trial comparing octreotide to octreotide/IFN-α showed no survival advantage between the two arms [4]. It is hypothesized that the difference in the results lies on the heterogeneity of the disease and further larger trials are required. At present, we do not have enough statistical evidence for an upfront use of the combination of IFN-α and SSA in patients with NETs, but we have some clinical evidence coming from non-randomized studies and the sub-analysis of randomized trials that would justify the sequential use of the two drugs or the combination after progression to single-agent therapy [33].

Head to head comparisons of octreotide/IFN-α to other biological combinations have not been performed yet. A randomized phase III trial is currently recruiting patients in order to compare the efficacy of octreotide/IFN-α2b and octreotide/bevacizumab in metastatic or locally advanced, high-risk NETs (clinical trial link: http://clinicaltrials.gov/show/NCT00569127). This trial was based on a previous phase II study showing better PFS and objective responses of octreotide/bevacizumab compared to octreotide/PEG-IFN [116].

18.5.2.3 Targeted Therapies

The more thorough understanding of signaling pathways implicated in tumor cell proliferation and angiogenesis underlining the pathogenesis of NETs has prompted the application of small molecules targeting these pathways such as everolimus and sunitinib.

Everolimus

Based on preliminary observations, a phase II clinical trial conducted by J. Yao at the MD Anderson Cancer Center studied everolimus, an oral inhibitor of mTOR, and octreotide LAR in advanced low-grade neuroendocrine carcinomas [115]. A total of 60 evaluable patients were treated in two cohorts; patients received either everolimus at 5 or 10 mg/day. Out of the 60 treated patients, 30 had carcinoid and 30 islet cell tumors. Sixty-five percent of the patients were in progression at the time of study entry. Overall, 12 (20 %) patients were reported to have partial response (PR; 4 carcinoids and 8 islet cell tumors, respectively), 43 (72 %) had stable disease (SD; 25 in carcinoids and 18 in islet cell, respectively), and 5 (8 %) had progressive disease (PD; 1 in carcinoids and 4 in islet cell, respectively). Overall median progression-free survival (PFS) was 59 weeks (64 weeks in carcinoids and 50 in islet cell tumors, respectively). Median OS was not reached, with a 2-year survival rate of 78 %. The combination of everolimus and octreotide LAR 30 mg appears to have been well tolerated. The most common toxicity reported was mucositis. Grade 3/4 toxicities observed included anemia, thrombocytopenia, aphthous ulcer, diarrhea, edema, fatigue, hypoglycemia, nausea, pain, and rash.

Another phase II open-label, parallel group study was conducted in patients with advanced PNETs. Patients received either everolimus 10 mg daily as monotherapy (stratum 1; $n=115$) or everolimus 10 mg daily in combination with ≤30 mg octreotide LAR q 28 days (stratum 2; $n=45$), based on prior octreotide LAR treatment [114]. In stratum 1, there were 11 patients with PRs (9.6 %), 78 patients with SD (67.8 %), and 16 patients with PD (13.9 %); median progression-free survival was 9.7 months. In stratum 2, there were two patients with PRs (4.4 %), 36 patients (80 %) with SD, and no patient with PD; median PFS was 16.7 months.

Following a recently completed phase III trial, the RADIANT-3 [117], that showed activity in a wide range of NETs, everolimus was granted approval by the FDA and EMEA authorities for patients with progressing advanced well-/moderately differentiated PNETs. The RADIANT-3 involved 410 patients with advanced well-/moderately differentiated, radiologically progressing PNETs receiving 10 mg everolimus daily continuously or placebo. The results of this study show prolongation of PFS from 4.6 months in the placebo arm to 11.4 months in the everolimus arm. Grade 1–2 toxicity including stomatitis or aphthous ulceration (64 %), diarrhea (34 %), fatigue (31 %), and infections (23 %) primarily of the upper respiratory tract was mainly observed [117]. Other adverse events include noninfectious pneumonitis and laboratory abnormalities such as anemia, neutropenia, thrombocytopenia, hypercholesterolemia, hypertriglyceridemia, and hyperglycemia [117]. A phase II study investigating the role of another mTOR inhibitor, temsirolimus, was negative

for activity in advanced NETs, while the toxic effects were fatigue (78 %), hyperglycemia (69 %), and rash/desquamation (64 %) [24]. A recently published study investigating safety and tolerability of pasireotide LAR in combination with everolimus in patients with advanced NETs showed promising antitumor activity and is further being investigated [14].

Sunitinib

Sunitinib is an oral multi-tyrosine kinases inhibitor that has demonstrated both antiangiogenic and antiproliferative activity by blocking a range of signaling pathways and receptors such as VEGFR-1 to VEGFR-3, stem cell factor receptor, platelet-derived growth factor receptor (PDGFR)-α and PDGFR-β, RET, colony-stimulating factor-1 receptor, and fetal liver tyrosine kinase receptor 3 (FLT-3) [29]. It was originally approved for the treatment of advanced renal cell carcinoma (RCC) and gastrointestinal stromal tumor (GIST) refractory to imatinib treatment. Sunitinib was recently granted authorization for the treatment of advanced well-differentiated progressing PNETs. The exact mechanism underlining the antiproliferative effect of sunitinib is unknown. However, in a NET mouse model, sunitinib has shown an antiangiogenic effect by inhibiting pericytes that support the endothelial cell function [85]. This is most probably achieved by the targeting of PDGFR-β, expressed on pericytes, that drives recruitment and maturation of vessels [6]. The efficacy of sunitinib has been demonstrated in two major clinical studies. The first was a phase II study involving 109 patients with advanced NETs where sunitinib was administered at 50 mg daily for four to six weeks [56]. Of 61 patients with PNETs, 16.7 % had an objective RR, and 68 % a prolonged period of SD. The median time to tumor progression was 7.7 months.

The second was a multinational, randomized, double-blind, placebo-controlled phase III trial comparing sunitinib and best supportive care with placebo and best supportive care. This study recruited patients with advanced well-differentiated PNETs, and sunitinib was delivered at 37.5 mg continuous daily dose [91]. The study was discontinued early as the monitoring committee observed more deaths in the placebo group and a statistically significant difference in progression-free survival (PFS) favoring sunitinib by 6 months [91]. The most frequent adverse events in the sunitinib group were diarrhea (59 %), nausea (44.6 %), vomiting (33.7 %), asthenia (33.7 %), and fatigue (32.5 %). As it results from previous studies, the proactive assessment of other sunitinib-related side effects, such as anorexia, oral changes, hand–foot syndrome and other skin toxicities, thyroid dysfunction, myelotoxicity, hypertension, cardiotoxicity, and thromboembolic events, is critical to ensure optimal treatment benefit by allowing appropriate drug dosing and prolonged treatment periods [51, 107]. Additionally, the objective RR observed in the phase III study was low as it regarded only 9.3 % in the sunitinib group. It is important to identify those patients who may benefit by sunitinib therapy. To this end, a study entitled "Predictive Biomarkers of Response to Sunitinib in the Treatment of Poorly Differentiated NEURO-Endocrine Tumors (NET)" is currently underway in France (clinical trial link: http://clinicaltrials.gov/show/NCT01215578).

18.5.2.4 Chemotherapy

Over the last three decades, the applied cytotoxic agents for NETs included DNA-damaging agents such as the antimetabolite 5-fluorouracil (5-FU); the alkylating agents streptozotocin (STZ), dacarbazine (DTIC), and temozolomide (TMZ); the topoisomerase inhibitors doxorubicin (Dox) and etoposide; and the DNA cross-linker platinum derivatives, such as cisplatin (CDDP), carboplatin (CBDCA), and oxaliplatin. Single-agent chemotherapy has not shown any real benefit [69], and the newer chemotherapy regimens based on the combination of these active drugs show low RRs, which set the need to improve the results of the medical treatment of these malignancies [108].

Chemotherapy has heterogeneous results in NET patients mainly due to the large variability of biological features among the disease subgroups. For example, chemotherapy in functioning advanced NETs has limited efficacy with a RR of 15 % [102], whereas PNETs have a RR of 30–40 % although with significant toxicity [26, 67]. One of the earliest observations regarding these tumors was that differentiation grade represents one of the most important predictive markers of tumor response to chemotherapy. Patients with metastatic disease of well- and moderately differentiated NF-PNETs are expected to respond better to STZ-based and targeted therapy, whereas those of high-grade NF-PNETs with Ki-67 LI above 20 % are likely to benefit from platinum-based treatment. More specifically, an early report from the Mayo Clinic suggested that anaplastic NETs independently of the primary site location are responsive to combination chemotherapy including etoposide and cisplatin [70]. Conversely, well-differentiated tumors were unresponsive to this regimen [70]. Overall, the population with anaplastic disease had an overall RR of 67 %, a median time to progression (TTP) of 8 months and a median survival of 19 months. Although toxicity of this regimen was severe with vomiting, leukopenia, thrombocytopenia, anemia, alopecia, and neuropathy, the efficacy of this regimen remains unsurpassed by any other treatment until today as there is no unequivocal evidence of survival improvement. Similarly to the grade of differentiation, recent evidence suggests that a careful evaluation of the mitotic index and the Ki-67 assessment can provide further information on the overall prognosis and the degree of response to systemic cytotoxic treatment. For instance, highly proliferative (Ki-67 >30 %) tumors with aggressive behavior such as the PNETs are more sensitive to cytotoxic therapy with cisplatin/etoposide [5]. On the other hand, tumors originating from the small intestine have most commonly a low mitotic index and demonstrate poor response to cytotoxic chemotherapy [5].

One of the first tested combinations was STZ/5-FU in the 1970s and remained a standard treatment until now [68]. There have been very few phase III trials in NETs with conflicting results mainly due to the heterogeneity of the population taken in consideration. The first comparing 5-FU/STZ to Dox showed no statistically significant difference between the two arms [27], and the second comparing 5-FU/Dox to 5-FU/STZ showed the latter being statistically significant better [102]. For PNETs, there have been two phase III trials, one comparing STZ to STZ/5-FU that found no difference between the two arms [69] and a second one comparing chlorozotocin, STZ/5-FU, and STZ/Dox showing a statistically significant benefit in the last arm

[71]. During the past decade, there have been few chemotherapy studies in advanced well- and moderately differentiated NETs with encouraging results: (a) with STZ and Dox [21]; (b) with STZ, 5-FU, and Dox [54]; (c) with 5-FU, epirubicin, and dacarbazine [110]; and (d) with STZ, 5-FU, and cisplatin [106]. For the poorly differentiated NETs, the most effective combination chemotherapy remains cisplatin with etoposide [45]. Interestingly, it has been recently reported that gastroenteropancreatic carcinomas with Ki-67 <55 % had a lower RR to platinum-based chemotherapy while having a better prognosis compared with those having Ki-67 >55 % [100]. As a result, it is expected that patients with Ki-67 <55 % may respond to different chemotherapy schedules such as temozolomide, but this needs to be further investigated in the context of a clinical trial [53].

The very high response rates reported in the first studies with STZ-based chemotherapy were criticized since they derived in part from the historical use of nonstandard response criteria. Furthermore, STZ-related toxicity and not manageable schedule limited its use and prompted to newer and better manageable agents like TMZ.

An interesting novel combination chemotherapy is an oral regimen with capecitabine and TMZ that has been shown to exert high activity and low toxicity both in well-differentiated [101] and poorly differentiated neuroendocrine tumors [112]. However, a larger study comparing this regimen with STZ-based therapy or TMZ monotherapy is missing.

Based on the findings of a study assessing retrospectively the triplet capecitabine, STZ, and CDDP, the National Cancer Research Network approved NET01, a randomized phase II study comparing two chemotherapy regimens, capecitabine/STZ and capecitabine/STZ/cisplatin, in unresectable metastatic NETs of the foregut and pancreas and NETs of unknown primary site [106].

Another interesting approach with promising efficacy is the combination of targeted agents with chemotherapy. One such doublet is TMZ and the antiangiogenic agent bevacizumab that has been explored in two different TMZ schedules, a standard [15] and a low continuous dose [52]. A second doublet is TMZ with everolimus that has been tested in 40 patients with PNETs and is shown to achieve a PR in 40 % and a PFS of 15.4 months [13]. However, further studies evaluating the efficacy of these combination therapies compared to TMZ alone are warranted.

18.5.3 Peptide Receptor Radionuclide Therapy

Peptide receptor radionuclide therapy (PRRT) is a targeted radiation therapy involving an intravenously administered radiolabeled somatostatin analog designed to bind on type 2 SSTR overexpressed on PNET cells and deliver a direct and a bystander lethal effect.

Accumulating clinical experience indicates that this therapy achieves significant clinical responses with survival benefit and improved quality of life with a usually manageable toxicity in patients with G1–G2 NETs overexpressing SSTRs [58]. A committee of international experts under the auspices of the International Atomic

Energy Agency, in cooperation with the European Association of Nuclear Medicine and the American Society of Nuclear Medicine and Molecular Imaging, recently published the PRRT eligibility requirements such as disease inoperability and high SSTR expression determined by functional studies such as ^{111}In-pentetreotide (OctreoScan) or SPECT/PET with ^{68}Ga-labeled SSA [119].

The most commonly applied radiolabeled somatostatin analogs are the [^{90}Y-DOTA^{0}, Tyr^{3}] octreotide (^{90}Y-DOTATOC) and the [^{177}Lu-DOTA^{0}, Tyr^{3}] octreotate (^{177}Lu-DOTATATE), both emitting beta radiation with tissue penetration of 12 and 2 mm, respectively. Randomized studies of PRRT in NF-PNETs are lacking, but comparisons among different single-arm studies have shown that the median response duration for Yttrium- and Lutetium-labeled compounds is 30 and 40 months that compare favorably with other treatments for advanced disease [58].

In one of the largest retrospective PRRT studies including 504 patients of whom 310 GEP NET were treated with ^{177}Lu-DOTATE, median overall survival from treatment initiation was 46 months [57]. In the same cohort, 72 were NF-PNETs, of whom 4 achieved complete response (6 %), 26 PR (36 %), 13 minor response (18 %), and 19 SD (26 %). Of the four patients who originally had inoperable locally advanced disease and achieved complete response, three were successfully operated 6–12 months following treatment completion, suggesting a possible role of PRRT also in the neoadjuvant setting [57].

In a more recent study evaluating ^{177}Lu-DOTATE in 52 G1–G2 PNET patients of whom 46 were NF-PNETs, a statistically significant prolongation of survival was observed in patients receiving a total dose of 27.8 GBq as compared to those receiving 18.5 GBq due to kidney or bone marrow low reserve [95]. In the same study, the disease control rate was reported to be 81 % including a CR of 12 %, a PR of 27 %, and a SD of 46 % [95]. The same group published another study suggesting that FDG PET evaluation prior to PRRT is useful for predicting response to ^{177}Lu-DOTATE in patients with G1–G2 advanced PNETs [97].

18.5.4 Cytoreductive Therapies

Cytoreductive treatments include surgery, radiofrequency ablation, laser-induced thermotherapy or selective hepatic transcatheter arterial embolization, chemoembolization, and selective internal radiotherapy or sferes. There are no prospective comparative studies investigating the efficacy of the cytoreductive approaches, but several retrospective studies have validated their individual role in G1 and G2 NETs. The choice of any of these options depends on patient and tumor characteristics such as performance status (PS), comorbidities, number and size of metastases, as well as the availability of the specific approach. According to the most recent ESMO guidelines, general rules support the use of debulking surgery if >70 % of tumor volume can be excised as it can improve symptoms and be combined with systemic therapy but is mostly reserved for good PS patients with resectable liver metastases with or without locally advanced disease [83]. Radiofrequency ablation is reserved for a small number (and small size) of metastatic nodules, whereas arterial (chemo-)

embolization and sferes are reserved for patients with widespread metastatic disease in the liver. Herein is described the experience gained in NF-PNETs.

18.5.4.1 Surgery

There are no prospective trials investigating the role of surgical resection in patients with advanced or metastatic NF-PNETs, and information mainly comes from retrospective analysis of patients treated individually and not according to a specific protocol. One of the first studies from MD Anderson in 163 NF-PNETs indicated that patients with advanced disease who underwent a potentially curative resection had an improved survival [99]. A large retrospective SEER study including 2,158 NF-PNET patients indicated that those treated with resection of either the primary tumor or distant metastatic site had increased survival compared to those who were not operated [38]. A more recent retrospective study from the Mayo Clinic including 72 NF-PNETs patients with liver metastases indicated that surgical debulking of more than 90 % of tumor load leads to a similar patient prognosis when compared to completely resected patients [19]. In the same study, patients achieved a median overall survival of 55.2 months and a 5-year overall survival of 59.9 % that compare very favorably with historical survival data of patients with untreated liver metastasis (median survival 27 months; 5-year survival 29 %) [19].

18.5.4.2 Embolization and Chemoembolization

Hepatic arterial embolization (HAE) and chemoembolization (HACE) are two alternative therapeutic options for liver metastases of G1 and G2 tumors, not amenable to surgical resection, that have shown efficacy but have never been compared in NF-PNETs. HAE and HACE are delivered through percutaneous incanulation of the femoral artery, and when into the relevant hepatic vascular territory, an agent such as polyvinyl alcohol in HAE or a chemotherapeutic agent with lipiodol in HACE are injected in the engaged vessel to provoke stasis and ischemia in the downstream tissues. Regarding these procedures, several questions remain unanswered such as whether combining chemotherapy and which drug (5-FU, Dox, and mitomycin C) adds to the overall benefit of embolization and which are the appropriate dosage intervals and the timing of the procedure evaluation [30]. Both procedures are safe but are contraindicated in patients with portal vein thrombosis, borderline liver function, and previous Whipple procedure [83]. The most common side effect is the so-called postembolization syndrome that includes abdominal pain, fever, nausea, vomiting, and elevated liver enzymes that usually respond to symptomatic treatment and lasts for 3 days [61]. More severe side effects such as pancreatitis, liver and renal failure, sepsis, and myocardial infarction may be responsible for increased 30-day mortality rate and support the application of these methods only in high-volume centers [61].

A recent small prospective study compared 12 midgut NET patients receiving HACE to 14 patients receiving HAC and found equal tumor control in 95 % of cases and similar 2-year PFS [64].

From the published literature, it is noted that both procedures result in 50–90 % tumor marker responses and 30–50 % in imaging response; this smaller response in

imaging is due to the difficulty in assessing necrosis caused by embolization by RECIST [83].

18.5.4.3 Radiofrequency Ablation and Radioembolization

Radiofrequency ablation (RFA) is a procedure that can be applied during standard surgery, laparoscopically or percutaneously through an image-guided procedure, with probes that deliver a lethal hit by extreme heat directly in the lesion of interest. There are no prospective studies assessing RFA in a patient population diagnosed selectively with NF-PNETs.

In one of the largest prospective trials including 54 patients with liver metastases of NET, RFA performed laparoscopically with ultrasound guidance reported a median overall survival of 11.0 years, a median post-diagnosis of hepatic metastases survival time of 5.5 years, and a median of 3.9 years post-first RFA [65].

Radioembolization employs either biodegradable resin-based (SIR-Spheres; SIRteX Medical Limited, New South Wales, Australia) or nonbiodegradable glass-based (TheraSphere; MDS Nordion, Ottawa, ON, Canada) microspheres labeled with Yttrium-90 (Y-90). Most studies in NETs evaluate resin microsphere radioembolization, and there have been none analyzing only NF-PNETs. One of the largest studies with 184 patients treated with ^{90}Y resin microspheres in 10 centers reported a very low toxicity profile, mainly fatigue (6.5 %), a high disease response (CR in 2.7 %, PR in 60.5 %, SD in 22.7 %), and a median survival of 70 months [48].

Radioembolization with Y-labeled spheres is still considered investigational although several small studies in NET patients with liver metastases show high RRs [83]. Nevertheless, a randomized trial comparing HAE and ^{90}Yttrium-labeled microspheres is urgently needed.

18.6 Follow-Up

The role of surveillance after resection for PNETs is to evaluate the results of treatment and to identify early relapse for curative treatment. According to the recent ENETS guidelines [30], a follow-up program could be omitted in patients with localized G1 NF-PNET treated by radical pancreatectomy. For surgically removed G2 NF-PNETs, there are no studies evaluating the frequency of surveillance, but the recommendation is to follow up for disease recurrence [30, 83].

In R1 resected G1 and G2 NF-PNETs, it is recommended that follow-up is performed every 3–6 months [83].

Patients with G3 NF-PNETs are at high risk for early relapse even if they had a R0 surgery, and for advanced G1, G2, and G3 NF-PNETs, frequent follow-up every 2–3 months is required [30, 83].

Depending on each patient, the follow-up program includes clinical examination and routine investigations with full blood count, biochemistry, biomarkers (CgA or NSE), and imaging (ultrasound, CT, or MRI) [1, 3, 73]. If chromogranin A is not

elevated, NSE may be found abnormal and used as an alternative biomarker [83]. Other imaging procedures including endoscopy, endoscopic ultrasound, octreotide scintigraphy, and PET imaging with different tracer-bound compounds such as 68Ga-DOTATOC/-NOC/-TATE or ^{18}FDG [3, 83] are recommended every 18–24 months [83].

In the case of a patient with rapid tumor progression, it may become necessary to perform new CT- or ultrasound-guided biopsies of the metastases in order to evaluate whether the proliferative activity has changed [83].

18.7 Clinical Trials

Eagerly awaited are the results of the following clinical trials that are in progress or recently concluded:

1. A recently concluded phase II study by the H. Lee Moffitt Cancer Center and Research Institute, USA (NCT00609765): Avastin, fluorouracil, doxorubicin, and streptozocin in locally advanced and metastatic pancreatic endocrine tumors
2. A phase III multinational study sponsored by Ipsen (NCT00353496): Study of lanreotide autogel in nonfunctioning enteropancreatic endocrine tumors (CLARINET)
3. A phase III multinational study by Ipsen (NCT00842348): Study of lanreotide autogel 120 mg in patients with nonfunctioning enteropancreatic endocrine tumor (NET729)
4. A phase II study by Novartis (NCT01658436): BEZ235 phase II trial in patients with advanced pancreatic neuroendocrine tumors (pNET) after failure of mTOR inhibitor therapy
5. A phase II multinational study by Novartis (NCT01628913): Efficacy and safety of BEZ235 compared to everolimus in patients with advanced pancreatic neuroendocrine tumors (MACS 1938)
6. A phase II study from the University of Peking, China (NCT01480986; IPONEC Trial): Study on the efficacy and safety using sequential IP (irinotecan and cisplatin) therapy and octreotide LAR in the treatment of advanced GI NEC
7. A biomarker study from Assistance Publique Hôpitaux de Paris, France (NCT01215578): Predictive biomarkers of response to sunitinib in the treatment of poorly differentiated neuroendocrine tumors (NET)
8. A CALGB phase II study (NCT01229943): Everolimus and octreotide with or without bevacizumab in treating patients with locally advanced or metastatic pancreatic neuroendocrine tumors that cannot be removed by surgery
9. A phase II study from Hellenic Cooperative Oncology Group (HeCOG), Greece (NCT01648465): Study of everolimus treatment in newly diagnosed patients with advanced gastrointestinal neuroendocrine tumors
10. 68-Ga-labeled octreotide analogs PET in duodenal-pancreatic neuroendocrine tumors

11. A phase II study from Stanford University (NCT01525082): Capecitabine, temozolomide, and bevacizumab for metastatic or unresectable pancreatic neuroendocrine tumors
12. A phase I/II study from Northwestern University, USA (NCT01465659): Temozolomide and pazopanib hydrochloride in treating patients with advanced pancreatic neuroendocrine tumors that cannot be removed by surgery

Conclusions

Pancreatic neuroendocrine tumors are relatively rare and heterogeneous malignancies, with an increasing incidence. After around three decades from the approval of STZ, two biological agents, everolimus and sunitinib, showed a clinical and statistical benefit in terms of PFS, and they have been approved for patients with progressing advanced well-/moderately differentiated PNETs. However, the prognosis of poorly differentiated PNETs remains dismal even when treated with chemotherapy. A vital challenge remains the recognition of key molecular pathways for the development of targeted therapies that could improve the prognosis of these patients

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Gastroenteropancreatic Neuroendocrine Carcinoma

19

Halfdan Sorbye

19.1 Background

Extrapulmonary neuroendocrine carcinoma (EP-NEC) has been found in most organs, but the most common sites are the gastrointestinal tract, cervix uteri, urogenital tract and breast [1, 2]. No primary site can be identified in up to 30 % of cases [3]. In the prior 2000 WHO classification, these tumours were known as poorly differentiated endocrine carcinoma (PDEC) [4]. In the 2010 WHO classification, the nomenclature of grade 3 (G3) neuroendocrine tumours (NET) has been altered to neuroendocrine carcinoma (NEC) [5]. NEC has a much higher proliferation rate than well-differentiated neuroendocrine tumours (G1–G2); by definition the proliferation rate (Ki-67) is above 20 %. In devising treatment strategies for EP-NEC, many authors refer to the extensive literature surrounding high-grade NEC of the lung [2, 6]. Several series have however questioned the rationale for this and pointed out many differences between pulmonary small-cell carcinoma and extrapulmonary small-cell neuroendocrine carcinoma (EP-SCNEC) [6–9]. EP-SCNEC has less smoking history and a much lower frequency of brain metastases; a better survival and survival is site specific, i.e. gynaecologic and head and neck cancers have better outcomes than gastrointestinal (GI) primaries. Furthermore, there are molecular differences, e.g. Bcl-2 overexpression is less common in EP-SCNEC than in lung. Studies reporting results from mainly pulmonary NEC or EP-NEC outside the GI tract will therefore not be covered in this chapter. This chapter will focus on gastrointestinal NEC (GI-NEC).

Clinical data on GI-NEC is sparse. Many cases have been misdiagnosed as poorly differentiated adenocarcinoma. When diagnosed, data are often presented together with results from well-differentiated NETs or together with other

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extrapulmonary NEC cases. There has been a lack of epidemiological, clinical and treatment data for GI-NEC patients, and little has been known on possible prognostic and predictive factors. Recently however, several studies with novel treatment data on GI-NEC patients have been published [10–12].

19.2 Epidemiology

Gastrointestinal (GI) primary tumours account for 35–55 % of all NEC outside the lung [13, 14]. They can originate anywhere in the GI tract, but are mainly located in the oesophagus, pancreas and large bowel [10, 15]. A primary tumour of uncertain origin will usually be considered GI if the metastatic load is dominating in the liver and abdomen [16]. Poorly differentiated NETs account for about 20 % of malignant digestive NETs [17]. In the SEER database, colorectal NEC has an incidence of 2/1,000,000 inhabitants, and distant disease at diagnosis was present in 62 % of patients [18]. Data from SEER on 4,054 patients with poorly differentiated NET showed a median survival of 34 months in patients with localised disease, 14 months with regional disease and 5 months with distant disease [19]. Amongst 94 GI-NEC patients in the national cancer registry of Spain, 59 % presented as distant metastatic disease, median survival was 1.2 months and 44 % were alive at 5 year [20]. Colorectal small-cell carcinoma (SCC) patients have a median survival of 10 months with 1-, 2- and 3-year survival rates of 46, 26 and 13 % [21]. Advanced gallbladder small-cell carcinoma treated with combination chemotherapy has a median survival of 8 months, with 1- and 2-year survival rates of 28 and 0 % [22]. In the EUROCARE study, 5-year survival rates for poorly differentiated NEC varied between 6 and 11 % according to European region [17]. The number of patients with NEC seems to be increasing [10], probably due to better pathology workup. There are primary site-specific differences in survival. Patients with a primary pancreatic tumour have more often somatostatin receptor scintigraphy positive tumours, more often a Ki-67 <55 % and a longer survival than other GI primaries and may constitute a specific subgroup within GI-NEC with a better prognosis [10]. Median survival for advanced pancreatic NEC is 15–21 months, compared to 11 months concerning all GI-NEC [1, 10].

19.3 Diagnosis

19.3.1 Histology

A gastrointestinal NEC has been defined as a poorly differentiated, high-grade malign cancer composed of small cells or large to intermediate cells. These tumours may contain different tumour components; often a mixture of neuroendocrine and adenocarcinoma parts can be seen. By definition the neuroendocrine component has to exceed 30 % to be classified as an NEC [5]. Up to 40 % of NEC contain elements of non-NEC, usually adenocarcinoma [23]. If the tumour also consists a gland

forming epithelium exceeding 30 %, it is classified as MANEC (mixed adenoneuroendocrine carcinoma) [5]. Reclassification of 39 grade 3 colon NEC classified 1/3 of them as MANEC, but no differences in clinical features, (features, Ki-67 etc) Ki-67 level or survival was seen between NEC and MANEC [24]. GI-NEC contains marked nuclear atypia, multifocal necrosis and a high mitotic rate defined as >20 mitotic figures per 10 high-power fields or a Ki-67 >20 % (Fig. 19.1). Most NECs exhibit substantially more mitoses than these thresholds, typically in the range of 40–70 mitoses per high-power fields, and with a Ki-67 between 50 and 100 %. The Ki-67 index seems to be associated with the location of the primary tumour. In the

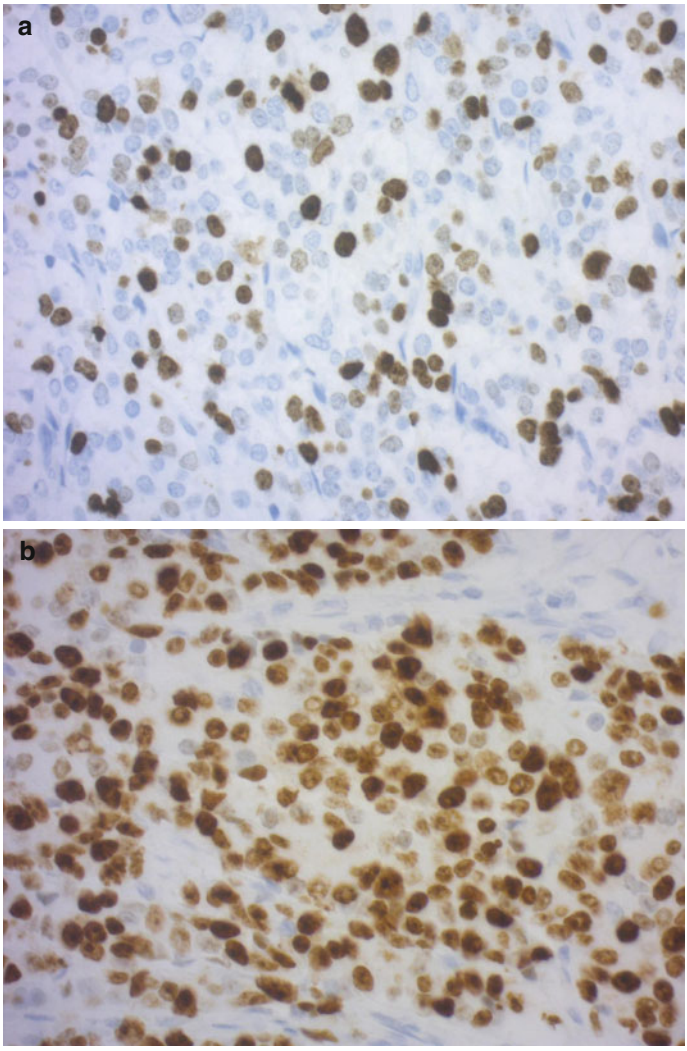

Fig. 19.1 (**a**) Colon NEC with a Ki-67 index of 30 %. (**b**) Colon NEC with a Ki-67 index of 90 %

NORDIC NEC study, Ki-67 was usually >55 % in primary tumours from the oesophagus, colon and rectum, whereas in primary tumours from the pancreas, only 30 % had a Ki-67 >55 % [10]. Patients with a Ki-67 range of 20–55 % had a better survival, but were less responsive to chemotherapy than patients with a Ki-67 >55 %. This may have consequences for future classification and treatment of NEC.

NEC encompasses mainly two histological entities: small-cell neuroendocrine carcinoma (SCNEC) resembling small-cell carcinoma of the lung and a large-cell pleomorphic carcinoma (LCNEC) [5] (Fig. 19.2). LCNEC is morphologically distinguished from SCNEC by cytological features of a non-small-cell carcinoma,

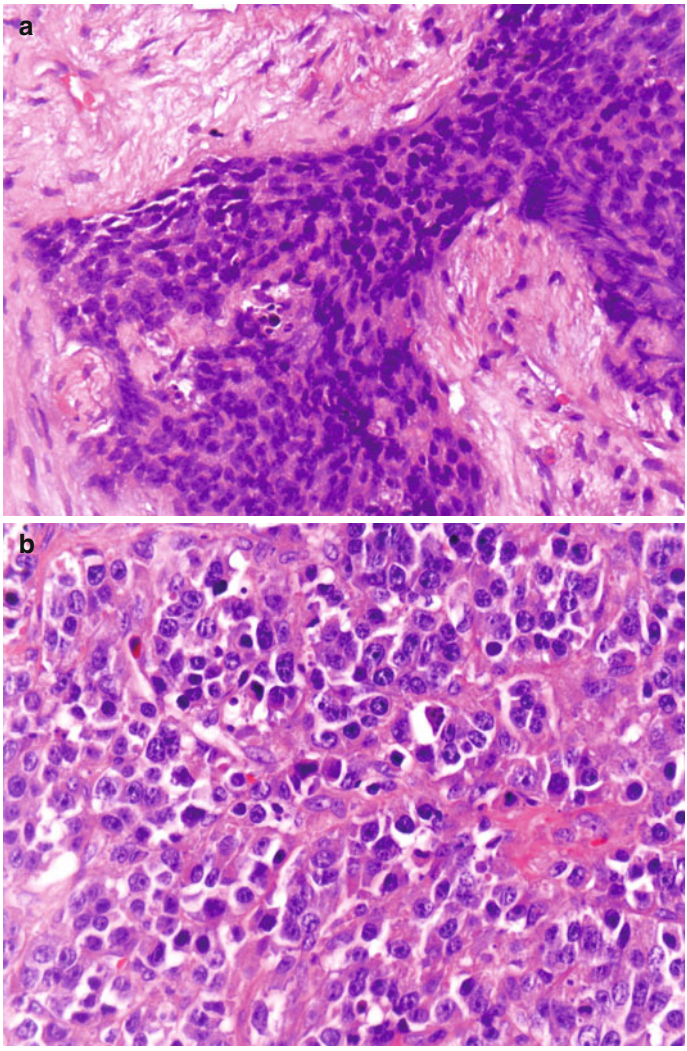

Fig. 19.2 (**a**) Small-cell NEC from pancreas. (**b**) Large-cell NEC from colon

including large cell size, low nuclear-to-cytoplasm volume ratio, coarse chromatin and frequent nucleoli. There is small-cell histological preponderance in the squamous GI tract (oesophagus and anus) and large-cell carcinomas in the glandular parts [23]. Awareness of the latter is essential, as these tumours often are indistinguishable from poorly differentiated non-NET carcinomas. In a study on 87 high-grade GI tract tumours, no significant difference in survival was detected amongst small cell, mixed and large cell subtypes, nor were there any differences between tumours of the upper GI tact versus the lower GI tract [23]. In pancreatic NEC, small-cell NEC was genetically similar to large-cell NEC, and these genetic changes were distinct from pancreatic neuroendocrine tumours pNET [25]. There seems to be no significant differences in survival or response to chemotherapy based on histological subtypes [10, 21, 23]. The terms poorly differentiated high-grade NET and NEC have been used synonymously. However, recently data suggest that amongst large-cell NEC, there are tumours that are well differentiated [26, 27]. Survival was longer in well-differentiated NEC than in poorly differentiated NEC (40 months vs. 17 months) [26]. The presence of well-differentiated tumours in NEC needs to be validated in larger series, as it may have consequences for future classification and treatment.

NEC is characterised by immunohistochemical markers of neuroendocrine differentiation with synaptophysin, neuron-specific enolase, chromogranin A (CgA) and CD56 being the primary stains. Several pathologists advocate for routine staining with synaptophysin and CgA for all tumours initially classified as poorly differentiated gastrointestinal adenocarcinoma. Usually synaptophysin immunohistochemistry staining is positive, whereas staining for CgA may be negative. A strong positive staining for CgA was found in 45 % of patients with advanced GI-NEC, but the intensity of CgA staining was not correlated to survival or location of the primary tumour [10]. It is debated if a positive staining for synaptophysin or CgA is necessary to classify the tumour as an NEC. The presence of CgA indicates a more mature tumour, and the presence of both markers may be a good prognostic sign [11, 28]. In a study of 39 colorectal NEC patients, CD177 immunoreactivity (a stem cell marker) was found in 54 % of the tumours and was together with vascular invasion an independent prognostic marker for survival [24]. Achaete-scute homolog 1 has been proposed as a diagnostic marker of poor differentiation and may help to differentiate NEC from NETs in difficult cases [29].

19.3.2 Diagnostic Procedures

Diagnostic workup should include a CT scan of the abdomen and thorax. Location of metastases at the time of diagnosis of advanced disease in 305 NEC patients was in the liver (68 %), lymph nodes (62 %), lung (15 %), bone (15 %), brain (4 %) and other locations (27 %) [10]. PET is usually positive in NEC and could be of prognostic value [30]. PET/CT may be especially indicated for patients with apparently localised disease, to locate possible metastatic disease and avoid unnecessary surgery. CgA measurement has been assumed to be normal in GI-NEC patients;

however, CgA was recently shown to be elevated in two third of 188 patients with advanced GI-NEC disease [10]. CgA could therefore be a useful tumour marker for NEC patients. Neuron-specific enolase has only been tested in patients with mainly pulmonary NEC [31].

The relevance of somatostatin receptor scintigraphy (SRS) in NEC patients is uncertain. In 14 patients with a Ki-67 >15 %, 69 % had a positive SRS [32]. In another study a high uptake on SRS was found in 69 of 182 patients (37 %), but SRS was less frequently performed in patients with a high Ki-67 [10]. A high uptake on SRS was more often found in primary pancreatic tumours (46 %) compared to primary tumours from other locations (0–25 %). In two small second line studies, 20–40 % of NEC patients had a high uptake on SRS [11, 12]. An SRS may be clinically relevant if the Ki-67 is below 40 %, as peptide receptor radionucleotide therapy (PRRT) then may be a treatment option [33–35]. Routine measurement of U-5HIAA does not seem meaningful, as hormone-related symptoms are rare and urinary 5-HIAA elevated in few patients [10].

Several baseline laboratory abnormalities are correlated with poor survival in NEC: low haemoglobin level and elevated levels of platelets, white blood cell count, lactate dehydrogenase (LDH), alkaline phosphatase and C-reactive protein (CRP), with LDH and platelet count as the strongest factors [10]. It is increasingly recognised that variations in outcome are not solely determined by the characteristics of the tumour, but also by host response factors. Markers of a systemic host inflammatory response, e.g. elevated platelets and leucocytes, have a prognostic value in a variety of common solid tumours [36]. LDH may be a biomarker for hypoxia and highly angiogenetic tumours, and high LDH-5 is directly related to an up-regulated Hypoxia-induced Factor (HIF) pathway and linked with an aggressive tumour phenotype [37].

19.4 Treatment

Treatment of this very aggressive disease remains a challenge for clinicians. NEC is characterised by a high proclivity for metastatic dissemination even in patients with clinically localised tumours. Extensive disease is almost invariably treated by systemic chemotherapy. In contrast, the treatment approach for limited disease, a potentially curable condition, is presently neither consistent nor uniform. Retrospective studies have shown that surgery alone is rarely curative [7]. Based on the treatment paradigm for limited-stage small-cell lung cancer, chemotherapy and local therapy (surgery or radiation) can be considered in many patients with locoregional EP-NEC. Adjuvant or neoadjuvant chemotherapy is usually given with cisplatin/carboplatin and etoposide, based on the effect seen in metastatic setting.

19.4.1 Surgery and Neoadjuvant (Perioperative) Treatment

Despite rare reports of long-term survivors, surgery alone is usually inadequate therapy even for apparently localised disease. Adjuvant radiotherapy and chemotherapy are widely recommended, although the effectiveness of a combined

modality approach has not been firmly established. Casas and colleagues highlighted the role of systemic chemotherapy and local treatment in oesophageal SCC. In a retrospective series of 199 patients, improved survival from 5 to 20 months was reported in patients with localised disease who received multimodal therapy, i.e. surgery, radiation therapy and systemic treatment [38]. Local therapy only (e.g. surgery alone) was the single most powerful predictor of poor prognosis, whereas chemotherapy was strongly associated with improved survival. Median survival was 20 months after induction chemotherapy followed by consolidative chemoradiation for 25 patients with limited disease oesophageal SCNEC [39]. Surgery might therefore not be necessary as part of initial therapy for oesophageal NEC if complete response is achieved after chemoradiation. A study on GI SCC however suggests a potential role for surgery for limited disease with pre- or postoperative chemotherapy, as half of the operated patients retained locoregional control [15]. Altogether, most data support that with limited disease, a combination of systemic platinum-based chemotherapy with local treatment consisting of radiotherapy, surgery or both offers the best chance for long-term survival. Several authors suggest that a sequence of neoadjuvant chemotherapy followed by definitive surgery seems appropriate [8]. Potential advantages to a neoadjuvant approach include earlier treatment of micrometastatic disease, better compliance to treatment, determination of responsiveness to chemotherapy and avoidance of unnecessary surgery for patients with early systemic disease progression.

In contrast to metastatic NETs with a low Ki-67, debulking surgery and surgery for liver metastasis are generally not recommended in NEC patients. Surgery or locoregional treatment for metastatic disease after neoadjuvant chemotherapy can however in selected patients give long-time survival (Fig. 19.3) [40, 41].

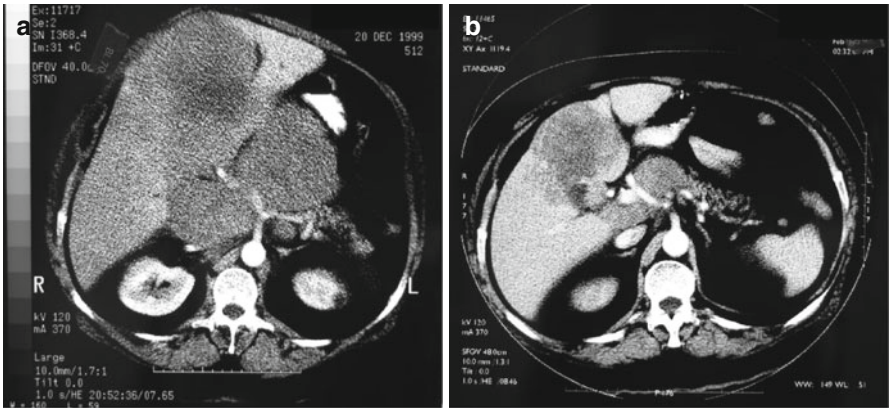

Fig. 19.3 Baseline (**a**) and partial response of liver metastases and lymph node metastases after 4 months of neoadjuvant chemotherapy with cisplatin/etoposide (**b**) before radical surgery and long-term survival

19.4.2 Postoperative Adjuvant Chemotherapy

Although there are no studies examining postoperative adjuvant chemotherapy in NEC, their aggressive behaviour warrants consideration of adjuvant therapy in most cases. The North American Neuroendocrine Tumor Society (NANETS) recommends 4–6 cycles of cisplatin or carboplatin and etoposide as adjuvant therapy for resected patients [1]. Several treatment schedules are recommended by the National Comprehensive Cancer Network (NCCN) and the European Neuroendocrine Tumor Society (ENETS) [42, 43]. A commonly used schedule is etoposide 100 mg/m^2 for 3 days and cisplatin at a dose of 45 mg/m^2/day on days 2 and 3, every 4 weeks [43]. As rapid recurrence often occurs after surgery, postoperative radiological staging should be performed before initiation of adjuvant chemotherapy.

19.4.3 Palliative Chemotherapy

Advanced GI-NEC is an aggressive disease where rapid referral to an oncologist is necessary, some advocate referral within a week. Patients should be referred for possible treatment before the performance status (PS) deteriorates to the extent that that the patient is no longer fit to receive chemotherapy. Median survival in 53 patients not given chemotherapy was only 1 month after diagnosis of advanced disease [10]. Patients not receiving palliative chemotherapy were older and had a worse PS, less positive SRS, less intense CgA staining and more often elevated white blood cell count and C-reactive protein levels. The very short median survival for patients not receiving chemotherapy indicates that the benefit from palliative chemotherapy is probably substantial.

19.4.3.1 First-Line Chemotherapy

Guidelines for advanced extrapulmonary NEC advocate the use of platinum-based chemotherapy combined with etoposide [1, 42–44], although until recently only few and small first line studies have been reported. In the study of Moertel et al., 18 predominantly GI-NEC patients were treated with cisplatin/etoposide [45]. Etoposide was given as 130 mg/m^2 iv days 1–3 and cisplatin 45 mg/m^2 days 2–3 every 4th week. Median number of courses was 5, 67 % had objective tumour regression, time to progression was 8 months and median survival was 19 months. Mitry et al. treated 41 poorly differentiated NEC; 27 patients were GI-NEC or CUP with predominantly GI metastases [16]. They were treated with etoposide 100 mg/m^2 days 1–3 and cisplatin 100 mg/m^2 day 1 every 3rd week. Median number of courses was 4, response rate 42 %, complete response (CR) 10 %, partial response (PR) 32 %, and stable disease (SD) 34 %. Progression-free survival (PFS) was 9 months and median survival 15 months. Toxicity was however high probably due to the 1-day cisplatin schedule. A 3-drug regimen combining carboplatin/etoposide with paclitaxel was given to 78 patients with NEC with mainly unknown primary tumour [46]. Although response rate was high (53 %), median survival was only 14.5 months and as grade 3–4 toxicity was frequent this schedule is not widely used. Although treatment with cisplatin/etoposide often induces initial tumour reduction, progressive disease usually occurs rapidly (Fig. 19.4).

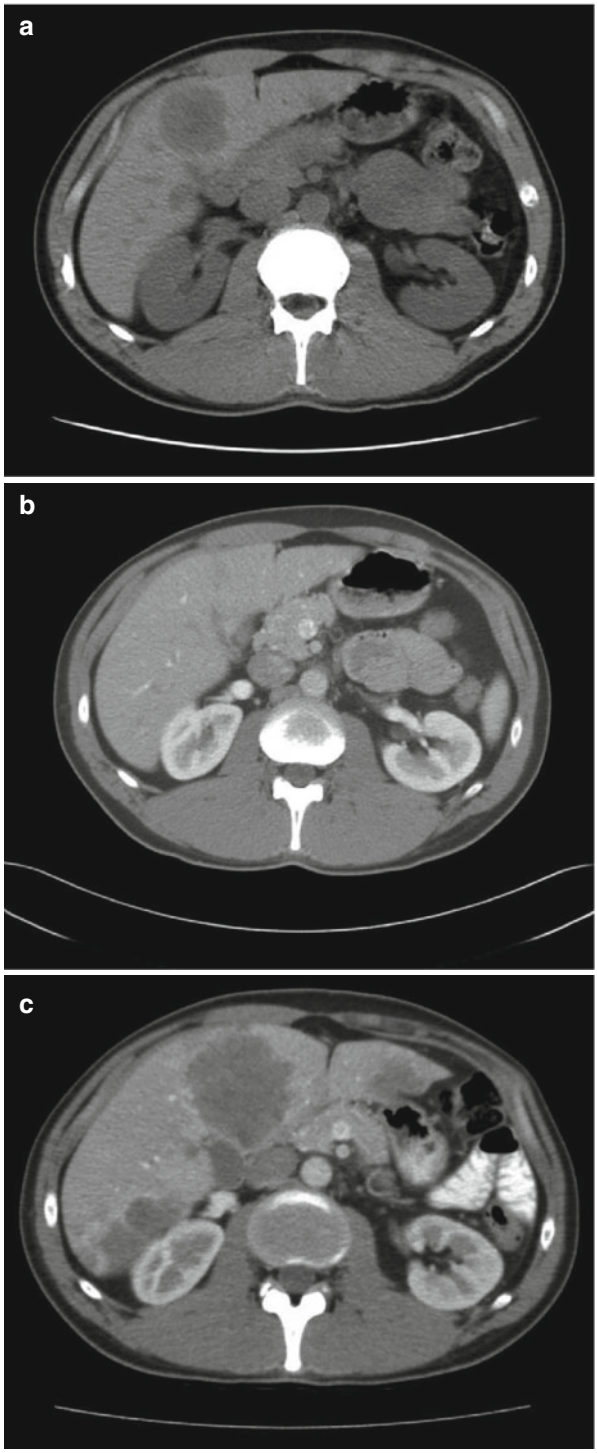

Fig. 19.4 Baseline (**a**) and partial response after 6 months of cisplatin/etoposide treatment (**b**), but massive progression of disease after a 2-month treatment break (**c**) in a 39-year-old patient with primary rectal NEC

Recent data suggests that response rates and survival of GI-NEC patients might be lower than previously reported. In a retrospective study palliative chemotherapy was given to 252 patients with advanced GI-NEC. They were mainly treated with cisplatin/etoposide or carboplatin/etoposide and some with carboplatin/etoposide/vincristine. The median number of courses was 4; response rate was 31 % (2 % CR, 29 % PR) and disease control rate 64 %. PFS was 4 months and median survival 11 months with 2- and 3-year survival of 14 and 9.5 %, respectively. No differences were seen according to chemotherapy schedule regarding response rates, PFS and survival (Fig. 19.5a), indicating that in GI-NEC cisplatin could probably be replaced by carboplatin. No further benefit was seen by adding vincristine to the etoposide/platinum combination. Multivariate analyses of the clinical and pathological features of this patient cohort identified several variables associated with response to treatment and survival such as performance status (PS), proliferation index, platelet count, lactic dehydrogenase levels and primary site of origin [10]. Median survival was only 8 months in primary colon tumours treated with chemotherapy, significantly shorter than the 15 months for patients with primary pancreatic tumours (Fig. 19.5b). Performance status was a strong prognostic factor for survival and varied from a median survival of 18 months with PS 0–5 months with PS 2 (Fig. 19.5c). Patients with PS 2 had a higher percentage of immediate disease progression compared to PS 0 (61 % vs.26 %). A relevant question is whether GI-NEC patients with a poor PS will benefit from a potentially toxic platinum-based treatment. However, in PS 2 patients who achieved a partial response after chemotherapy (25 %), PFS and median survival were 5 and 11 months, respectively. Patients with a PS 2 at the time of diagnosis should therefore not be excluded from receiving palliative chemotherapy. An ROC analysis was done in this study to investigate a possible correlation between response rate and Ki-67 level. The best cut-off value concerning response rate for Ki-67 was 55 %. Tumours with Ki-67 <55 % were less responsive to platinum-based chemotherapy (response rate 15 % vs. 42 %) (Table 19.1, Fig. 19.6). Patients with Ki-67 <55 % had a significantly longer survival compared to patients with higher Ki-67 levels (14 vs.10 months) and may thus constitute a different disease entity (Table 19.1, Fig. 19.7). Thirty months after chemotherapy initiation, 23 % of patients with a Ki-67 <55 % were alive compared to only 7 % when Ki-67 ≥55 %.

The present WHO classification of GI-NEC seems insufficient, classifying all tumours with a Ki-67 above 20 % as one single disease entity. One possibility would be to divide the existing G 3 group into a 3A and 3B group. Platinum-based chemotherapy may furthermore not be the optimal chemotherapy schedule when Ki-67 <55 %. When temozolomide-based chemotherapy was given to GI-NEC after 1st-line platinum-based chemotherapy, patients with Ki-67 <60 % had a better response to treatment [11]. Clinical trials with drugs traditionally used for G2 NET tumours (sunitinib, everolimus and temozolomide) have now started in patients with advanced G3 NEC tumours.

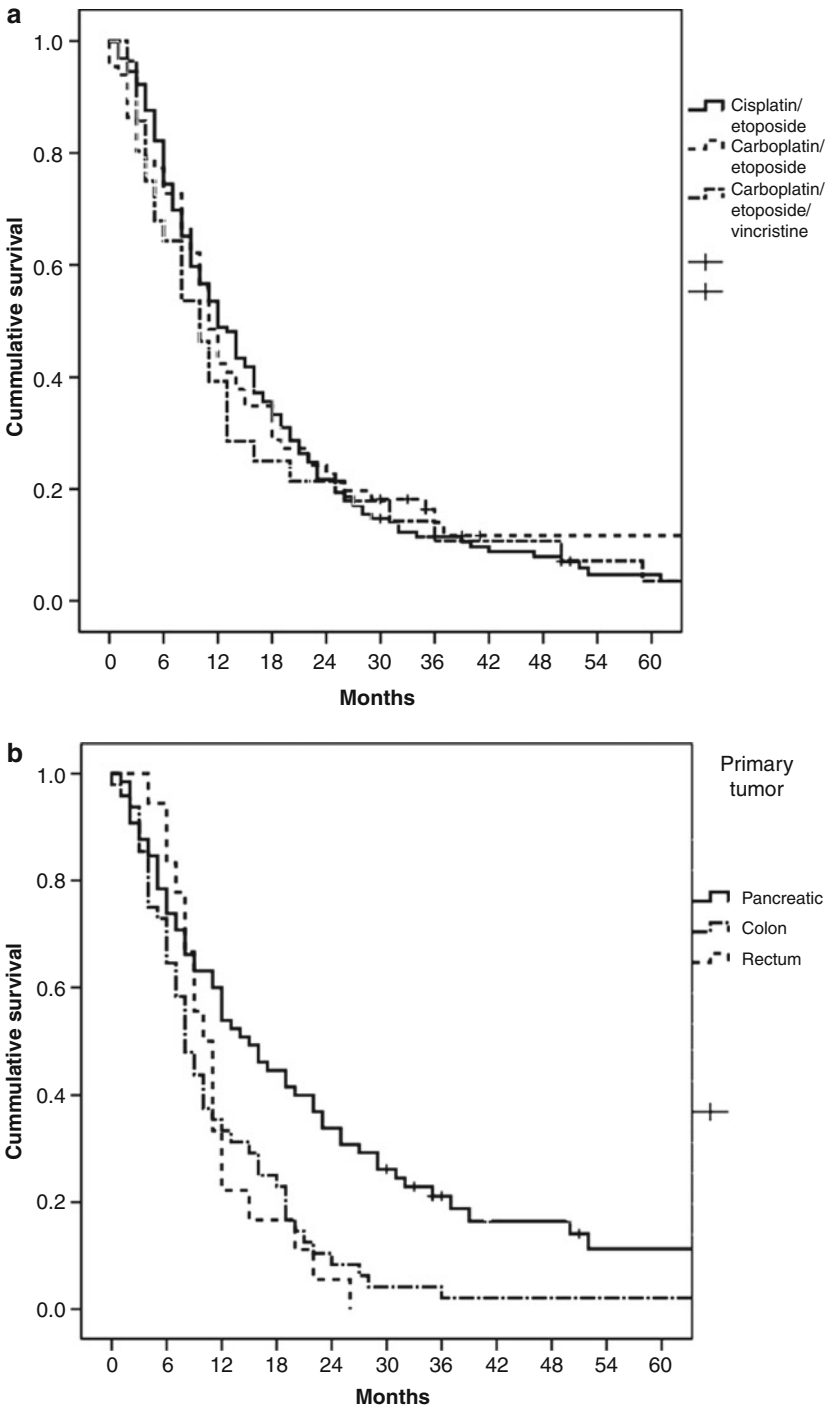

Fig. 19.5 Survival in patients treated with chemotherapy according to (**a**) chemotherapy schedule, (**b**) location of primary tumour and (**c**) performance status (From Sorbye et al. [10])

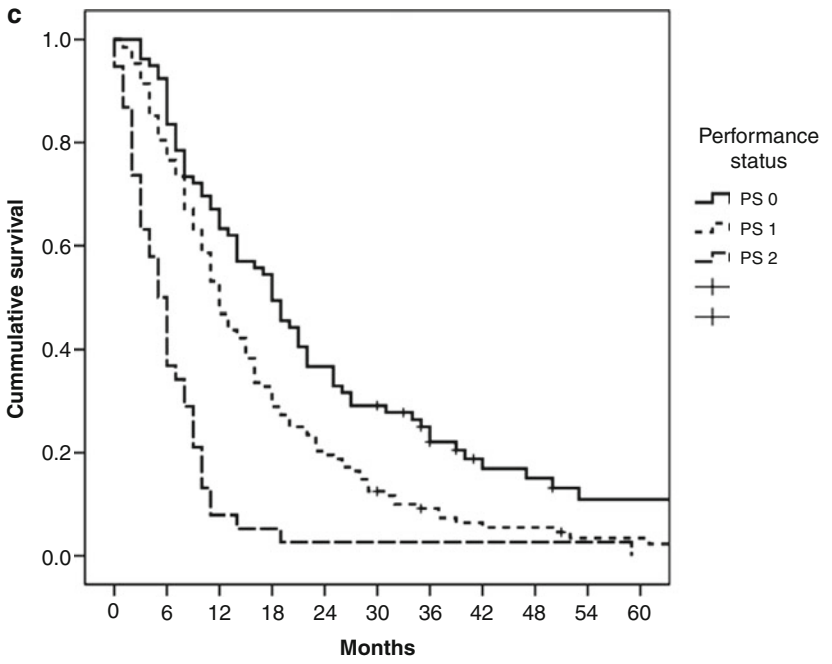

Fig. 19.5 (continued)

19.4.3.2 Second-Line Chemotherapy

After first line treatment, no further standard therapy has been established in GI-NEC. Reintroduction of cisplatin/carboplatin and etoposide after a treatment break resulted in a response rate of 15 % and another 27 % achieved SD [10]. These data indicate that retreatment with cisplatin/carboplatin and etoposide is a valid option, as 42 % had a re-stabilisation of the disease.

Not much has been known about the possible benefit from second line treatment; however, recently several small retrospective studies have been published. Welin et al. collected data from 25 patients with G3 NEC, all of whom had progressed after cisplatin-based chemotherapy [11]. The patients received temozolomide 150–200 mg/m^2 for 5 days every 4 weeks. The majority of the patients also received capecitabine 750–1,000 mg/m^2, and a smaller subset received a three-drug regimen including bevacizumab every 2 weeks. The results of this treatment were encouraging, with a response rate of 33 % with a median duration of response of 19 months. Median PFS was 6 months, and median survival from start of second line chemotherapy was 22 months. Interestingly, a Ki-67 value of <60 % seemed predictive of response. Epigenetic silencing of O6methylguanidine-DNA methyltransferase (MGMT) has been shown to predict benefit of temozolomide treatment. It does not seem to play a major part in NEC as only 1 of the 17 patients investigated displayed methylation of this gene. In another retrospective second line study, NEC patients had received temozolomide 200 mg/m^2 for 5 days every 4 weeks [47]. The results were less encouraging with no objective responses; 38 % had SD and 62 % progressive disease (PD).

Table 19.1 Response rates to systemic chemotherapy in NEC according to Ki-67

Ki-67	PR/CR (%)	SD (%)	PD (%)	PFS (95 % CI)	OS (95 % CI)
<55 % ($n=136$)	15	47	38	4 m (3.2–4.8)	14 m (10.7–17.3)
≥55 % ($n=154$)	42	24	34	4 m (3.1–4.9)	10 m (8.4–11.6)

Adapted from Sorbye et al. [10]

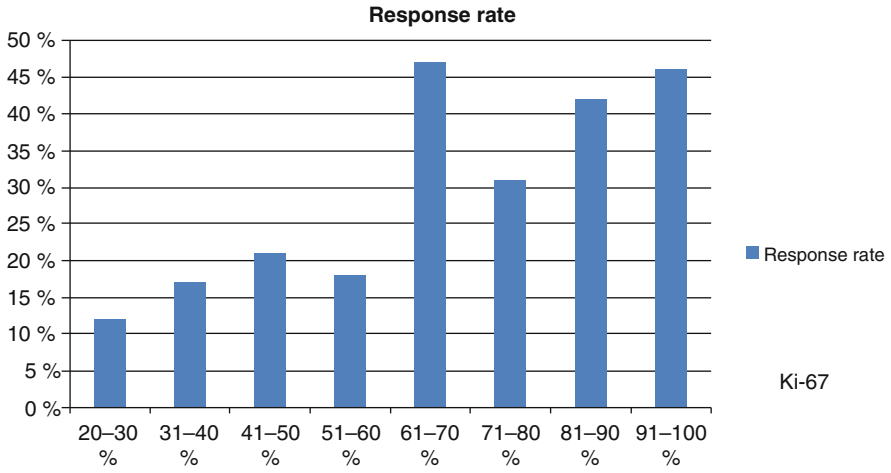

Fig. 19.6 Response rates to systemic chemotherapy in NEC according to Ki-67 (Adapted from Sorbye et al. [10])

However, many patients had a poor performance status at start of treatment. Median survival was only 3.5 months, with a median PFS of 2.4 months, although patients with pancreatic primaries had better results. Patients with a Ki-67 in the lower range (20–55 %) had a longer median survival (10.9 vs. 2.7 months).

Irinotecan and 5-flourouracil (5-FU) was given as FOLFIRI to 19 GI-NEC patients after etoposide-platinum treatment in a retrospective study [12]. Median number of cycles was 6, response rate was 31 % and median PFS was 4 months. Overall survival from date of initial diagnosis was 18 months. FOLFIRI is therefore an option as second-line chemotherapy after failure of etoposide-platinum. In the NORDIC NEC study, second-line chemotherapy had been given to 100 patients; of these 35 received temozolomide-based chemotherapy and 20 taxan-based chemotherapy [10]. Response rate was 18 %, 33 % had SD and 49 % PD. Third-line chemotherapy was given to 31 patients. The response rate was 7 %, 34 % had SD and 59 % PD. The data illustrate that many GI-NEC patients benefit from further lines of chemotherapy.

19.4.4 Other Treatments

Somatostatin analogues are used for symptom control and have an anti-proliferative effect in intestinal NET where Ki-67 is typically 2–4 %. There are no data supporting the use of somatostatin analogues in NEC [48]. Peptide receptor radiotherapy

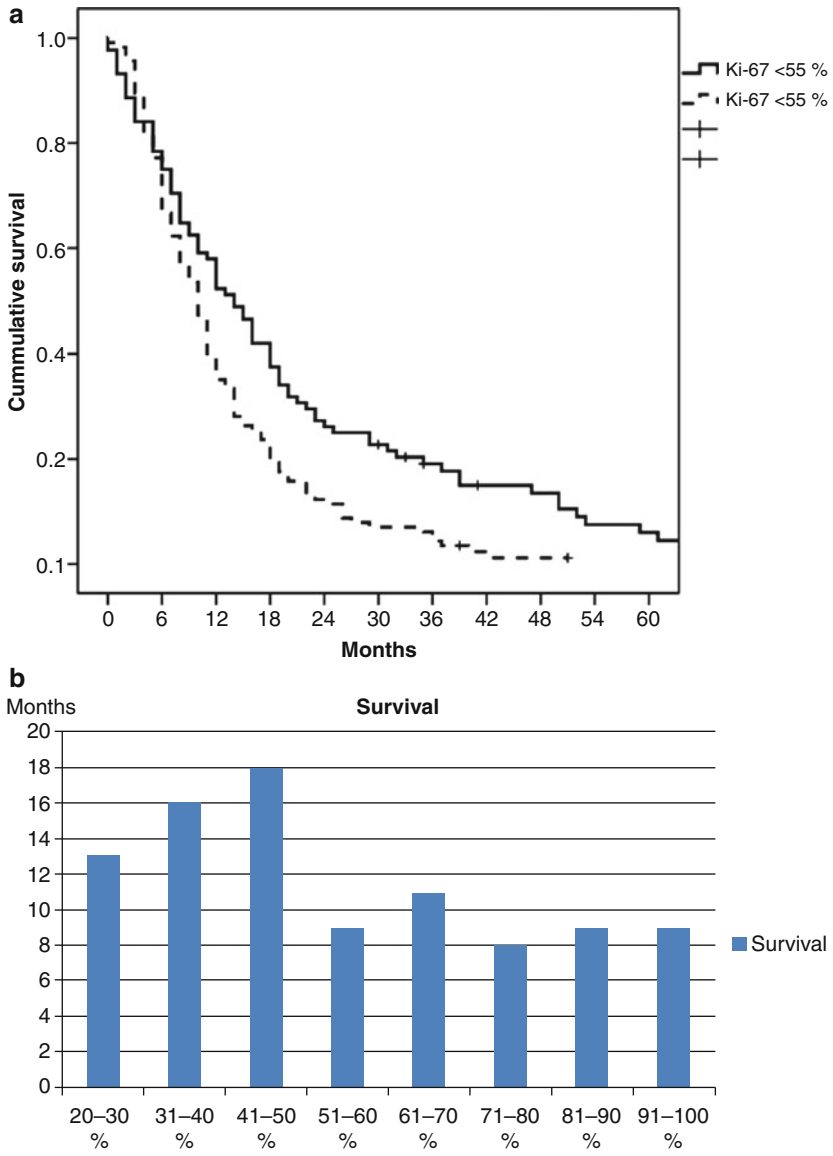

Fig. 19.7 Survival according to Ki-67 in chemotherapy-treated patients ($N = 252$). (**a**) Survival by Ki-67 estimated by the Kaplan-Meier method. (**b**) Survival by Ki-67 in 10 % percentiles (From Sorbye et al. [10])

(PRRT) is regularly used for G1–G2 NET tumours with a high uptake on SRS. The benefit of PRRT in patients with a higher proliferation rate is unknown. Case reports have shown long-lasting partial responses to PRRT treatment in some NEC patients [33–35]. PPRT treatment may therefore be a treatment option for NEC patients with

a high SRS uptake with a Ki-67 in the lower range. The optimal timing would probably be as a consolidation treatment after initial chemotherapy [34]. Palliative external beam radiation may be useful, especially for painful bone metastasis.

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The Multiple Endocrine Neoplasia Syndromes

20

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20.1 Introduction

The term multiple endocrine neoplasia (MEN) refers to hereditary neoplastic disorder involving more than one endocrine organ and includes the MEN type 1 (MEN1), the MEN type 2 (MEN2), and the familial medullary thyroid carcinoma (FMTC). The estimated frequency of each of these syndromes is around 1 in 30,000 and is characterized by complete penetrance with variable expression [1, 2]. The recent progress in our knowledge of both the molecular and the clinical genetics of these syndromes has improved the clinical management of the patients [3]. On the one hand, the MEN1 gene, a tumor suppressor gene responsible of MEN1 syndrome, is involved in the regulation of several cell functions (e.g., DNA replication and repair and transcriptional machinery). On the other hand, the RET proto-oncogene encodes for a receptor tyrosine kinase protein whose expression is fundamental for appropriate migration, development, and differentiation of neuroendocrine cells originating from neural crest [4]. Currently, DNA testing allows the early identification of germline mutations in presymptomatic mutant gene carriers in the MEN syndromes [5]. Consequently, an early identification of the MEN-associated neoplasms and the genotype-phenotype correlation improve the outcome and the quality of life for affected subjects [6].

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20.2 Multiple Endocrine Neoplasia Type 1 (MEN1, Wermer's Syndrome)

MEN1 is a rare hereditary endocrine cancer syndrome involving the occurrence in the same patient of tumors of the parathyroid glands, the anterior pituitary, and the pancreatic islet cells. Whereas firstly described in the early twentieth century, it was not until 1954 when Wermer described familial occurrence in which a father and four of the nine offspring were affected [7]. The disease was initially described as a multiple endocrine adenomatosis, and it has subsequently become known as multiple endocrine neoplasia type 1 (MEN1).

Patients with MEN1 characteristically have tumors of the parathyroid glands (95 %), the anterior pituitary (15–90 %), and the pancreatic islets (30–80 %) [5, 8] (major criteria for diagnosis); less commonly, they may have some other endocrine tumors (adrenal cortical adenomas and more rarely carcinoma, thyroid tumors, usually follicular adenomas, neuroendocrine tumors involving organs other than the pancreas such as duodenal gastrinoma and gastric, thymic, and bronchial carcinoids). Non-endocrine neoplasms can also occur: visceral and cutaneous lipomas, meningiomas, facial angiofibromas, and collagenomas [9–15] (minor criteria for diagnosis). The phenotype of MEN1 is extremely diverse, with over 20 different combinations of endocrine and non-endocrine manifestations being described to date. MEN1 should be suspected in patients with an endocrinopathy of two of the three pathognomonic/major affected organs or with an endocrinopathy of one of these organs plus two minor manifestations or with a first-degree relative affected by MEN1 syndrome [16].

The overall estimated incidence of MEN1 is 0.25 %; estimated incidences are 1–18 % in primary hyperparathyroidism, 16–38 % in gastrinomas (pancreatic neuroendocrine tumors secreting gastrin), and <3 % in pituitary tumors. The reported age range is 5–81 years; however, 80 % of patients develop clinical manifestations of the disorder by the fifth decade [17].

20.2.1 MEN1-Associated Primary Hyperparathyroidism (PHPT) (Table 20.1)

20.2.1.1 Clinical Features and Diagnosis

PHPT is the most common and frequently the earliest manifestation in MEN1 mutation carriers and occurs in ~95 % of cases [9, 13]. The PHPT in MEN1, as compared to the sporadic form, has an earlier age of onset (20–25 years, compared to 55–60 years), exhibits an equal male to female ratio (compared to a female preponderance in sporadic PHPT), and involves all four parathyroid glands (diffuse hyperplasia or multiple adenomas), rather than a single one (solitary adenomas) [8, 9]. Noteworthy is the increased frequency of supernumerary (up to 20 %) and ectopic parathyroid glands in MEN1 patients, usually localized within the thyroid gland, in the anterior mediastinum, or exceptionally in the pericardium [18]. Although the MEN1 gene is a tumor suppressor, parathyroid carcinoma may be diagnosed in a small percentage of patients [19].

Table 20.1 Clinical manifestations in MEN1

	Organ involved		Specific tumor (prevalence by 40 years)	Clinical presentation
MEN1	Parathyroid disease (95 %)		Diffuse hyperplasia	Symptoms related to hypercalcemia or hypercalciuria
			Adenoma, multiple	
	NETs	PNETs (30–80 %)	Gastrinomas (>50 %)	ZES, diarrhea, abdominal pain
			Insulinomas (10–30 %)	Whipple triad
			Glucagonomas (~3 %)	Necrolytic migratory erythema, weight loss, anemia, stomatitis
			VIPomas (extremely rare)	Verner-Morrison syndrome
			NF PNETs (20–100 %)	Asymptomatic, but with malignant potential
			Somatostatinomas (extremely rare)	Somatostatinomas syndrome, rare
			Other (e.g., GHRH-secreting)	Rare, increased GH and IGF1 levels
		Foregut NETs (2–10 %)	Thymic, gastric, bronchial NETs	Organ specific
	Pituitary tumors		Prolactinomas (20 %)	Oligomenorrhea, galactorrhea, infertility in woman; impotence and infertility in men
			Other: ACTH, TSH, GH+PRL, GH, NF (each 2–9 %)	Hormone-dependent
	Other endocrine manifestations		Benign adrenocortical tumors (73 %)	Most nonfunctioning
			Adrenocortical carcinoma (13 %)	Hormone-dependent
			Pheochromocytomas (<1 %)	Rarely described, mainly asymptomatic
			Thyroid adenomas, goiter, and carcinoma (25 %)	Usually incidental finding
	CNS tumors		Ependymomas, schwannomas, meningiomas (1 %)	Mainly asymptomatic
	Cutaneous manifestations		Multiple subcutaneous lipomas (33 %); visceral, pleural, or retroperitoneal lipomas (rare)	
			Facial angiofibromas and collagenomas (up to 88 %)	

NETs neuroendocrine tumors, *PNETs* pancreatic neuroendocrine tumors, *ZES* Zollinger-Ellison syndrome, *VIPomas* vasointestinal polypeptide-secreting tumors, *NF PNETs* nonfunctioning pancreatic neuroendocrine tumors, *GHRH* growth hormone-releasing hormone, *IGF1* insulin growth factor 1, *ACTH* adrenocortical hormone, *TSH* thyroid-stimulating hormone, *PRL* prolactin, *NF* nonfunctioning, *CNS* central nervous system

PHPT is a progressive disease in MEN1: whereas asymptomatic hypercalcemia is the most common manifestation, patients may present with symptoms (e.g., fatigue, weakness, polydipsia, polyuria, myalgias, or abdominal pain) or signs (nephrolithiasis or osteitis fibrosa cystica) related to the progressive increase in serum and/or urinary calcium and disease duration.

A major clinical manifestation of MEN1-associated PHPT is progressing demineralization with severe osteopenia (T score < –2.0) in ~ 44 % of patients with uncontrolled disease, by the age of 35 [20]. Recurrent kidney stones are less frequently described in MEN1 families, and it is unusual for these patients to develop chronic renal failure as a result of nephrolithiasis [21, 22].

The diagnosis of PHPT is based upon demonstrating elevated serum levels of calcium and parathyroid hormone (PTH). Mild hypercalcemia with normal/increased range of serum PTH concentration can usually be detected during the second decade of life [23]. Total serum calcium concentration corrected for albumin level is considered the screening test for PHPT in MEN1 [24]. Noteworthy, identifying the "subclinical" stage of hyperparathyroidism has not been considered essential until recently, when an increased cardiovascular risk has been reported in patients with mild normocalcemic hyperparathyroidism [25]. However, the performance of serum PTH measurement as a screening test remains controversial. Diagnosis of hyperparathyroidism requires levels of PTH inadequately high for the concomitant calcium levels. At present, different kits for assessment of PTH are available. Second generation of kits, measuring concentrations of the so-called intact PTH (iPTH), is in general usage [26]. Routine testing for MEN1 mutations in young patients with primary hyperparathyroidism is not recommended, as these mutations are rare in unselected patients even below 40 years of age [27]. Such testing should be considered in patients with multiple gland involvement, coexistence of other tumors characteristic for MEN1, or family history of hyperparathyroidism or MEN1 tumors.

20.2.1.2 Treatment of MEN1-Associated PHPT

In contrast to single-gland resection in sporadic cases, a more complex surgical approach, including total parathyroidectomy and thymectomy with autotransplantation of parathyroid tissue or excision of 3.5 parathyroid glands, is indicated for PHPT in MEN1 [18, 28, 29]. Moreover, postoperative hypoparathyroidism and higher rates of recurrent or persistent disease are more frequent in MEN1-associated PHPT than in the sporadic form of the disease [28]. Recurrence is usually located in preserved/residual parathyroid tissue [30], and it is strongly influenced by the surgeon's experience and the possibility to perform intraoperative PTH determinations [28]. Intraoperative PTH assessment is mandatory in these patients to confirm the removal of all functional parathyroid tissue before parathyroid autotransplantation.

In MEN1 patients with preclinical/eucalcemic PHPT, the approach is still uncertain: early parathyroidectomy is recommended by some experts to control even mild hyperparathyroidism and to prevent the progressive decline of bone mineral density and the increased risk for kidney stones [31, 32].

In MEN1-associated PHPT patients in whom surgery is not possible or ineffective, medical treatment with calcimimetic drugs emerged as an effective, though expensive, option [33, 34]. Cinacalcet therapy is well tolerated by most of the patients; rarely, gastrointestinal side effects may induce discontinuation of the therapy [34, 35]. Interestingly, the administration of the somatostatin analogue octreotide LAR controlled hypercalcemia and hypercalciuria in two-thirds of patients with MEN1-related PHP, this effect being most probably mediated by the octreotide LAR binding to somatostatin receptors on parathyroid tumor cells in patients with MEN1-related PHP [36].

20.2.2 MEN1-Associated Pancreatic Islet Cell Neuroendocrine Tumors (PNETs)

Pancreatic and duodenal neuroendocrine tumors represent the second most frequent manifestation in MEN1 (occurring in 30–80 % of cases) and continue to be the primary cause of tumor-related death in these patients [10, 37]. Although PHPT is usually the first manifestation of MEN1 syndrome, the penetrance of MEN1-related PNETs is similar to that of parathyroid tumors [38]. In contrast with the sporadic PNETs, MEN1-related PNETs are characterized by an early onset, multifocality, variable expression of the tumors, and propensity for malignant transformation; both the histology and the size of the tumor are predictors of the malignant potential [5, 39–41]. Some of these tumors secrete gastrin, insulin, glucagon, vasoactive intestinal polypeptide (VIP), etc., and are associated with distinct clinical syndromes, whereas most PNETs are clinically silent tumors (nonfunctioning, secreting pancreatic polypeptide, PP) [5]. Given that MEN1-associated PNETs are usually multiple and of a possible malignant behavior, their timely diagnosis and management are challenging and of major importance.

20.2.2.1 Gastrinomas

Clinical Manifestations and Diagnosis

Gastrin-secreting tumors (gastrinomas) are the most frequent functioning duodenopancreatic NETs in patients with MEN1 (>50 %), and ~ 20 % of patients with gastrinomas will have MEN1 [42–44]. Gastrinomas occur more often in patients with MEN1 who are older than 30 years; are associated with marked gastric acid secretion and recurrent and severe peptic ulcerations (the Zollinger-Ellison syndrome, ZES), which may perforate; and are usually metastatic at diagnosis [45, 46]. These features are responsible for the major morbidity (peptic ulcer disease, diarrhea and steatorrhea, cachexia, and abdominal pain) and mortality associated with MEN1 [47, 48].

In contrast to the sporadic gastrinomas that occur predominantly in the pancreas, in MEN1, most of these tumors develop in the proximal duodenum [49]. Gastrinomas are usually small (<5 mm) and multiple, growing slowly deep into the mucosa and metastasizing frequently to the peripancreatic lymph nodes and

further to the liver [50]. Pancreatic gastrinomas or other extra-pancreatic, extra-duodenal (e.g., ovary, liver, bile duct, pylorus, spleen, mesentery), and extra-abdominal (e.g., cardiac intraventricular septum) locations in MEN1 patients are extremely rare [51, 52]. When found in the pancreas, it may be difficult to distinguish gastrinomas from concomitant nonfunctioning PNETs, and in this eventuality selective arterial secretagogue injection test (SASI test) may help in localizing the gastrin-secreting tumor [43, 53].

The diagnosis is established by demonstration of an increased fasting serum gastrin concentration (found to be elevated in 90–98 % of ZES patients) after stopping antisecretory drugs (proton pump inhibitors, PPIs, at least 1 week; histamine receptors 2 blockers, H2R, for at least 2 days) [54], in association with increased basal gastric acid secretion (gastric pH < 2) [52, 55]. However, stopping antisecretory drugs in ZES patients is often impossible due to severe symptoms of peptic disease and diarrhea and may be hazardous due to possible perforation or bleeding. Occasionally, iv provocative tests with either secretin (2 U/kg) or calcium infusion (4 mg Ca^{2+}/kg·h for 3 h) are required to distinguish patients with ZES from other patients with hypergastrinemia (e.g., those with antral G-cell hyperplasia) [50]. In two-thirds of patients with ZES, fasting serum gastrin levels overlap with values seen in other conditions. In these patients, gastrin provocative tests are needed to establish the diagnosis of ZES [56]. For unknown reasons, secretin stimulates the release of gastrin by gastrinoma cells, and therefore patients with these tumors have a dramatic rise in serum gastrin in response to a secretin infusion. In contrast, normal gastric G cells are inhibited by secretin, and therefore serum gastrin concentrations do not rise in patients with other causes of hypergastrinemia. The secretin stimulation test is performed by administering 0.4 μg of secretin per kg body weight (RepliGen Corporation, Waltham, MA) intravenously over 1 min; a baseline serum gastrin is measured twice before the secretin is administered and classically 2, 5, 10, 15, and 20 min later. An increase of ≥120 pg/mL in gastrin levels is highly suggestive of gastrinoma [56].

In some patients with MEN1, the diagnosis of ZES may be difficult, as it does not appear to develop in the absence of primary hyperparathyroidism, whereas hypergastrinemia has also been reported to be associated with hypercalcemia [54]. Moreover, successful treatment of MEN1-associated PHPT with restoration of normocalcemia is known to ameliorate clinical symptoms and biochemical abnormalities in ~ 20 % of MEN1 patients with ZES [44].

Tumor localization and determination of the tumor extent are essential to the proper management for MEN1 gastrinomas: endoscopic ultrasonography, CT, MRI, selective abdominal angiography, and mainly somatostatin-receptor scintigraphy/Ga^{68}-DOTA-TATE/-TOC/-NOC PET-CT are very helpful in localizing the tumor [53, 57]. Moreover, the combination of intra-arterial calcium injections with hepatic venous gastrin sampling has been shown to regionalize the gastrinomas [58]. Importantly, these tumors are often occult to conventional exploration, and their detection requires duodenotomy and meticulous evaluation of the mucosa by eversion and direct palpation [59].

Management of MEN1-Associated Gastrinomas

Control of Gastric Hypersecretion

The management of patients with ZES is directed toward reducing basal acid output to less than 10 mmol/l and includes acute- and long-term control of gastric acid hypersecretion by parietal cell H^+-K^+-adenosine triphosphatase (proton pump) inhibitors, PPIs (omeprazole, lansoprazole, pantoprazole, rabeprazole, and esomeprazole), starting a dose equivalent to 60 mg/day of omeprazole [54]; the dosage should be increased to b.i.d. in patients with severe gastroesophageal reflux disease (GERD) symptoms, with previous Billroth II or gastroenterostomies, or with hypercalcemia with MEN1 [52]. Long-term PPI treatment is safe and without loss of efficacy; no gastric carcinoid development associated to PPI-induced hypergastrinemia was demonstrated in humans [52]. Some patients may also require additional treatment with the histamine H2 receptor antagonists, cimetidine or ranitidine [5].

Surgical Management of Gastrinoma

In patients with MEN1 and ZES, correction of the PHPT by parathyroidectomy can significantly decrease basal acid output and fasting gastrin level and increase sensitivity to gastric antisecretory drugs [60]. The role of surgery in the treatment of the gastrinoma in MEN1/ZES patients is controversial [61, 62]: these patients cannot be cured as they usually have multiple duodenal tumors with frequent lymph node metastases. In addition, they usually have concomitant nonfunctional PNETs. As development of liver metastases, associated with a poor prognosis, is more frequent in the presence of large tumors, exploration is recommended whenever a PNET of >2 cm is found [62]. Surgical resection of duodenal MEN1-associated gastrinomas, based on preoperative and intraoperative (e.g., transillumination of the duodenum) tumor regionalization, may be considered in specialized centers. Due to increased morbidity and potential mortality after extensive resections, the surgical excision has to be individualized based on preoperative findings, patient history (e.g., preexisting diabetes), and preference [37]. Interestingly, the presence of lymph node metastases in 50–60 % of patients with MEN1-related duodenal gastrinomas is not usually associated with a decreased survival [60].

Management of Metastatic MEN1 Gastrinoma

Long-acting somatostatin analogues (SSAs) such as octreotide LAR, lanreotide SR, or the lanreotide Autogel alone or in combination with interferon are recommended as initial therapy [52, 63]. The main effect of these agents is tumoristatic effect, with disease stabilization observed in ~ 40–70 % of patients, whereas decrease in tumor size (partial response) is reported in only 10–20 % of patients [64]; these agents are particularly effective in slower-growing PNETs [65]. Chemotherapy with streptozotocin and 5-fluorouracil, hepatic artery embolization, PRRT, or removal of all resectable tumor have been occasionally successful in patients with disseminated MEN1 gastrinomas [66, 67].

20.2.2.2 Insulinomas

Clinical Manifestations and Diagnosis

Insulinomas represent between 10 and 30 % of all MEN1-related PNETs, and rarely (in 10 % of patients with MEN1), they may coexist with a gastrinoma or with other nonfunctioning PNETs at the time of diagnosis, in the same patient [9, 68]. In MEN1 patients, insulinomas occur more often at an age younger than 40 years (in many patients even younger than 20 years and in some as young as 5 years), in contrast to the sporadic insulinoma patients in whom the disease occurs usually after the age of 40 years [9, 69]. Insulinoma is the first manifestation of MEN1 in 10 % of patients, whereas ~4 % of patients with insulinomas will have MEN1 [5, 66]. The clinical presentation, biochemical and anatomical diagnosis of MEN1-related insulinomas, and the medical and surgical treatment of these patients are similar to those in the sporadic insulinoma patients and are addressed in detail in the "Insulinomas" chapter of this textbook [70, 71].

20.2.2.3 Glucagonomas

Clinical Manifestations and Diagnosis

Glucagon-secreting pancreatic tumors occur in fewer than 3 % of patients with MEN1 (Table 20.1) [5, 50], are highly malignant, and frequently are localized at the tail of the pancreas [5, 72]. Importantly, in MEN1-associated glucagonomas, the characteristic clinical manifestations (such as the necrolytic migratory erythema, weight loss, anemia, or stomatitis) [73, 74] may be absent, and the tumor may be detected in a completely asymptomatic patient with MEN1 undergoing routine pancreatic imaging or detected by glucose intolerance and concomitant increase in glucagon levels [75, 76].

Management of MEN1-Associated Glucagonomas

Surgical removal is the treatment of choice. However, up to 80 % of glucagonoma patients have metastatic disease at the time of diagnosis [77], and therefore systemic therapy using SSAs, PRRT, chemotherapy (streptozotocin and 5-fluorouracil), or hepatic artery embolization has been successful in some patients [63, 78–81].

20.2.2.4 VIPomas

Clinical Manifestations and Diagnosis

The vasoactive intestinal peptide (VIP) secreting tumors (VIPomas) are rare in MEN1, occur commonly in the tail of the pancreas, and produce a clinical syndrome (Verner-Morrison syndrome) with watery diarrhea, hypokalemia, and achlorhydria (WDHA) [80, 82]. The diagnosis requires excluding laxative and diuretic abuse, demonstration of a large fasting stool volume (0.5–1 l/day) during a fast, and an elevated serum VIP concentration [50].

Management of MEN1-Associated VIPomas

Surgical excision of VIPomas is the main therapeutic intervention in these patients, with a curative purpose. However, in patients with unresectable or metastatic tumor, systemic treatment with SSAs, chemotherapy (streptozotocin and 5-fluorouracil), corticosteroids, indomethacin, metoclopramide, lithium carbonate, and hepatic artery embolization has been used with different efficacy [37, 38].

20.2.2.5 Nonfunctioning PNETs (NF PNETS)

Clinical Manifestations and Diagnosis

NF PNETs represent a heterogeneous group of tumors being increasingly identified as the result of increasing sensitivity of radiological screening methods [40, 41, 83]. Importantly, NF PNETs have been reported to occur in young asymptomatic patients who are even less than 15 years of age. NF PNETs are not associated with a clinical syndrome and may be associated with minor elevation of specific pancreatic hormones (e.g., pancreatic polypeptide) but without clinical symptoms [38].

Identification of NF PNETs is of outmost importance, as they are the most common NETs occurring in the MEN1 setting, they have a well-recognized malignant potential, they are associated with a worse prognosis, and they are the most common cause of death in MEN1 patients [47, 55, 84, 85]. In the absence of specific clinical and biochemical abnormalities, the diagnosis of NF PNETs may be delayed, and therefore radiological screening for enteropancreatic NET in MEN1 is mandatory and should begin at the age of 10 years. The optimal screening method and its timing interval are still controversial and depend on method availability and skills of the performer [48, 83]: endoscopic ultrasound is probably the most sensitive, whereas somatostatin-receptor scintigraphy/gallium-68-DOTA-TATE/-TOC/-NOC PET-CT is the most specific method for detecting metastatic disease. The clinical significance of small NF PNETs (e.g., <1 cm) in asymptomatic individuals is still not fully understood [86].

Management of MEN1-Associated NF PNETs

Surgical Therapy of MEN1-NF PNETs

The treatment approach of asymptomatic NF PNETs is controversial. To date, treatment decision is based on tumor size, as the risk of metastases is thought to be higher in patients with larger tumors [87, 88]. Some reports suggest that early and aggressive surgery might reduce the risks for malignant progression [89]. However, other studies have not confirmed this association [45, 90], and therefore, there is no consensus to date regarding the indications and timing of surgery. The latest clinical guidelines [37] suggest considering surgical resection for NF PNETs that are more than 1 cm in size which may be successful in up to 80 % of MEN1 patients [91, 92]; however, other centers recommend surgery only for tumors of more than 2 cm [87]. For tumor size less than 1 cm, surgical resection should be considered for tumors with a size doubling time of a 3- to 6-month interval [37]. However, there are many

factors influencing the decision on surgery in these patients, such as the increased morbidity of pancreatic surgery (as complications such as diabetes mellitus, steatorrhea, dumping syndromes are not uncommon) [38], the possible presence of occult metastatic disease at the time of initial diagnosis, and the high risk for disease recurrence in the remnant pancreatic tissue after surgery [91–93].

Medical Therapy of MEN1-NF PNETs

PNET cells may express different kinase receptors, such as tyrosine kinase receptors (TK-R), vascular endothelial growth factor receptors (VEGF-R), and platelet-derived growth factor receptors (PDGF-R), whereas some exhibit over-activation of the mammalian target of rapamycin (mTOR) signaling pathway, which stimulates cell proliferation and angiogenesis. Recently, systemic therapy with tyrosine kinase receptor inhibitors (TKI; sunitinib malate) or with mTOR inhibitors (mTORi; everolimus) was reported as significantly increasing time to tumor progression in patients with locally progressive or metastatic PNETs [94, 95]. Moreover, all the other treatment modalities considered for functioning PNETs such as chemotherapy (the classic streptozotocin and 5-FU combination or the new combination of temozolomide and capecitabine), SSAs, peptide receptor radioligand therapy, hepatic artery chemoembolization, radio-frequency ablation, etc., may be individually used in treating patients with metastatic NF PNETs [96–100].

20.2.2.6 Other PNETs

Rare PNETs ectopically secreting growth hormone-releasing hormone (GHRH) have been reported in some patients with MEN1 [66, 101]. They may occur in the lungs, pancreas, or the small intestine and should be suspected when elevated levels of growth hormone (GH) and GHRH are found in the serum of the patient or when the patient has clinical acromegaly.

About 0.65 % of MEN1-related PNET may produce somatostatin and are defined as somatostatinomas, rare tumors which are usually nonfunctioning or, in 10 % of cases, associated with a clinical syndrome of hyperglycemia, cholelithiasis, low acid output, steatorrhea, diarrhea, abdominal pain, anemia, and weight loss (the somatostatinoma syndrome) [72, 102].

Surgical removal is the treatment of choice for these rare tumors.

20.2.3 MEN1-Associated Pituitary Tumors

Patients affected by the MEN1 syndrome display a high incidence (~42 %) of pituitary adenomas [103–105], which may occur as early as 5 years of age and as late as the ninth decade and have been reported to occur more frequently in women than men (Table 20.1) [103]. The molecular pathways involved in their development seem different from the sporadic tumors [106–108]; approximately 40 % of patients with MEN1 have pituitary adenomas and 17 % present with a pituitary tumor [109].

All types of adenomas can be found in MEN1 patients with a predominance of prolactinoma and macroadenoma (i.e., diameter greater than 1 cm) compared to a control

population. Acromegaly is seen in 9 % of cases and has a greater female preponderance in MEN1 than in sporadic disease [105]. Nonsecreting adenomas, thyroid-stimulating hormone (TSH)-secreting adenomas, and adrenocorticotropic hormone (ACTH)-secreting adenomas do occur in MEN1 patients, in decreasing frequency [105]. MEN1-related pituitary tumors may exhibit immunoreactivity to several hormones, with a higher occurrence of somatolactotrophinomas [103]. Interestingly, plurihormonal expression is frequently observed in MEN1-associated pituitary tumors compared with sporadic pituitary tumors [105]. The MEN1 tumors seem to have a more aggressive behavior, to be more invasive (with infiltration of tumor cells into surrounding normal pituitary tissue) and more resistant to treatment, therefore requiring a long and careful life follow-up [106, 110]. However, no increased prevalence of pituitary carcinoma is observed in MEN1 (63). These tumors may occur in the pediatric population and may be the first and only manifestation of MEN1 for some years [106, 111].

20.2.3.1 Clinical Manifestations and Diagnosis

Clinical manifestations of MEN1-associated pituitary tumors are similar to those in sporadic pituitary tumors [103]. They depend on the hormone secreted (e.g., symptoms of increased prolactin, such as oligomenorrhea, galactorrhea, infertility in woman, impotence, and infertility in men; symptoms of acromegaly due to increased GH/IGF-I secretion; symptoms of Cushing's disease due to increased cortisol secretion, etc.) and on the size of the tumor. Enlarging pituitary tumors may compress adjacent structures such as the optic chiasm or normal pituitary tissue and may cause visual disturbances and/or hypopituitarism [106].

In an MEN1 patient, periodic biochemical monitoring, including measurement of serum prolactin and IGF-I levels, and MRI [83] of the pituitary are indicated, and further hypothalamic-pituitary hormonal testing should be performed in patients with abnormal results [37].

20.2.3.2 Treatment of MEN1-Related Pituitary Tumors

Treatment of these tumors is similar to the one in patients with sporadic tumors and includes medical therapy (specifically dopamine agonists for prolactinoma or somatostatin analogues for GH-secreting adenoma together with surgical excision) and, if feasible, surgical excision, e.g., selective transsphenoidal tumor resection, with adjuvant radiotherapy in case of residual unresectable tumor tissue [105]. Noteworthy, as a result of the aggressive tumor behavior, MEN1 patients with pituitary tumors are less responsive to medical or surgical therapy, with a hormone normalization rate of 42 % compared with 90 % in patients with sporadic tumors, after therapy [103, 110, 112].

20.2.4 Other MEN1-Associated Tumors

MEN1 patients frequently develop tumors involving tissues other than the parathyroid glands, pancreas, and pituitary gland, such as neuroendocrine tumors (carcinoids), adrenal cortical tumors, thyroid tumors, facial angiofibromas, collagenomas, lipomatous tumors, and meningiomas [113, 114] (Table 20.1).

20.2.4.1 MEN1-Related Neuroendocrine Tumors (NETs, Carcinoids)

Clinical Manifestations and Diagnosis

NETs occur in ~3 % of MEN1 patients (Table 20.1), with the lungs, intestinal tract, or thymus being frequent sites of origin. In MEN1 patients, these tumors may be gender related, with a woman predominance for lung NETs and a male predominance for thymic NETs, cigarette smokers having an increased risk for developing these tumors [66, 115, 116]; moreover, there are no hormonal or biochemical abnormalities consistently reported in MEN1 patients with thymic or lung NETs. Importantly, MEN1-associated thymic NETs seem to be particularly aggressive, with a median survival time of approximately 9.5 years and with 70 % of patients dying as a direct result of the tumor [116].

Screening for these NETs is of major importance and their diagnosis depends on the imaging method used and the skills of the radiologist; CT and MRI are sensitive methods, whereas somatostatin-receptor scintigraphy may be useful, although there is still insufficient evidence to recommend its routine use [116, 117]. The current guidelines for the early detection of MEN1-related thymic and lung NETs recommend the use of CT or MRI imaging every 1–2 years (in favor of MRI, as repeated CT exposure to repeated doses of ionizing radiation may be harmful) [117, 118]. Noteworthy, thymic NETs have occurred also after prophylactic thymectomy, suggesting that surveillance imaging is still required [119] even after tumor excision.

Type II gastric NETs are associated with MEN1 and Zollinger-Ellison syndrome, are usually multiple and smaller than 1.5 cm, and may be detected incidentally during endoscopy in these patients [119].

Treatment of MEN1-Associated NETs

Surgical excision is the treatment of choice [119]. For unresectable or metastatic disease, treatment with somatostatin analogues, such as octreotide or lanreotide, has resulted in improvement in symptoms and tumor regression [119, 120]. In addition, chemotherapeutic agents (e.g., cisplatin, etoposide) or radiotherapy may be used in patients with aggressive tumors [121].

20.2.4.2 MEN1-Related Adrenal Tumors

Clinical Manifestations and Diagnosis

Adrenocortical tumors occur in up to 73 % of the patients with MEN1 (Table 20.1) [83, 122–124] and include adenomas, hyperplasia, cysts, or carcinomas. Most are nonfunctioning [122], whereas functional tumors are reported to occur in less than 10 % of patients, with primary hyperaldosteronism and ACTH-independent Cushing's syndrome most commonly reported [122]. Pheochromocytomas are rarely described in MEN1 patients, whereas adrenocortical carcinoma is reported to occur in up to 13 % of MEN1 patients, in parallel with a tumor size of more than 1 cm [122].

Biochemical investigation (e.g., plasma renin and aldosterone concentrations, low-dose dexamethasone suppression test or 24-hour urine collection for free cortisol, urinary catecholamines and metanephrines, or plasma metanephrines) should be performed as routine for adrenal tumors. It is recommended that MEN1 patients

Table 20.2 Clinical manifestations in MEN2

		Organ involved	Specific tumor/ manifestation (prevalence by 40 years)	Clinical presentation
MEN2	MEN2A (>75 %)	Thyroid	MTC (90 %)	Neck mass or neck pain, diarrhea, flushing
		Adrenal	Pheochromocytomas (50 %)	Subtle symptoms, usually bilateral
		Parathyroid	Multigland adenoma/ hyperplasia (20–30 %)	Usually asymptomatic
		Skin	CLA	Pruritic, lichenoid skin lesion in the upper portion of the back
		MEN2A genetically related	HSCR	Bowel enlargement and constipation/obstipation
			PTC	Concomitant PTC and MTC
	FMTC (10–20 %)	Thyroid	MTC (100 %)	≥4 individuals with MTC in the same family in the absence of pheochromocytoma or parathyroid adenoma/ hyperplasia
	MEN2B (5 %)	Thyroid	MTC (90 %)	Very aggressive, usually metastatic
		Adrenal	Pheochromocytomas (50 %)	Usually multiple/bilateral (50 %)
		Other	Mucosal neuromas, frequent	Tongue, palate, or pharynx; eyelid
			Ocular signs, frequent	Inability to cry tears, thickened corneal nerves, ptosis, eyelid eversion
			Diffuse ganglioneuromatosis of the GIT (40 %)	Abdominal distension, megacolon, constipation, or diarrhea
			Marfanoid habitus (75 %)	Associated with kyphoscoliosis or lordosis, joint laxity, decreased subcutaneous fat, proximal muscle wasting, weakness

FMTC familial medullary thyroid carcinoma, *MTC* medullary thyroid carcinomas, *CLA* cutaneous lichen amyloidosis, *HSCR* Hirschsprung's disease, *PTC* papillary thyroid carcinoma, *GIT* gastrointestinal tract

with adrenal tumors should undergo an annual imaging screening (CT or MRI) of the adrenal glands (Table 20.2) [122, 124].

Treatment of MEN1-Related Adrenal Tumors

The treatment of MEN1-related adrenal tumors is similar to that for tumors occurring in the sporadic context. Surgical excision is recommended for adrenal tumors

that are more than 4 cm in diameter, or less than 4 cm but with suspicious radiological features, or for tumors with significant growth over a 6-month interval [124].

20.2.4.3 MEN1-Related Central Nervous System (CNS) Tumors

Different CNS tumors have been reported in MEN1 patients, including ependymomas, schwannomas, and meningiomas (Table 20.1). The majority of meningiomas are asymptomatic and do not enlarge. Their follow-up and treatment are similar to that occurring in non-MEN1 patients [125].

20.2.4.4 MEN1-Related Thyroid Tumors

Thyroid adenomas, nodular goiter, and thyroid carcinoma have all been reported in more than 25 % of MEN1 patients. However, the occurrence of thyroid abnormalities in these patients may be incidental, as these disorders are also frequent in the general population. The treatment of MEN1-related thyroid tumors is similar to that for sporadic cases [50].

20.2.4.5 MEN1-Related Cutaneous Manifestations

Multiple subcutaneous lipomas are reported in more than 33 % of MEN1 patients (Table 20.1). Rarely, visceral, pleural, or retroperitoneal lipomas may also occur [50]. They are usually treated conservatively but may be excised for cosmetic reasons. Facial angiofibromas and collagenomas may occur in up to 88 % of MEN1 patients (Table 20.1), and usually no treatment is required [126].

20.2.5 Genetic Testing and Screening in MEN1

20.2.5.1 MEN1 Gene

The MEN1 gene is located on chromosome 11q13 and consists of 10 exons, which encode menin, a 610-amino-acid protein [127, 128], and a tumor suppressor that regulates transcription, genome stability, cell division, and proliferation [66, 129]. Inheritance of a germline MEN1 mutation predisposes an individual to develop a tumor that arises after an additional somatic mutation of the normal allele, which may be a point mutation or a deletion, leading to loss of heterozygosity (LOH) in the tumor DNA (the Knudson two-hit hypothesis) [66].

20.2.5.2 MEN1 Germline Mutations

Over 1,336 mutations of the MEN1gene have been characterized [66]. About 75 % of the MEN1 germline mutations are inactivating, consistent with those expected in a tumor suppressor gene. There are nine sites in the MEN1 gene accounting for over 20 % of all the germline mutations [66]. Noteworthy, it appears that there is no clear-cut correlation between MEN1 mutations and MEN1 clinical manifestations. The apparent lack of genotype-phenotype correlation, together with the wide diversity of mutations in the 1830-bp coding region of the MEN1 gene, renders mutational analysis more difficult [76, 129]. Importantly, more than 10 % of MEN1 germline mutations arise de novo [129]. Up to 25 % of MEN1 patients may not harbor germline mutations in the MEN1 gene coding region, probably related to

whole or partial gene deletions, or mutations in the promoter or untranslated regions [129, 130]. Phenocopies may mimic MEN1 either by occurrence of a single sporadic endocrine tumor in a patient with familial MEN1 or occurrence of two endocrine abnormalities associated with different etiologies. Phenocopies arose in >5 % of MEN1 families and awareness of them is important in the clinical management of MEN1 and other hereditary disorders [131].

20.2.5.3 MEN1 Gene Polymorphisms

A gene is said to be polymorphic if more than one allele occupies that gene's locus within a population [132]. Twenty-four different polymorphisms of the MEN1 gene have been reported [129]. The recognition of these polymorphisms is important as they may occasionally help in families without any MEN1 mutation identified [37].

20.2.5.4 MEN1 Tumor Somatic Mutations

More than 90 % of the tumors originating in MEN1 patients exhibit LOH on 11q13, consistent with the Knudson's two-hit (second-hit) hypothesis [129]. Besides the LOH mechanism, the second-hit hypothesis may result also from intragenic deletions and point mutations.

In clinical practice, the mutational analysis of tumor DNA to identify somatic mutations adds little value as LOH involving chromosome 11q13 in the MEN1 location has been observed in up to 50 % of sporadic endocrine tumors [129, 133].

20.2.5.5 MEN1 Variants

In rare families with MEN1 variants, only some of MEN1 characteristic clinical manifestations may be observed. Familial isolated hyperparathyroidism (FIHP) is a condition characterized by the development of parathyroid tumors as the sole endocrinopathy, being reported to date in 42 FIHP kindreds: 38 % developed missense mutations, and fewer than 31 % presented with nonsense or frameshift mutations [129, 134]. Another MEN1 variant is the Burin or prolactinomas variant, characterized by a high occurrence of prolactinomas and a low occurrence of gastrinomas [135–137] as result of nonsense mutations (Tyr312Stop and Arg460Stop). Another example is Tasmanian MEN1 kindred in whom the absence of somatotropinomas was observed as a result of a splice site mutation [138].

20.2.5.6 MEN1 Mutational Analysis in Clinical Practice

MEN1 mutational analysis is important for confirmation of the clinical diagnosis, for the identification of family members who require screening for tumor detection and appropriate treatment, as well as for the identification of the 50 % of family members who do not harbor the familial germline MEN1 mutation and can therefore be reassured [50].

MEN1 mutational analysis should be performed in the following circumstances [27, 139, 140]:

- In an index case with ≥2 classic MEN1-associated endocrine tumors (i.e., parathyroid, pancreatic, or pituitary tumors)
- In asymptomatic first-degree relative of a known MEN1 mutation carrier

- In a first-degree relative of an MEN1 mutation carrier having symptoms, signs, biochemical, or radiological evidence for one or more MEN1-associated tumors
- In patients with suspicious or atypical MEN1, including individuals with parathyroid adenomas diagnosed before the age of 30 years
- In patients with multigland parathyroid disease, gastrinoma, or multiple pancreatic NETs at any age
- In individuals who have two or more MEN1-associated tumors not part of the classical triad of parathyroid, pancreatic islet, and anterior pituitary tumors
- In those individuals presenting at an early age with a single, apparently sporadic MEN1-associated tumor

20.2.5.7 MEN1 Tumors Identification

As MEN1 syndrome is associated with a complex spectrum of clinical, diagnostic, and therapeutic aspects, it is recommended for these patients and their families to be followed by teams with expertise in the management of MEN1, including an endocrinologist experienced in MEN syndromes [50]. As the MEN1 gene mutation appears to be nonpenetrant in individuals younger than 5 years, but with a >50 % penetrance by 20 years of age and >95 % by 40 years, a timely biochemical and imaging screening in asymptomatic members of MEN1 families is imperative, as earlier diagnosis and treatment may reduce the morbidity and mortality related to these tumors [5, 39, 66, 141].

PHPT-induced hypercalcemia is usually the first manifestation of MEN1 and therefore has become an easy biochemical screening investigation. In addition, hyperprolactinemia, usually asymptomatic, may represent the first manifestation in approximately 15 % of patients and may be easily detected [66]. PNETs may be detected in asymptomatic individuals by measuring fasting plasma concentrations of gastrin, pancreatic polypeptide, glucagon, and chromogranin A and by abdominal imaging [142, 143].

The latest guidelines [50] suggest that mutant MEN1 gene carriers should undergo biochemical screening (including evaluation of serum calcium, PTH, gastrin, insulin with a fasting glucose, glucagon, VIP, pancreatic polypeptide, chromogranin A, prolactin, and IGF-I) at least once a year. Baseline pituitary and abdominal imaging (e.g., MRI or CT) should be performed and then repeated at 1–3 years' interval, as well as imaging for thymic and bronchial carcinoids using CT or MRI every 1–2 years. Screening should possibly commence in early childhood (e.g., by the age of 5 years) and should be repeated throughout life [50].

20.3 Multiple Endocrine Neoplasia Type 2 (MEN2, Sipple Syndrome)

Type 2 multiple endocrine neoplasia (MEN2) is a rare familial cancer syndrome caused by mutations in the RET proto-oncogene. The association between thyroid cancer and pheochromocytoma was first described by Sipple in 1961 [144]. In 1965,

the thyroid cancer occurring in association with pheochromocytoma was defined as medullary thyroid carcinoma (MTC), characterized by stromal amyloid. Since 1968, the familial constellation of MTC in conjunction with pheochromocytoma and with parathyroid hyperplasia was recognized as MEN2. Although patients with mucosal neuromas were already identified, the distinction between MEN2A and MEN2B subtypes was only in 1975 [145].

MEN2 is an autosomal dominant syndrome classified into main two variants: MEN2A and MEN2B [146]. All variants of MEN2 show a high penetrance for MTC, which is diagnosed in as much as 90 % of MEN2 carriers (as a palpable thyroid nodule or an increase in blood calcitonin levels, CLT) [146, 147].

All MEN2 subtypes are caused by a germline mutation in the RET proto-oncogene and specifically with selected RET codon mutations [148, 149].

20.3.1 The Clinical Presentation of MEN2 Syndromes (Table 20.2)

20.3.1.1 Clinical Presentation of MEN2A

MEN2A is characterized by the development of MTC in 90 % of adult gene carriers, unilateral or bilateral pheochromocytoma in 50 %, and multigland parathyroid adenoma/hyperplasia with primary hyperparathyroidism (PHPT) in 20–30 % [150–152]. MEN2A accounts for over 75 % of MEN2 [153] and may present as rare variants including familial MTC (FMTC) [151, 154], MEN2A with cutaneous lichen amyloidosis [155, 156], and MEN2A or FMTC with Hirschsprung's disease [157].

MEN2A-Related MTC

Hereditary MTC is a rare calcitonin (CLT)-producing tumor developing from the parafollicular or C cells of the thyroid gland [158, 159]. Multifocal C-cell hyperplasia (CCH) is the precursor lesion to hereditary MTC, and the age of transformation from CCH to microscopic MTC varies with different germline RET mutations and may take years [160, 161]. MTC is generally the first manifestation of MEN2A, and the patients typically present with a neck mass or neck pain, usually before age 35 years, with cervical lymph node metastases in up to 70 % [162]. Diarrhea, the most frequent systemic manifestation (~30 % of patients with advanced MTC), occurs in affected individuals with a plasma calcitonin concentration of >10 ng/mL and implies a poor prognosis [163]. Approximately 10 % of MTC patients have flushing often induced by alcohol ingestion, calcium infusion, or pentagastrin injection [164]. All individuals with an MTC-predisposing mutation who have not undergone prophylactic thyroidectomy demonstrate biochemical evidence of MTC by age 35 years [147]. Metastatic spread may occur locally (to the central, lateral, cervical, and mediastinal lymph nodes) or distantly (to the lungs, liver, or bones) [165].

MEN2A-Related Pheochromocytomas

Pheochromocytomas usually present concomitantly or after MTC; however, they may be the presenting symptom in up to 27 % of individuals with MEN2A [166–168]. MEN2-related pheochromocytomas are usually diagnosed earlier, have subtler

symptoms, and are more likely to be bilateral than sporadic tumors [169, 170]. Pheochromocytomas in MEN2 patients entail a distinct biochemical phenotype in that they consistently produce epinephrine or epinephrine and norepinephrine [171]. Malignant transformation was reported in ~4 % of cases [172].

MEN2A-Related Primary Hyperparathyroidism (PHPT)

MEN2A-related PHPT is usually mild, being diagnosed many years after the diagnosis of MTC, with an average age of onset ~38 years [173]. Most individuals are asymptomatic; however, hypercalciuria with renal calculi may occur. In case of long-standing or underdiagnosed disease, hypercalcemia-related symptoms may develop and become severe, in parallel with disease progression [174].

MEN2A-Related Cutaneous Lichen Amyloidosis (CLA)

Pruritic CLA, a lichenoid skin lesion usually located over the upper portion of the back, has been reported in a small number of families with MEN2A carrying codon 634 mutations [175, 176].

20.3.1.2 Clinical Presentation of FMTC

FMTC is historically diagnosed in families with four or more cases of MTC in the absence of pheochromocytoma or parathyroid adenoma/hyperplasia [153, 154] and comprises approximately 10–20 % of cases of MEN2. Because *RET* accounts for all clinical subtypes of MEN2, FMTC may be viewed as MEN2A with reduced organ-specific penetrance. The age of onset of FMTC is later, and the penetrance of MTC is lower than what is observed in MEN2A and MEN2B [177, 178].

20.3.1.3 Clinical Presentation of MEN2A Genetically Related (Allelic) Disorders

Hirschsprung's Disease (HSCR)

HSCR is a genetic disorder characterized by aganglionosis of the gut, likely due to absent gut ganglia and typically resulting in bowel enlargement and constipation/obstipation in neonates [179]. Up to 50 % of familial cases and up to 35 % of simplex cases (i.e., a single occurrence in a family) of HSCR are caused by germline loss-of-function mutations in the RET proto-oncogene [180, 181].

Papillary Thyroid Carcinoma (PTC)

Up to 40 % of PTCs are associated with somatic gene rearrangements that cause juxtaposition of the tyrosine kinase domain of RET to various gene partners [182, 183]. A few families with rare RET exon 13 and exon 14 germline mutations have demonstrated concomitant MTC and PTC [184, 185].

20.3.1.4 Clinical Presentation of MEN2B

MEN2B comprises approximately 5 % of cases of MEN2, and it is the most aggressive variant of MEN2 syndrome, characterized by MTC and pheochromocytoma, together with a marfanoid habitus and mucosal (lips and tongue) and intestinal ganglioneuromatosis, but not PHPT [186, 187].

MEN2B-Related MTC

MEN2B-related MTC is the most aggressive form of MTC in all affected individuals and metastasizes in all MEN2B carriers who do not undergo thyroidectomy at an early age (<1 year). Before intervention with early prophylactic thyroidectomy, the average age of death in individuals with MEN2B was 21 years [188].

MEN2B-Related Pheochromocytomas

Pheochromocytomas occur in 50 % of individuals with MEN2B; approximately half are multiple and often bilateral [189]. Most commonly, these patients complain of palpitations, anxiety spells, or headache early in their disease; however, in many patients whose tumors were found by family screening, there are no symptoms. Hypertension spells may not be noted until a crisis is induced by an operation or delivery [190].

MEN2B-Related Other Clinical Presentations

- *Mucosal neuromas* (Figure 20.1) may be identified in early childhood in MEN2B carriers and develop on the anterior dorsal surface of the tongue, palate, or pharynx, together with a distinctive facial appearance. The lips become prominent (or "blubbery") over time as a result of submucosal nodules present on the vermilion border of the lips. Eyelid neuromas may cause thickening and even eversion of the upper eyelid margins [191].
- *Ocular Signs of MEN2B*: children with MEN2B have the inability to cry tears, and this is an important clinical symptom for early diagnosis preceding the development of metastatic MTC [192]. Other ocular signs develop during childhood and include prominent thickened corneal nerves (seen by slit lamp examination) [193, 194], eyelid neuromas, lid margin eversion or thickening, subconjunctival neuromas, or ptosis [195–197]. There are usually no fundal findings on examination, but in patients presenting already with MTC, choroidal metastases have been observed [198].
- *Diffuse ganglioneuromatosis of the gastrointestinal tract* is reported in ~ 40 % of affected individuals, and it is associated with early childhood symptoms of aganglionic megacolon [199].

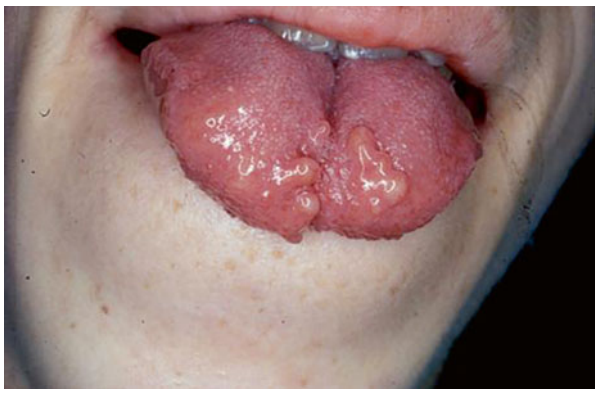

Fig. 20.1 MEN2B-associated oral mucosal neuromas

- *Marfanoid habitus*, often with kyphoscoliosis or lordosis, joint laxity, decreased subcutaneous fat, proximal muscle wasting, and weakness, can also be seen in ~ 75 % of affected individuals [200].

20.3.2 The Diagnosis of MEN2 Syndrome Manifestations

20.3.2.1 MTC Diagnosis

CLT values (basal or stimulated by pentagastrin, calcium, or both) are nearly always elevated in MTC [201], being a specific and a sensitive marker, and elevation after surgery suggests persistent or recurrent disease [202]. In provocative testing, plasma CLT concentrations are measured before and 2 and 5 min after intravenous administration of the CLT secretagogue. Reference levels for basal calcitonin vary across laboratories, and usually levels of <10 pg/mL for adult males and <5 pg/mL for adult females are typically considered normal. Furthermore, a basal or stimulated calcitonin level of ≥100 pg/mL is considered an indication for surgery [203, 204]. Preoperative calcitonin testing may be useful for assessing CCH (C-cell hyperplasia) or tumor spread and should be considered when deciding the extent of surgery for MEN2A MTC [205].

20.3.2.2 Pheochromocytoma Diagnosis

Pheochromocytoma should be suspected whenever elevated levels of catecholamines and/or their metabolites (i.e., norepinephrine, epinephrine, metanephrines, and vanillylmandelic acid) are demonstrated in plasma or 24-h urine collections [170, 206]. Noteworthy, in MEN2, pheochromocytomas consistently produce epinephrine or epinephrine and norepinephrine [206]. Abdominal magnetic resonance imaging (MRI) and computed tomography (CT) are performed whenever a pheochromocytoma is suspected clinically or biochemically, with the MRI being more sensitive than CT in detection of these tumors. [^{18}F]-fluorodopamine positron emission tomography (PET) is the best overall imaging modality in the localization of pheochromocytomas, and if unavailable, then ^{123}I- or ^{131}I-metaiodobenzylguanidine scintigraphy (MIBG) should be used for further tumor localization and staging [207].

20.3.2.3 PHPT Diagnosis

The diagnosis of PHPT is made when simultaneously elevated serum concentrations of calcium and elevated or high-normal parathyroid hormone are demonstrated on biochemical screening. For adenoma localization, either pre- or postoperatively (in case of recurrence/persistence), localizing studies with [99] mTc-sestamibi scintigraphy or three-dimensional single-photon emission CT may be used [208].

20.3.3 Management of MEN2 Manifestations

20.3.3.1 MEN2-Related MTC Treatment

The main therapeutic approach is surgery; successful excision is dependent upon the adequacy and timing of the initial operation [209–212]. Surgery for MTC should be performed, if possible, before the age of the possible malignant progression, and the likelihood of metastasis is higher if the first operation is performed in an adult [213]. If the basal or stimulated CLT values are elevated, a minimum surgical approach should include total thyroidectomy with central lymph node dissection [214]. Total thyroidectomy with central and complete bilateral neck dissection should be performed routinely in all patients with hereditary MTC, even in those with small thyroid tumors—a contralateral neck dissection may be avoided only in sporadic MTC patients with unilateral involvement of the thyroid gland in the absence of central and ipsilateral neck involvement [214]. If after the primary thyroid surgery the basal (or stimulated) plasma CLT levels are high, it is important to define the extent of the residual disease [215]; if there is no evidence of distant spread and local disease is found, then reoperation is recommended [216, 217]. Surgical intervention is not indicated if distant metastases are found, unless the patient develops respiratory symptoms or secretory diarrhea, for which tumor debulking may help [218]. For systemic disease, standard chemotherapeutic regimens have not proven beneficial, have considerable toxicity, and are therefore not recommended [203, 219, 220], whereas the benefit of external beam irradiation therapy is controversial [221]. In recent years, several kinase inhibitors (including axitinib, cabozantinib (XL-184), lenvatinib (E7080), motesanib, pazopanib, sorafenib, sunitinib, and vandetanib) have been evaluated in phase I and II clinical trials [222], and two of these drugs, vandetanib and cabozantinib, have completed phase III clinical trials. Vandetanib (300 mg/day) prolonged median progression-free survival (PFS) from 19 months in the placebo arm to a predicted median of 31 months in the vandetanib arm [223] and was therefore approved by the FDA in April 2011 and by the EMEA in February 2012. Cabozantinib (175 mg/day) prolonged PFS prolongation from 4 to 11 months and was approved by the FDA in November 2012 [224]. However, taking into account that the toxicity of these drugs is significant, with dose reduction or treatment withdrawal in a significant proportion of patients, and that there is currently no evidence for a higher treatment efficacy at an earlier disease stage nor survival benefit, these treatments should be initiated only in patients with documented tumor progression [225].

20.3.3.2 MEN2-Related Pheochromocytomas Treatment

Pheochromocytomas are usually removed by adrenalectomy, which may be performed by video-assisted laparoscopy [226]. Before operation, antihypertensive treatment should be instituted with initial α-adrenergic blockade, and if tachycardia ensues, β-adrenergic receptor blockade can be added [170, 227]. Because of an

increased risk of adrenal insufficiency after bilateral adrenalectomy, most experts recommend to date unilateral adrenalectomy in unilateral tumors and cortical-sparing adrenal surgery with close monitoring of the remnant tissue in persons with one remaining adrenal gland or bilateral pheochromocytoma [166].

The presence of a pheochromocytoma has to be excluded in any individual with MTC or MEN2 before any surgery, as patients with undiagnosed tumors may die from an uncontrolled hypertensive crisis perioperatively [228]. If detected, pheochromocytoma excision should precede any other surgical intervention to avoid an intraoperative crisis [229].

20.3.3.3 MEN2-Related Primary Hyperparathyroidism (PHPT) Treatment

Once the biochemical diagnosis of PHPT is confirmed in an MEN2 patient, the indications for surgical excision are similar to those in patients with sporadic PHPT and include symptomatic or marked hypercalcemia, nephrolithiasis, major hypercalciuria, and evidence of bone loss [230]. The presence/absence of pheochromocytoma should be established before the parathyroid surgery in order to avoid a life-threatening pheochromocytoma crisis.

One of the important concerns in the management of MEN2-related PHP is the ability to identify single versus multiple gland disease and subsequently decide on the appropriated surgical procedure in order to prevent recurrences. Initially, treatment of MEN2-related PHPT included subtotal parathyroidectomy or total parathyroidectomy with forearm autograft [173]. However, it appears that sporadic PHPT and MEN1- and MEN2A-related PHPT are three distinct entities as is reflected preoperatively by differences in gender, age at diagnosis, and calcium and PTH levels; MEN2A patients are very similar to sporadic PHPT with respect to operative approach and findings [231]. Minimally invasive procedure is the treatment of choice for both, with low rates of persistent and recurrent PHPT and a low complication rate. The percentage of multiglandular disease and recurrences are significantly higher in MEN1 patients, demonstrating the need for a different approach [231].

Individuals with PHPT who have undergone prior thyroidectomy should have preoperative localization with excision of the localized hypertrophied parathyroid glands and forearm autotransplantation. Therapy with medications to control PHPT should be considered in individuals with a high risk of surgical mortality, limited life expectancy, or persistent or recurrent PHPT after one or more surgical attempts [173].

20.3.4 Prevention of MEN2-Related Primary Manifestations

- *Prophylactic thyroidectomy*, a major preventive measure for all age groups of carriers of a germline RET mutation [162, 203], is a reasonably safe decision when performed by experienced surgeons. To date, there is still controversy about the timing of the surgery [232] and it is recommended to be guided by the codon position of the RET mutation [203]. Thyroidectomy before progression to invasive MTC may allow thyroidectomy with sparing of lymph nodes [233]. For all individuals with a RET mutation who have not underwent a thyroidectomy, annual

biochemical screening is recommended, with immediate thyroidectomy if results are abnormal [234]. Annual serum CLT screening should start at age 6 months for MEN2B children and at age 3–5 years for those with MEN2A or FMTC. Importantly, caution should be used in interpreting calcitonin results for children younger than 3 years and mainly in children younger than 6 months [203].

20.3.5 Molecular Genetic Testing for MEN2 Patients

MEN2 is associated with activating mutations in the RET proto-oncogene, and therefore RET molecular genetic testing is indicated in all individuals with a clinical diagnosis of MTC, MEN2, or primary CCH [5].

It is recommended that all MTC patients, including those with clinical/family suspicion for MEN2, should undergo germline RET analysis for exons 10, 11, and 13–16, as ~98 % of MEN2A patients have a mutation in exon 10 or 11 [153, 203] and ~95 % of FMTC patients have a mutation in exons 10, 11, and 13–16 [235]. Importantly, if exon-specific testing is negative, then sequencing of the entire RET coding region should be completed in the setting of an MEN2A.

In case of MEN2B suspicion, targeted mutation analysis or sequencing of exons 16 and 15 to detect the p.M918T and p.A883F mutations should be firstly performed, and if negative, testing for p.V804M in exon 14 followed by sequencing of the entire RET coding region should be done [170]. Moreover, in the subset of patient with apparently isolated adrenal pheochromocytoma, RET as well as VHL and succinate dehydrogenase (SDH) molecular genetic testing should be considered [236–238].

20.3.5.1 MEN2 Genotype-Phenotype Correlation

MEN2 RET genotype-phenotype correlations were the first to be found in inherited neoplasia syndromes [153], and the gain-of-function mutations affected several hot spot codons (the great majority mutating cysteine residues in exons 10 and 11) [153].

Notably, mutations of codon 634 in exon 11 are highly associated with the full-blown phenotype of MEN2A (high prevalence of pheochromocytoma and hyperparathyroidism), with p.C634R being the most common, the most highly penetrant, and the most fulminant and aggressive [1, 239]. Twenty-five percent of FMTC kindreds harbor a mutation in codon 634 (commonly p.C634Y) [235].

RET germline p.M918T mutations are associated with MEN2B; however, somatic mutations at this codon are frequently observed in MTC in individuals with no known family history and being overrepresented in individuals with sporadic MTC who have a particular germline RET variant [240].

Mutations involving the cysteine codons 609, 618, and 620 in exon 10 of RET are associated with MEN2A or FMTC together with HSCR [241]. Mutations at codons 768, 804, and 891 that were initially only associated with FMTC have subsequently been found in rare families with MEN2A [242–244].

Phenotypic expression of mutations at codon 804 has been shown to be highly variable, even within the same family [245, 246]: some individuals have MTC at age 5 years and fatal metastatic MTC at age 12 years, whereas other individuals

have been shown to have normal thyroid histology at age 27 years, normal biochemical screening at age 40 years, and no clinical evidence of MTC at age 86 years [247]. Interestingly, it was suggested that in addition to their association with MTC, mutations in codons 790 or 804 may be associated with PTC [185].

The American Thyroid Association Guidelines Task Force has classified mutations based on their risk for aggressive MTC, and this risk classification may be used in the decision for the age of prophylactic thyroidectomy or when to begin biochemical screening for pheochromocytoma and hyperparathyroidism [153].

20.3.6 MEN2 Patient Surveillance

20.3.6.1 Medullary Thyroid Carcinoma

Recurrent disease is expected in as much as 50 % of MTC patients following total thyroidectomy and neck dissections [162], and therefore, continued monitoring for residual or recurrent MTC is indicated for life. The screening protocol for MTC includes an annual measurement of serum calcitonin; a more frequent follow-up (3–6 months) is recommended for those with residual disease and should include biochemical analysis for calcitonin, together with appropriate imaging (neck ultrasound, high-resolution CT, or [^{18}FDG]-PET-CT in case of elevated tumor markers) [5].

20.3.6.2 Pheochromocytoma

If the initial screening for pheochromocytoma is negative, then annual biochemical screening is recommended, followed by MRI and/or CT if the biochemical results are abnormal [153]. Women with MEN2 should be screened for pheochromocytoma before a planned pregnancy. Annual biochemical screening starting at the age of 8 years has been recommended for individuals with MEN2A caused by mutations of codons 630 and 634 and at age 20 years for mutations in all other codons, whereas MEN2B carriers should begin screening for pheochromocytoma at age 8 years [153].

20.3.6.3 PHPT

Annual serum calcium and parathyroid hormone concentrations are recommended for at-risk individuals who have not had parathyroidectomy [248]. In MEN2A, screening should begin at age 8 years for individuals with mutations of codons 630 and 634 and by age 20 years for individuals with other RET mutations [153]. Screening is likely unnecessary in MEN2B [153].

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Thyroid Cancer

21

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21.1 Introduction

Thyroid carcinoma is the most common endocrine malignancy (95 % of all endocrine malignancies) accounting for approximately 2 % of all cancers diagnosed worldwide [1]. Recent reports describe a continuous increase in thyroid cancer incidence worldwide; this increase is as high as 250 % in some areas, especially in the United States of America (USA) [2]. The American Cancer Society (ACS) estimates that there will be about 60,220 new thyroid cancer cases, and 1,850 people will die of thyroid cancer in 2013, in the USA [3]. Despite the generally good prognosis of thyroid carcinoma, 5–10 % of patients will die of the disease [4, 5]. Thyroid carcinoma is responsible for the 66 % of the deaths due to endocrine cancers.

Thyroid cancer is two- to threefold more common in females than males, although the latter gender dies of cancer more often than women (45,310 and 14,910 new thyroid cancer cases and 810 and 1,040 deaths from thyroid cancer in women and men, respectively, estimated in 2013 in the USA) [3]. It is now the fifth most

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common cancer (6 % of all female cancers) among women in the USA [3]. Papillary cancer is the most common type in the USA and has excellent prognosis.

The peak incidence of thyroid cancer diagnosis is 45–49 years in women and 65–69 years in men; it also affects young people. Thyroid cancer accounts for approximately 10 % of malignancies diagnosed in individuals aged 15–29 years [6].

Thyroid carcinoma can arise from either follicular or nonfollicular thyroid cells. Follicular cancers include papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC), Hürtle cell cancer or oncocytic cancer (HCC), and anaplastic thyroid cancer (ATC). Medullary thyroid cancer (MTC) arises from nonfollicular thyroid cells (other names are parafollicular C cells and calcitonin-producing cells) and accounts for 5 % of thyroid cancers. PTC and FTC are commonly referred together as differentiated thyroid cancer (DTC) which account for 94 % of thyroid cancers. Less than 1–3 % of thyroid cancers are ATC [7, 8].

21.2 Etiology

21.2.1 External Radiation

The only well-established risk factor for differentiated thyroid cancer, specifically PTC, is external head and neck radiation during infancy, childhood period, and young adulthood [6, 7]. The younger the patient was at the time of exposure, the higher the risk of developing cancer. Iodine deficiency increases the risk for radiation-induced thyroid cancer [9]. From the 1920s to 1960s, external low-dose radiation therapy had been used for the treatment of a variety of benign diseases such as acne, tonsillitis, enlarged thymus, and fungal disease of the scalp [10–12]. Treatment of malignant diseases with high-dose radiation therapy to the head and neck (>20 Gy), such as Hodgkin's disease, non-Hodgkin's lymphoma treated with mantle-field irradiation, Wilms' tumor, and neuroblastoma, especially at young ages, is also together with increased benign and malign thyroid neoplasm [10, 13]. Epidemiologic studies show that between 7 and 9 % of patients who received 5–10 Gy external radiation develop thyroid cancer [14]. Relative risk is linearly related to exposure dose, starting as low as 0.1 Gy and at least up to 30 Gy [14]. Exposure to ionizing radiation results in a 30 % risk for thyroid cancer.

The average time duration between irradiation and thyroid tumor is 10–20 years; however, periods from 5 to 50 years have been reported [14–16]. The majority of cases occur between 20 and 40 years after exposure. Exposure to the external sources of radiation, radioisotopes of iodine after atomic bombing, and the Chernobyl nuclear accident led to an increased incidence of PTC in fallout regions, especially in younger children [17]. In some regions of Japan, the incidence of thyroid cancer in a screened population is as high as 0.1 %, 10-fold higher than expected on US incidence rates. Within the first decade after the Chernobyl accident, some regions of Belarus showed a 100-fold increase in thyroid cancer in individuals, below the age of 15 at the time of exposure [18]. In one of the last publications from Japan, the excess thyroid cancer risk associated with childhood exposure has persisted for >50 years after atomic-bomb exposure [19].

There was no evidence that the diagnostic use of ^{131}I increased the risk of thyroid cancer. The therapeutic use of ^{131}I for ablation of thyroid tissue to treat hyperthyroidism seems to be associated with a very modest increased risk of thyroid cancer [20].

21.2.2 Dietary Factors

Thyroid cancer incidence is different in different geographic areas. Dietary influences have primarily focused on the level of iodine in the diet [21]. In some regions with high dietary iodine intake due to shellfish, such as Iceland, Norway, and the Pacific Rim, PTC incidence is higher. However, more recent data suggest that the relatively elevated levels of fish consumption do not appreciably increase thyroid cancer risk [22]. Iodine deficiency is associated with follicular and anaplastic thyroid carcinomas.

21.2.3 Prolonged Thyroid-Stimulating Hormone (TSH) Stimulation

The association of thyroid cancer and Hashimoto's thyroiditis, Graves' disease, and multinodular goiter has been reported. However, any causative relationship between these diseases remains poorly documented [7]. There is no increased incidence of thyroid cancer among patients with primary hypothyroidism.

21.2.4 Familial-Hereditary Factors

Familial cases have been reported in 5 % of all patients with PTC, and they have more aggressive disease course [7]. Epidemiologic studies have demonstrated a four- to tenfold increased risk of DTC in first-degree relatives of the patients [23]. DTC can both be inherited in an autosomal dominant fashion as the main part of some syndromes (PTC with papillary renal neoplasia, familial non-medullary thyroid carcinoma, and familial thyroid tumors with cell oxyphilia) or have an increased incidence in other tumor susceptibility syndromes (familial adenomatous polyposis, Cowden disease, and Carney complex 1) [6, 7, 24]. Family history is important for MTC. Multiple endocrine neoplasia (MEN) 2A and 2B syndromes and familial MTC (FMTC) syndrome associated with germline mutation of *RET* proto-oncogene. In these syndromes, prophylactic thyroidectomy should be undertaken in at-risk individuals at young ages [25].

21.2.5 Other Factors

Oral contraceptive use, late menarche, late age at first birth, and benign thyroid nodules have been studied. But the results are mixed, and there is no clear association [26, 27].

21.3 Pathology

There are four major types of thyroid carcinomas according to morphology and biological behavior [6, 7]: papillary, follicular, medullary and anaplastic carcinomas. Papillary and follicular carcinomas are called as DTC. The WHO (World Health Organization) classification of thyroid carcinomas is shown in Table 21.1 [28].

21.3.1 Differentiated Thyroid Carcinomas (DTC)

88 % of all thyroid carcinomas are DTC. Papillary tumors arise from thyroglobulin (Tg)-producing follicular cells (thyrocytes), and 85 % of DTC are PTC in developed countries where sufficient iodine is present in the diet [6, 7, 29]. PTC is the most well-differentiated histology. It usually presents as multifocal lesions, with a high incidence of regional lymph node metastases [30]. They vary in size from

Table 21.1 Tumors of the thyroid according to the WHO classification, distribution, and survival [29, 60]

Tumors	Frequency %	10-year survival (%)
Papillary carcinoma	80	93
Classic papillary		
Follicular variant		
Macrofollicular variant		
Oncocytic variant		
Clear cell variant		
Diffuse sclerosing variant		
Solid variant		
Cribriform carcinoma		
Papillary carcinoma with focal insular component		
Papillary carcinoma with squamous cell and mucoepidermoid carcinoma		
Papillary carcinoma with spindle and giant cell carcinoma		
Combined papillary and medullary carcinomas		
Papillary microcarcinomas		
Follicular carcinoma	11	85
Minimal and widely invasive		
Oncocytic variant (Hürtle cell)	3	76
Clear cell variant		
Poorly differentiated carcinoma		34
Undifferentiated (anaplastic) carcinoma	2	No data
Medullary thyroid carcinoma	4	75
Follicular adenoma		

microscopic cancer to large tumor. They may invade the thyroid capsule and contiguous structures and lymphatics. But blood vessel invasion is rare. Cystic changes, calcifications, and even ossification may be identified. Psammoma bodies, calcified scarred remnants of tumor papillae, are commonly seen in about 50 % of PTC and pathognomonic for PTC [6, 7, 29, 30]. Pure PTC has very good prognosis. But the insular, tall cell, columnar cell, and diffuse sclerosing variants are more aggressive forms of PTC [28] (Table 21.1). PTC less than 1 cm are often referred to as microcarcinomas [6, 7, 29]. Papillary thyroid carcinoma seems closely related to the activation of *trk* and *RET* proto-oncogenes. The *trk* proto-oncogene codes for the tyrosine kinase receptor; the ret shows a paracentric inversion of chromosome 10 and 11 in 30–35 % of the cases. Activating *RET* mutations may be the result of ionizing radiation [31]. *RET*/*PTC* gene rearrangements or *RAS*, *BRAF*, or *MEK-ERK* pathway mutations are present in 70 % of PTCs, and the upregulation of vascular endothelial growth factor (VEGF) signaling is also common in metastatic disease [6, 7, 32]. *RET*/*PTC* genetic alterations are the most common mutation found in the Chernobyl radiation-induced thyroid carcinomas [33, 34]. The most common were *RET*/*PTC1* and *RET*/*PTC3*, and the latter was associated with the more aggressive form of PTC. *BRAF* mutation is the most common genetic alteration in thyroid cancer, particularly in PTC. *BRAF* mutation is associated with poor clinicopathologic characteristics of PTC. Detection of *BRAF* mutation on FNA specimen before surgery is recommended as a useful diagnostic marker and prognostic indicator for PTC and thus influences the surgeon's decision on the management of PTC [35]. The prevalence of genetic alterations in patients with various thyroid carcinomas is outlined in Table 21.2 [36].

FTC is 12 % of DTC. True FTC is an unusual tumor comprising approximately 5–10 % of thyroid malignancies in non-endemic goiter areas of the world [29]. Prior to the introduction of iodinated salt, follicular carcinoma was much more frequently diagnosed. Follicular tumors although frequently encapsulated, microscopically vascular and capsular invasion commonly present. This histologic finding distinguishes benign neoplasms from malignant follicular neoplasm. Cytology alone cannot be used to diagnose follicular carcinoma [6, 7]. FTC may be associated with *RAS* mutations and mutations on chromosome 3 (PAX*8*-*PPAR*) [6, 36] (Table 21.2).

Table 21.2 Prevalence of genetic alterations in patients with various thyroid carcinomas [36]

Altered gene	Poorly differentiated thyroid carcinoma (%)	Papillary thyroid carcinoma (%)	Follicular thyroid carcinoma (%)	Anaplastic thyroid carcinoma (%)
RET/PTC	0	20	0	0
TP53	20–30	0	0	65–70
BRAF	15	45	0	20–25
RAS	30–35	10–15	4	50–55
Beta-catenin	20–25	0	0	65
PAX8-PPARγ	0	0	35	0

HCC or oncocytic carcinoma is a histological variant of FTC often of more aggressive behavior and accounts for 3 % of DTCs [6, 7].

Many tumors have both papillary and follicular elements histologically, and they are called follicular variants of papillary carcinoma and classified as papillary carcinoma. Because the clinical behavior of these tumors are not different from pure PTC [7, 37]. DTCs most often secrete thyroglobulin, and it can be used as a tumor marker.

Poorly differentiated thyroid carcinoma (PDTC) was introduced and defined by Sakamoto et al. and Carcangiu et al. in the 1980s [38, 39]. PDTC has aggressive histologic behavior with necrosis, increased mitotic rate, and vascular invasion. They account for 4–7 % of all thyroid cancers [36]. *RET*/*PTC* genetic alterations are 0 %, and *TP53* mutations are present in 20–30 % of cases [36] (Table 21.2). PDTC is more aggressive than DTC and less aggressive than ATC.

Other very rare forms of PTC and FTC are seen in Table 21.2.

21.3.2 Medullary Thyroid Carcinoma (MTC)

MTCs are derived from parafollicular cells or calcitonin-secreting C cells. RET proto-oncogene mutations are characteristic, with germline activating *RET* mutations as seen in FMTC and MEN 2A predisposing factor. Calcitonin and carcinoembryonic antigen (CEA) can be used as tumor markers [6, 7, 36, 37] (Table 21.2).

21.3.3 Anaplastic Thyroid Cancer (ATC)

ATC is the most aggressive form of thyroid carcinomas and typically the cause of death within 6 months. One-year survival is about 20 %. This type is less than 3 % of thyroid malignancies in the USA [6, 7, 37]. ATC can occur de novo or has arisen from more differentiated cancer that went undiagnosed for many years. In some cases there is a spectrum from papillary to anaplastic cancer [40]. ATC is distinguished from poorly differentiated (grade 3) thyroid carcinoma in part by loss of TTF-1 expression and abnormalities in *p53* signaling pathway [6, 36] (Table 21.2). *BRAF* and *RAS* mutations are also shown in ATC [36] (Table 21.2).

21.3.4 Other Cancers of the Thyroid Gland

Thyroid lymphoma (5 %), thyroid sarcoma (<1 %), and squamous carcinoma of the thyroid (<1 %) are the other very rare cancers of the thyroid gland [6, 7, 29, 30, 37]. Breast, lung, and kidney cancers are the most frequent cancers that metastasize to the thyroid.

21.4 Diagnosis

The majority of patients with thyroid cancer present with a clinically or ultrasonographically (USG) detectable solitary nodule. All thyroid nodules in the general population have a 5–8 % chance of malignancy [6, 7, 29, 41, 42]. History, physical examination, USG, and FNA are the four most important diagnostic methods. History is very important: history of dyspnea, dysphagia, and persistent dysphonia and male sex, age <14 years or >70 years, personal thyroid cancer history with lobectomy, radiation exposure in childhood or adolescent period, first-degree relative with thyroid cancer or MEN2, personal history of familial adenomatous polyposis, Carney complex, Cowden syndrome, FDG avid on PET scan are the high risk clinical features for malignancy. Firm and hard fixed nodule/s or rapidly growing nodules on physical examination and cervical lymph node/nodes palpation are important findings for malignancy [6, 7, 29, 30, 37, 42, 43].

Neck and thyroid USG and fine needle aspiration (FNA) are very important in the differential diagnosis of thyroid nodules: sonographic features and thresholds for FNA, according to the National Cancer Center Network (NCCN) guideline, are outlined in Table 21.3 [42]. The rate of carcinoma of a suspicious nodule is about 20 %. The false-positive and false-negative FNA cytology rates for all nodules are less than 5 % [7, 29, 37]. The Bethesda system suggests a six-category classification system to report thyroid FNA biopsy (FNAB) results [44]. In one of the last studies, the authors reviewed eight published studies including 25,445 thyroid FNABs [45]. Twenty-five percent of the patients (6,362 pts) subsequently underwent thyroidectomy. The final pathology results were compared to the FNAB results.

Table 21.3 Thyroid carcinoma: nodul evaluation according to the sonographic features and thresholds for fine niddle aspiration [42]

Sonographic features	Threshold for FNA
Solid nodule	
With suspicious sonographic features (hypoechoic, increased central vascularity, infiltrative margins, microcalcifications, taller than wide in transverse plane)	≥1.0 cm
Without suspicious sonographic features	≥1.5 cm
Mixed cystic-solid nodule	
With suspicious sonographic features (hypoechoic, increased central vascularity, infiltrative margins, microcalcifications, taller than wide in transverse plane)	≥ 1.5–2.0 cm
Without suspicious sonographic features	≥2.0 cm
Spongiform nodule (aggregation of multiple microcystic components in more than 50 % of the volume of the nodule)	≥2.0 cm
Purely cystic nodule	Not indicated except as therapeutic modality
Abnormal cervical lymph nodes	FNA node ± FNA-associated thyroid nodule(s)

NCCN thyroid cancer guideline, v.2.2013

1. Nondiagnostic/unsatisfactory: 13 % of all FNABs; of those 16.8 % were cancer at final pathology
2. Benign/noncancerous: 59 % of all FNABs; of those only 3.7 % were cancer at final pathology
3. Indeterminate: 9.6 % of all FNABs; of those 15.9 % were cancer at final pathology
4. Suspicious for follicular cancer: 10.1 % of all FNABs; of those 26.1 % were cancer at final pathology
5. Suspicious for cancer: 2.6 % of all FNABs; of those 75.2 % were cancer at final pathology
6. Positive for cancer: 5.4 % of all FNABs; of those 98.6 % were cancer at final pathology

As a result, in patients with benign or inadequate FNAB results, other factors, such as USG findings (Table 21.3), personal history, and nodule size, should be considered for the decision to do surgery. The author researchers recommend to repeat FNAB when the diagnosis is indeterminate [45]. In this group, molecular markers (*BRAF*, *RET*/*PTC*, *RAS*, *PAX8*/*PPAR* mutations) (Table 21.2) may help to find out in which patients surgery is necessary [35, 46, 47]. Gene mutations are very rare in benign FNABs (approximately ≤5 %).

All patients suspicious for cancer category should undergo surgery because of the very high (75 %) possibility of cancer [41, 45].

Papillary, medullary, and anaplastic carcinomas can be easily diagnosed with FNA cytology, but it is difficult to distinguish benign from malignant follicular lesions. Histologic examination showing capsular and vascular invasion is necessary to classify a lesion as malignant for follicular and Hürtle cell neoplasia [7, 29, 37]. Molecular diagnostics may be useful to allow reclassification of follicular lesions, such as follicular neoplasm or follicular lesions with undetermined significance [48]. In the diagnosis of follicular lesion with undetermined significance (other terms are atypia of undetermined significance, rule out neoplasm, atypical follicular lesion, and cellular follicular lesion, and the estimated risk of malignancy is 5–10 %), FNA can be repeated or surgery can be considered according to the clinical and USG findings [42].

Another diagnostic method is radionuclide scan: In the last years, it is not used in the initial evaluation of thyroid nodule, except for suppressed TSH levels. In this situation, radionuclide scan is done to assess for a functioning (hot) adenoma. Malignant lesions usually are documented as hypofunctioning or cold lesions. The overall incidence of cancer in a cold nodule is 12–15 %, but it is higher in people younger than 40 years and in people with calcifications present on preoperative USG [49, 50].

Serum calcitonin measurement may be helpful for the diagnosis of MTC [51]. According to the American Association of Clinical Endocrinologist/Associazione Medici Endocrinologi/European Thyroid Association (AACE/AME/ETA) guideline, assessment of serum thyroglobulin is not recommended in the differential diagnosis of thyroid nodules. In patients undergoing surgery for malignancy, serum

thyroglobulin (Tg) measurement may be useful to detect potential false-negative results. Measurement of basal serum calcitonin level may be a useful test in the initial evaluation of thyroid nodules [43].

21.5 Staging and Prognosis

There are many prognostic factors for DTC. Age, sex, histologic type, tumor size, tumor grade, multicentricity, extrathyroidal extension, type of surgery, ploidy, lymph node metastases, vascular invasion, and ^{131}I RAI ablation therapy are important prognostic factors [6, 7, 29, 30, 37, 52–55]. Also there are multiple prognostic scoring systems for DTC. These are AGES (age, grade, extent, size), AMES (age, metastases, extent, size), DAMES (ploidy + AMES), MACIS (metastases, age, completeness of resection, invasion, size), and the AJCC-TNM staging system 2010 7th edition (age, stage) (Tables 21.4 and 21.5) [52, 56–59]. Prognoses according to the type of cancer and stage are outlined in Table 21.6 [59, 60]. Age, size, and extent of the tumor are present in all prognostic scoring systems. Adverse prognostic factors included age older than 45 years, follicular histology, primary tumor size larger than 4 cm, extrathyroidal extension, and distant metastases.

21.5.1 Age and Sex

Age is the most important prognostic factor for thyroid cancer mortality [6, 7, 29, 37, 52, 56–59]. Thyroid carcinoma is more lethal in patients older than 40 years. The mortality rate increases dramatically after the age of 60. Recurrence frequencies are highest (40 %) in patients younger than 20 years or older than 60 years. Thyroid cancer is more aggressive in men than in women. The five-year survival rate is 85 % for females and 74 % for males [60].

21.5.2 Tumor Characteristics

The most important prognostic tumor characteristics are tumor histology, tumor size, vascular invasion, necrosis, *BRAF* mutation, local invasion, and metastases.

PTC has the most favorable prognosis. But tall cell, columnar cell, and diffuse sclerosing variants and anaplastic tumor transformation have worse prognosis. The follicular variant of PTC has the same prognosis with pure PTC [6, 7, 28, 29, 37].

FTC is more aggressive than PTC. Vascular invasion is a bad prognostic factor. Many FTCs are minimally invasive tumors, exhibiting only slight tumor capsule penetration without vascular invasion. They are less likely to produce distant metastases and prognosis is good. Highly invasive FTCs are much less common. Up to 80 % make metastases and cause death in about 20 % of the patients [6, 7, 29, 37, 52, 61].

Table 21.4 American Joint Committee on Cancer (AJCC) 2010: thyroid cancer TNM staging [59]

	Primary tumor (T)
Tx	All categories may be subdivided into (s) solitary tumor and (m) multifocal tumor
T0	Primary tumor cannot be assessed
T1	No evidence of primary tumor
T1a	Tumor 2 cm or less in greatest dimension, limited to the thyroid
T1b	Tumor 1 cm or less, limited to the thyroid
T2	Tumor more than 1 cm but not more than 2 cm in greatest dimension, limited to the thyroid
T3	
T4a	Tumor more than 2 cm but not more than 4 cm in greatest dimension, limited to the thyroid
T4b	
T4a	Tumor more than 4 cm in greatest dimension, limited to the thyroid, or any tumor with minimal extrathyroidal extension (e.g., extension to the sternothyroid muscle or perithyroid soft tissues)
T4b	
	Moderately advanced disease
	Tumor of any size extending beyond the thyroid capsule to invade the subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve
	Very advanced disease
	Tumor invades the prevertebral fascia or encases the carotid artery or mediastinal vessels
	All anaplastic carcinomas are considered T4 tumors
	Intrathyroidal anaplastic carcinoma
	Anaplastic carcinoma with gross extrathyroidal extension
	Regional lymph nodes (N)
NX	Regional lymph nodes are the central compartment, lateral cervical, and upper mediastinal lymph nodes
N0	Regional lymph nodes cannot be assessed
N1	No regional lymph node metastasis
N1a	Regional lymph node metastasis
N1b	Metastasis to level VI (pretracheal, paratracheal, and prelaryngeal/Delphian lymph nodes)
	Metastasis to the unilateral, bilateral, or contralateral cervical (levels I, II, III, IV, or V) or retropharyngeal or superior mediastinal lymph nodes (level VII)
	Distant metastases (M)
M0	No distant metastasis
M1	Distant metastasis

Table 21.5 Pathologic TNM staging system for thyroid cancer (AJCC) [59]

Stage	Papillary or follicular Age < 45	Papillary or follicular Age > 45	Medullary carcinoma, any age	Anaplastic carcinoma, any age
I	M0	T1, N0, M0	T1, N0, M0	
II	M1	T2, N0, M0	T2, N0, M0 T3, N0, M0	
III	–	T3, N0, M0 T1–3, N1a, M0	T1–3, N1a, M0	
IVA IVB IVC	–	T4a, N0–1a, M0 T1–3; N1b, M0 T4a, N1b, M0 T4b, any N, M0 Any T, any N, M1	T4a, N0–1a, M0 T1–3; N1b, M0 T4a, N1b, M0 T4b, any N, M0 Any T, any N, M1	T4a, any N, M0 T4b, any N, M0 Any T, any N, M1

Table 21.6 Five-year survival rates (%) for thyroid cancer per TNM stage [60]

Stage	Papillary	Follicular	Medullary	Anaplastic
I	100	100	100	–
II	100	100	98	–
III	93	71	81	–
IV	51	50	28	7

HCC is an aggressive variant of FTC. Vascular invasion and older age are together with worse prognosis. HCC more frequently metastasizes than FTC [62]. Ten-year survival rates were 85 % for FTC and 76 % for HCC [63].

PTCs smaller than 1 cm are termed microcarcinoma or incidentaloma [29, 30, 37]. The risk of recurrence ranges from 1 to 2 % in unifocal papillary microcarcinoma, and 4–6 % in multifocal papillary microcarcinoma [64, 65]. Their cancer-specific mortality rates are near zero. DTC tumors, with size less than 1.5 cm and confined to the thyroid, almost never cause distant metastases. Thirty-year recurrence rates are one third of >1.5 cm tumors; 30-year cancer-specific mortality is 0.4 % compared to 7 % for ≥1.5 cm tumors [52]. There is a linear relationship between tumor size and recurrence and mortality for both PTC and FTC [52, 56–58].

BRAF mutation is associated with poor clinical outcome in patients with PTC [35].

PDTCs are more aggressive than DTC and less aggressive than ATC [36, 38, 39]. They are typically diagnosed in patients between the age of 55 and 63 years, a 2/1 female predominance. Extrathyroidal extension and extensive local invasion are typically present at the diagnosis. Regional lymph node metastases (50–83 %) and distant metastases (36–85 %), most commonly to the lungs and bones, are frequently seen in PDTC. Five-, ten-, and fifteen-year survival rates are 50, 34, and 0 %, respectively [36, 66, 67].

MTC is the intermediate differentiated thyroid carcinoma (Table 21.1). Overall, the 5-year survival is 80–86 % and the 10-year survival is 75 % [59, 60] (Table 21.6). In patients with sporadic MTC, a somatic *RET* oncogene mutation confers an adverse prognosis [68].

ATC has the worst prognosis [6, 7, 29, 37, 40]. Median survival is 4 to 5 months after diagnosis. Favorable prognostic features seem to be unilateral tumors, tumor size less than 5 cm, no invasion of adjacent tissue, and absence of nodal involvement or distant metastases [69]. Older age and high white blood cell count are bad prognostic factors [29].

21.5.3 Tumor Invasion

Local tumor invasion are present in nearly 10 % of DTC patients. It has a worse prognosis [52, 70]. Local invasion may be microscopic or gross. Recurrence rates are 2 times higher with locally invasive tumors. One third of these patients die of cancer within 10 years [52].

21.5.4 Regional Lymph Node Metastases

The prognostic significance of lymph node metastases is controversial [71, 72]. Lymph node metastases are frequent in PTC, especially multifocal PTC. The lymph node metastases rate is 35 % in adults with PTC and 80 % in children with PTC. The lymph node metastases ratio is 15 % in FTC, and together with worse prognosis. Older patients (>45 years) with PTC and The lymph node metastases ratio also have decreased survival [73]. In one study, the size of the metastatic lymph nodes (>3 cm vs <1 cm) and number of metastatic lymph nodes (<5 vs >5–10 involved lymph nodes) were found significant for prognosis [74].

21.5.5 Distant Metastases

Distant metastases are the main cause of death from DTC [6, 7, 29, 30, 37, 61]. Ten percent of patients with PTC and 25 % of patients with FTC develop distant metastases. The lungs, bones, soft tissues, and central nervous system are the most frequent metastatic sites. The main predictors of outcome for patients with distant metastases are the patient's age, site of metastases, and ^{131}I RAI uptake of the metastatic site. Younger patients have better prognosis. Bone metastases have worse prognosis. RAI-refractory metastases have worse prognosis.

21.5.6 Extent of Surgery

The extent of surgery is one of the important prognostic factors for patients with >1 cm DTC [52, 55]. The relapse rate and cancer-specific mortality rate of subtotal thyroidectomy are 40 and 9 % respectively. These numbers are 26 and 6 % with near-total or total thyroidectomy and statistically significant.

21.6 Differentiated Thyroid Carcinoma

21.6.1 Treatment of Differentiated Thyroid Carcinoma

There are three major components in the treatment of DTC:

- Surgery
- Postoperative ^{131}I RAI ablation therapy
- TSH suppression with thyroid hormone therapy

External beam radiotherapy (EBRT) can be used in the adjuvant treatment of some patients and in the metastatic disease, such as bone and brain metastases.

21.6.1.1 Surgical Management of DTC

Total thyroidectomy is generally the standard surgical approach for most patients with DTC. Except for low-risk cancer found incidentally on the final pathology, most of the endocrine surgeons advocate for total thyroidectomy as the procedure of choice [50]. Arguments favoring total thyroidectomy are the presence of multiple foci in bilateral lobes in more than 60 % of patients, the adjuvant therapy using radioactive ^{131}I, and the specificity of serum Tg concentration as a tumor marker.

Some institutions still advocate unilateral surgery, especially for patients whose primary tumor is <1 cm, due to the lack of survival benefit with more extensive surgery and the apparent lower risk of hypoparathyroidism and recurrent laryngeal nerve injury; these latter two complications of thyroidectomy occur in 1 % or less of total thyroidectomies when done by experienced endocrine surgeons. However, many consensus guidelines state that a total thyroidectomy is indicated if the primary tumor is >1 cm, there are contralateral thyroid nodules, regional or distant metastases are present, the patient has a personal history of radiation therapy to the head and neck, or the patient has first-degree family history of DTC [55]. Older age (>45 years) may also be a criterion for recommending near-total or total thyroidectomy even with tumors <1–1.5 cm, because of higher recurrence rates in this age group [58, 75–78].

Increased extent of primary surgery may improve survival for high-risk patients [79–81] and low-risk patients [55]. Other studies have also shown that rates of recurrence are reduced by total or near-total thyroidectomy among low-risk patients [76, 82, 83].

Thyroid lobectomy alone may be a sufficient treatment for small (<1 cm), low-risk, unifocal, intrathyroidal papillary carcinomas in the absence of prior head and neck irradiation or radiologically or clinically involved cervical nodal metastases.

For patients with cytologically suspicious follicular neoplasm, a unilateral lobectomy and isthmusectomy are the initial procedures of choice. If a malignant follicular lesion is confirmed on histopathology, then a completion thyroidectomy is warranted to allow for treatment with radioiodine therapy. Nodal metastases represent an uncommon finding in follicular carcinoma, but when present may indicate decreased survival.

In patients with papillary thyroid cancer, 20–90 % have central (or level VI) lymph nodes involved at the time of initial presentation, even in low-risk patients [84–88]. The presence of lymph node metastases increases risk for disease recurrence; however, it is only a minor risk factor for mortality. The common practice is to perform therapeutic central lymph node dissection when these lymph nodes are involved. However, it is controversial to perform routine prophylactic central lymph node dissection during total thyroidectomy. Major doubts are focused on whether lymph node metastasis poses impact on overall survival, whether it can reduce the recurrence rate, and whether the extension of surgery may bring more complications. Therefore, several compelling reasons exist to consider the routine clearance of the central lymph nodes at the time of initial thyroid surgery, including improvement in disease-free survival, accurate staging, decreased local recurrence rate, and utilization as an indication for adjuvant ^{131}I ablative therapy [89]. According to data from

the Surveillance, Epidemiology, and End Results (SEER) program, lymph node metastasis increases mortality by 68 % in patients over 45 years of age with papillary thyroid cancer [90]. Accurate staging is important in treatment planning, and routine clearance of the central lymph nodes facilitates the nodal status determination.

As some reports have implicated, central lymph node dissection may increase morbidity such as transient hypoparathyroidism and recurrent laryngeal nerve injury [91, 92]. However, in experienced hands, it (therapeutic or prophylactic) can be achieved with low morbidity [93–97] and may upstage some patients from clinical N0 to pathologic N1a [98]. In addition, selective unilateral paratracheal central compartment node dissection increases the proportion of patients who appear disease-free with unmeasurable Tg levels 6 months after surgery [99]. Other studies of central lymph node dissection have demonstrated higher morbidity, primarily recurrent laryngeal nerve injury and transient hypoparathyroidism, with no reduction in recurrence [92, 100]. In another study, comprehensive (bilateral) central compartment dissection demonstrated higher rates of transient hypoparathyroidism compared to selective (unilateral) dissection with no reduction in rates of undetectable or low Tg levels [101]. Although some lymph node metastases may be treated with radioactive iodine, several treatments may be necessary, depending upon the histology, size, and number of metastases [102].

Lymph nodes in the lateral neck (compartments II–V), in level VII (anterior mediastinum), and rarely in level I may also be involved by thyroid cancer [85, 103]. Although unilateral and bilateral therapeutic lateral cervical lymph node dissection with curative intent is widely recognized, prophylactic lateral neck dissection is controversial. For those patients in whom nodal disease is clinically evident, surgical resection may reduce the risk of recurrence and possibly mortality [104–106]. Functional compartmental en bloc neck dissection is favored over isolated lymphadenectomy ("berry picking") with limited data suggesting improved mortality [107–110].

21.6.1.2 Postoperative RAI Ablation Therapy

Radioiodine therapy with iodine-131 isotope (^{131}I) is a well-established adjuvant therapy, which is performed after surgery for DTC patients. The principle of the therapy is that ^{131}I is preferentially taken up and trapped by thyroid follicular cells, with just the same mechanism as its chemical analogue nonradioactive (cold) iodine. Contrary to cold iodine, ^{131}I emits beta radiation as well as high energetic gamma radiation. Beta radiation from ^{131}I is responsible for its therapeutic effect, while imaging can be done with the use of its gamma radiation. Therefore, postoperative radioiodine allows the identification of residual regional and/or distant metastatic disease as well as it exerts therapeutic effect to destroy tumor foci by internal radiation exposure.

The major aims of postoperative radioiodine therapy are:

1. To destroy any postoperative microscopic residual tumor foci, with internal radiation exposure by beta particles. The destroy process of residual regional tissue is preferably called "ablation."
2. To ablate residual normal thyroid tissue and therefore to increase the specificity of subsequent ^{131}I scintigraphy in the follow-up to detect any recurrence or metastasis.

3. To provide the use of serum Tg as a tumor marker for thyroid cancer in the follow-up of patients. After ablation of residual tissue postoperatively, any increase in Tg level would represent the presence of recurrent or metastatic disease.
4. To assess the postoperative staging of the patient. Whole-body scintigraphic imaging after administration of high dose of ^{131}I for postoperative ablation makes it possible for the unexpected metastases to be detected easily. Therefore, the patients would be upstaged and additional ^{131}I or other treatment options could be planned sequentially.

Some previous retrospective studies conclude that remnant ablation reduces long-term, disease-specific mortality in patients with primary tumors which are 1 cm or larger in diameter, those with multicentric disease, and those in whom there is evidence of soft tissue invasion at initial presentation [52, 80]. A meta-analysis on the effectiveness of ablative therapy reported that the risk of 10-year locoregional recurrence was lowered from 10 to 4 % and the rate of distant metastases was decreased from 4 to 2 % following ^{131}I ablation therapy [111]. According to the results from a prospective study on 2936 patients in the National Thyroid Cancer Treatment Cooperative Study Group, postoperative ^{131}I therapy improved overall survival in patients with stage II and higher stages of the disease [112]. Despite these data, there are some recent studies which show that some low-risk patients may not benefit from ^{131}I therapy and that it should not be recommended for intrathyroidal solitary primary tumors <1 cm in diameter unless high-risk parameters or metastases exist. According to another recent study, the benefits of the therapy are more evident in tumors >1.5 cm or with residual disease following surgery, while patients with lower risk appear not to benefit [113].

Basically, the efficacy of ^{131}I therapy depends on several factors such as patient preparation, tumor characteristics, extent and sites of disease, and the therapeutic dose of ^{131}I. Iodine uptake by thyroid follicular cells, whether it is radioactive or not, is closely and directly related to the serum TSH level, while it is inversely related to the level of iodine pool within the body. Therefore, the patient should be off thyroxine (T4) at least for 3–4 weeks until the serum TSH level reaches 30 mU/l at minimum. To avoid some serious symptoms of hypothyroidism for especially older patients, liothyronine (T3) medication can be prescribed to be stopped 2 weeks before the administration of ^{131}I. During 2 weeks before ^{131}I administration, the patient is put on a low-iodine diet, in order to decrease the level of iodine pool, which has the potential to competitively inhibit ^{131}I uptake and decrease the treatment efficiency. Radiocontrast media and some other agents such as hair dyes, which are very rich in iodine, are strictly forbidden to avoid competitive inhibition with ^{131}I. Since ^{131}I is orally administered, the patient should be in a fasting state at least for 4 h and continue to fast a few hours to establish a good gastric absorption, following the oral administration of the therapeutic dose. Then, the patient is encouraged to be well hydrated, especially at the first day of the therapy, in order to both decrease radiation exposure from the unbound ^{131}I by increasing excretion and lower the glandular tissue exposure which may cause some side effects such as sialadenitis. To overcome such side effects, saliva stimulants like lemon juice or sour candies as well as antacids to protect the gastric mucosa are recommended.

The therapy dose varies between 30 and 100 mCi of ^{131}I for postoperative ablation setting. A short-term individual hospitalization of the patient in specifically radiation-protected (shielded) rooms is usually needed for radiation protection, although it depends on legislative and regulatory issues which change from one country to another. Whole-body scintigraphic imaging is performed at the day of discharge or within a week from dose administration, to detect ^{131}I uptake. Recently, SPECT/CT hybrid imaging has increased its use, given the advantages of providing sectional anatomic data of the sites with ^{131}I uptake.

^{131}I is known as a quite safe treatment option, with relatively low major side effects or complications [6, 7, 29, 37]. Short-term complications such as radiation thyroiditis, neck edema, sialadenitis, and tissue hemorrhage are rare and mostly occur in bulky residual tissue. Long-term complications are even more rare and increase with cumulative doses. These include xerostomia, lacrimal gland obstruction, pulmonary fibrosis if pulmonary metastases are present and treated with higher doses, and secondary malignancies, like leukemia and salivary gland, breast, and colon cancer, although there is conflicting data in literature. The only contraindication of ^{131}I therapy is pregnancy and most of centers recommend their female patients not to get pregnant for at least 6 months, to avoid possible teratogenic effects to fetus growth. Similarly, male patients are strongly encouraged to use birth control methods at least for 4–6 months, following treatment, to avoid potential teratogenic effects.

21.6.1.3 TSH Suppression with Thyroid Hormone Therapy

Patients have to receive thyroid hormone therapy after surgery and ^{131}I RAI ablation therapy for two reasons [7, 29, 37, 42, 50]: (a) for the correction of iatrogenic hypothyroidism and (b) because TSH is a trophic hormone that can stimulate the growth of cells derived from follicular epithelium. TSH suppressive therapy in patients with DTC has been shown to increase two-to threefold especially in high-risk patients. However, the optimal serum levels of TSH have not been defined because of a lack of specific data. The NCCN panel recommends tailoring the degree of TSH suppression to the risk of recurrence and death from thyroid cancer for each individual patient [42]. For low-risk patients and patients with excellent response to initial therapy, thyroid hormone should be given to suppress the TSH level to 0.1–0.5 mU/L. For high-risk patients and patients with known residual disease, the recommended TSH level is below 0.1 mU/L. The risk and benefit of TSH suppression therapy must be balanced for each individual patient. The average dosage needed to attain the serum TSH level in the euthyroid range is higher in patients with operated thyroid carcinoma (2.11 mcg/kg/day) than in patients with primary hypothyroidism (1.62 mcg/kg/day) [114]. Higher doses are necessary for TSH suppression. Osteopenia, possible cardiac hypertrophy, and atrial fibrillation are some of the complications of TSH suppression therapy. For patients whose TSH levels are chronically suppressed, particularly postmenopausal patients, an adequate daily intake of calcium 1200 mg/day and vitamin D 1000 units/day is recommended [42]. Excessive TSH suppression (into the undetectable level or thyrotoxic level) is not required to prevent disease progression in all DTC patients.

21.6.1.4 Adjuvant EBRT

There is no prospective randomized trial, but EBRT has a role in the treatment of locally invasive PTC [7, 29, 30, 37, 42]. Two retrospective studies have shown that it may be an effective adjuvant therapy to prevent local-regional recurrences in patients 45 years and older with locally invasive PTC (T4 tumor, N1 disease) [115, 116]. In the first study, 10-year local relapse-free rates were 93 to 78 %, and disease-specific survival rates were 100 % versus 95 % and were significantly improved in a subgroup of patients with microscopic disease treated with EBRT [115].

Doses in the range of 40–50 Gy EBRT may be included in the treatment of PTC patients who are older than 45 years, gross extrathyroidal extension, microscopic residual disease, and incomplete resection near the aerodigestive tract, to increase local-regional control. Patients younger than 45 years generally were not treated with EBRT because of their better prognosis and the possible late side effects, especially secondary malignancies [7].

21.6.2 Assessment and Management After Initial Treatment

After initial surgery and ^{131}I RAI ablation therapy, the patient needs lifelong follow-up with clinical and radiologic data. Serum Tg, neck USG, and whole-body ^{131}I RAI scan detect recurrent or residual disease in most patients who have undergone total thyroid ablation [7, 42, 50]. If the patients have no total thyroidectomy and remnant ablation, serum Tg and RAI scan is not useful for the detection of recurrent disease.

21.6.2.1 RAI Scan in the Follow-Up

The follow-up of patients after total thyroidectomy and radioiodine ablation includes clinical examination, laboratory, and imaging procedures. Many consensus guidelines recommend radioiodine scintigraphy 6–12 months after initial radioiodine ablation where radioiodine scintigraphy at the 6th month has been reserved only for patients with some poor prognostic factors or some suspicious clinical, laboratory, or ultrasonographic findings according to many recent guidelines [50, 117–119]. The predictive value for 10-year disease-free survival is reported to be approximately 90 %, if the first follow-up scintigraphy is normal. However, radioiodine scintigraphy after the first follow-up is not recommended routinely for all thyroid cancer patients and depends on patient characteristics as well as the center's preference [120]. A risk-stratified approach for the follow-up of thyroid cancer is recommended since low-risk thyroid cancer is associated with low recurrence rates and mortality compared to other groups, and for the patients younger than 45 years at diagnosis with stage 1 disease, there is no proven survival benefit from lifelong follow-up following primary treatment and first follow-up [121]. Neck ultrasonography is the imaging method of choice at long-term follow-up of all thyroid cancer patients, to detect recurrent disease and more importantly lymph node metastases even with millimetric size. Neck ultrasonography in the 3-to-12 month monitoring of the patients with extrathyroidal invasion and/or regional lymph node metastases is recommended on a routine basis in some recent consensus guidelines [42, 50,

117, 118]. Whenever a suspicious finding is encountered, fine needle aspiration biopsy is strongly advocated to confirm diagnosis. During follow-up, serum Tg and anti-Tg antibody levels should also be monitored. Any increase in serum Tg levels should be accepted as suspicious for recurrence or metastasis and should lead to other modalities.

Radioiodine scintigraphy can be performed by oral administration of either 2–5 mCi of ^{131}I or ^{123}I, which has some dosimetric and imaging advantages over ^{131}I, given that it only emits gamma radiation, despite its high cost and low availability as a disadvantage. On the other hand, the major disadvantage of ^{131}I is its potential for "stunning," defined as the phenomenon that the diagnostic dose of ^{131}I administered before the treatment decreases the therapeutic dose uptake of thyroid cancer cells, possibly as a result of the downregulation of the sodium/iodide symporter. However, there are some recent studies which conclude that when a low-activity diagnostic dose is used and therapeutic dose is given within the following 3 days, stunning does not occur, even if there is still controversy [122]. The patient needs to be off T4 medication for a period of 3–4 weeks for serum TSH level to be at least 30 mU/l. Alternatively, recombinant human TSH can be administered, without the need of ceasing T4 medication, especially for the patients who cannot tolerate hypothyroidism. Whole-body imaging and spot views from various body parts are acquired 4–24 and 48 h after ^{123}I and ^{131}I administration, respectively (Fig. 21.1). Additional delayed images are acquired when needed. Recently, SPECT/CT hybrid imaging modality has been introduced to provide cross-sectional lesion detection with a more precise anatomic localization at radioiodine scintigraphy.

21.6.2.2 Imaging Techniques

Neck USG

It is a useful method to detect the as small as 3 mm lesions. Neck USG contains thyroid bed and cervical lymph nodes. It can distinguish benign lesions from malign lesions; at that point, FNA is most helpful to definitively prove recurrent disease. Consensus guidelines recommend the routine use of neck USG in the 3–12-month monitoring of patients with extrathyroidal invasion or local-regional nodal metastases [42, 50, 123]. About half of the patients with recurrent disease with USG may have negative RAI scanning or may have undetectable serum Tg level.

Other Imaging Techniques

Other imaging techniques are CT scan or MRI (magnetic resonance imaging) of the neck, CT scan of the chest, chest X-ray, and FDG-PET imaging [6, 7, 42]. Chest X-ray may show macronodular lung metastases. Chest CT scan shows micronodular lung metastases. CT scan and MRI are not more sensitive than neck USG, but are much less operator dependent.

Positron Emission Tomography with F-18: Fluorodeoxyglucose (FDG-PET)

FDG-PET serves as a molecular in vivo imaging modality to show glycolytic activity, which is the preferred metabolic pathway for most of the tumors. Current hybrid

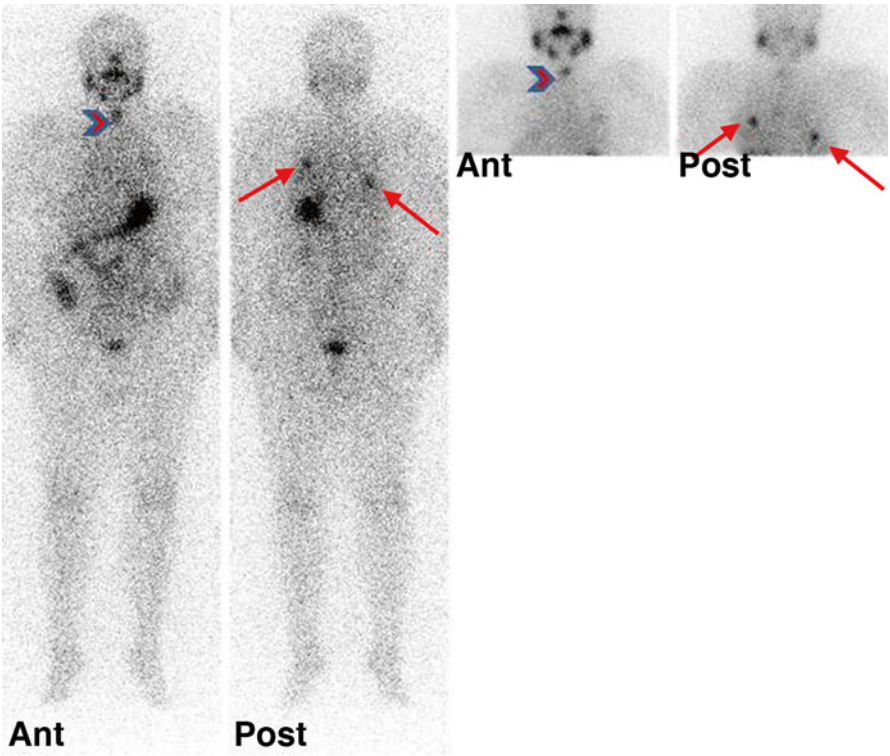
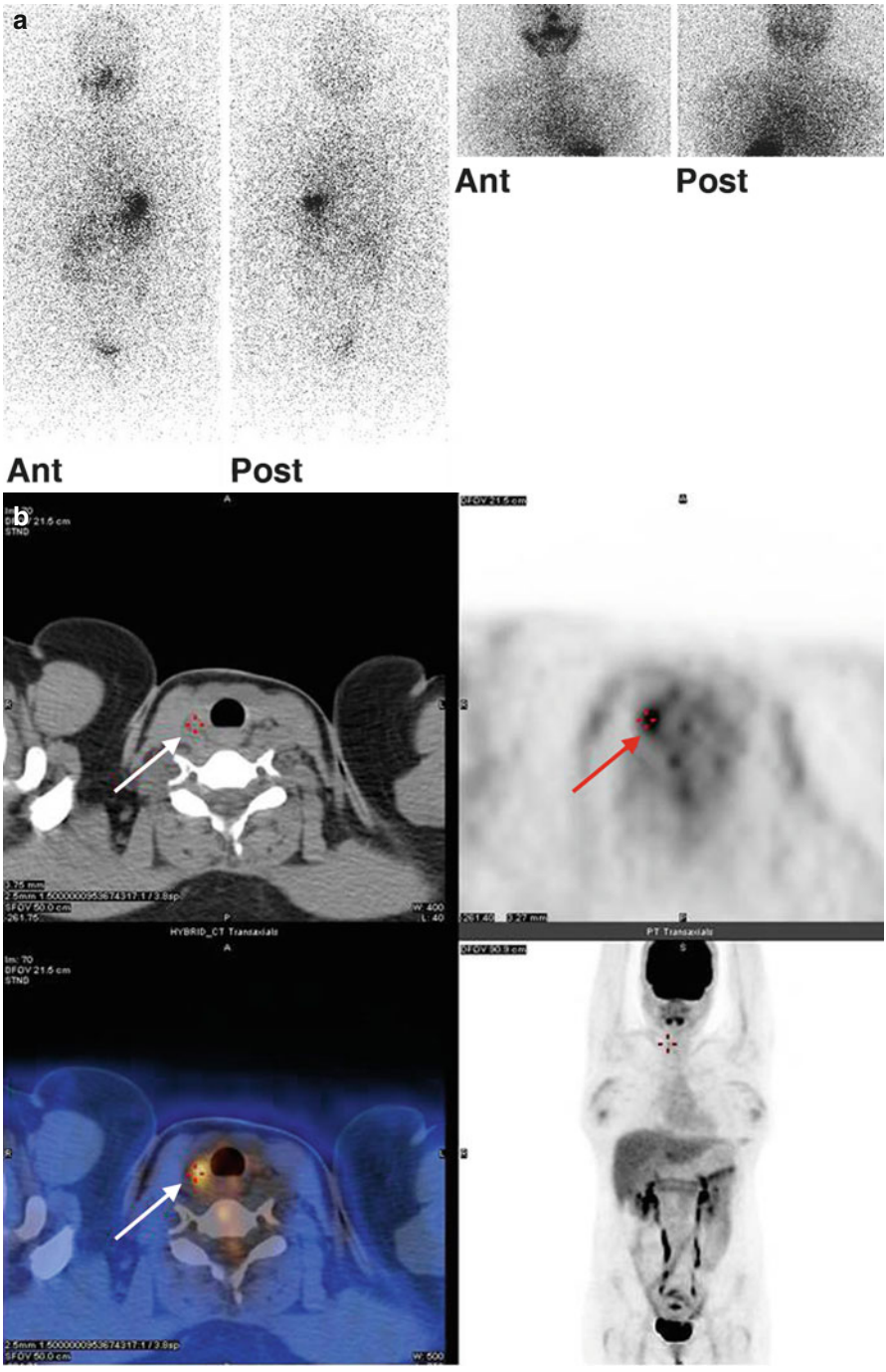


Fig. 21.1 Diagnostic I^{131} scintigraphy anterior and posterior whole-body and spot planar images of a 64-year-old female patient with papillary thyroid carcinoma (TSH, 98 uIU/ml; Tg, 278 ng/ml; anti-Tg Ab < 20 IU/ml) shows radioiodine uptake consistent with residual disease in the neck region (*arrowhead*) as well as bilateral lung metastases (*arrows*). *ant* anterior, *post* posterior

imaging systems combine PET with CT to provide the advantages of both functional and morphological imaging. In differentiated thyroid cancer, FDG-PET/CT is reserved only for patients with high serum Tg levels along with no uptake on radioiodine imaging during follow-up [124]. There is a "flip-flop mechanism" that FDG uptake in differentiated thyroid cancer cells increases as the tumor grade increases and as contrary radioiodine uptake increases as the grade decreases. Therefore, FDG acts as a marker of dedifferentiation and its degree of uptake correlates with poor prognosis. The possibility of detecting metastases on FDG-PET/CT increases as serum Tg increases; thus, it is generally recommended at a setting of serum Tg > 10 ng/l along with negative radioiodine scintigraphy (Fig. 21.2a, b). A positive FDG-PET/CT result can lead to surgery, especially when there are isolated lymph node or soft tissue metastases around the neck region, also allowing the use of PET probe-guided surgery, just like gamma probe-guided approaches. It also leads to other therapeutic modalities such as external radiation therapy for unexpected bone metastases which show no radioiodine uptake and some other systemic therapy options in advanced cases. The patient should be fasting for at least 4 h and the


a
Ant
Post
Ant
Post
b
HYBRID_CT Transaxialis
PT Transaxialis

serum glucose level should not be over 200 mg/dl on the day of FDG i.v. injection. Thyroid hormone withdrawal is encouraged by most of the literature data, given that the hypothyroid state increases glycolytic activity and increases the diagnostic efficacy of FDG-PET/CT imaging.

21.6.2.3 Monitoring Serum Thyroglobulin (Tg)

Thyroglobulin is a protein synthesized only by thyroid follicular cells (benign or malignant) [6, 7, 29, 37]. After total thyroidectomy and ablation therapy, Tg should be undetectable. Therefore, it is a good marker to assess the presence of recurrent, residual, or metastatic disease. The nadir should be reached within 3 months post-treatment, but it may take as long as 1–2 years. Tg and TSH must be measured together, because Tg production is dependent on TSH secretion. The sensitivity of Tg is only 50 % under TSH suppression therapy. After thyroid hormone withdrawal and TSH elevation, the sensitivity to detect cancer is increased to 85–95 % [42, 50]. Anti-Tg antibodies are present in nearly 25 % of thyroid carcinoma. The presence of antibodies after total thyroidectomy and RAI ablation therapy may be indicative of the presence of cancer, but it can take years for the antibodies to disappear even in the absence of cancer. Antibodies to Tg can falsely lower the Tg concentrations, and it is also important to measure Tg in the same laboratory with the same assay to avoid misinterpretations [125, 126]. If patients have negative neck USG, stimulated Tg level <2 ng/ml with negative anti-Tg antibodies and negative RAI imaging after RAI ablation therapy may be followed with unstimulated Tg annually and with periodic neck USG [42, 50].

21.6.3 Metastatic Disease

Nearly 15–20 % of patients with DTC will recur locoregionally (80 %) or have distant metastases (20 %). Five percent of the patients will die with the disease. There are different treatment options for metastatic DTC. These are surgery, RAI therapy, systemic therapy, and supportive therapy. Only about 50–80 % of primary tumors and their metastases take up ^{131}I RAI [6, 7, 29, 30, 37, 61].

Metastatic disease may be encountered as either local-regional recurrences, such as lymph node metastases or distant metastatic disease. The most common sites of metastases are the lungs (49 %), bones (25 %), bone and lung (15 %), and other soft

Fig. 21.2 (**a**) Follow-up diagnostic I^{131} scintigraphy of a 38-year-old female patient with the diagnosis of diffuse sclerosing type of thyroid cancer does not show any uptake consistent with recurrent or metastatic disease. (**b**) The same patient underwent FDG-PET/CT imaging since Tg is 46,7 ng/ml (anti-Tg Ab < 20 IU/ml, TSH > 100 uIU/ml) with a negative radioiodine scintigraphy, which displays focal FDG hypermetabolism of a middle-jugular lymph node (*arrow*). The biopsy was consistent with thyroid cancer metastasis and the patient was referred to surgery

tissues and rarely the brain (10 %). Other rare metastatic sites are the parapharyngeal region, parotid, breast, liver, renal and adrenal glands, ovary, muscle, and skin [127]. One of our patients presented with hemoptysis due to endobronchial metastases [128]. Older patients have a higher risk of distant metastases [129]. Lung metastases were most frequent in patients with HCC and were least common in patients with PTC. Pulmonary metastases may be micronodular or macronodular [130]. Macronodular metastases are more frequent and RAI uptake is usually not present. Micronodular metastases have diffuse reticular and miliary pattern and show diffuse RAI accumulation. These lesions predominantly localized in the lower lobes of the lungs. Patients with the age less than 40 years and RAI uptake in lung metastases have better prognosis (5-year survival 60 % for ^{131}I RAI-positive metastases and 30 % for ^{131}I RAI-negative metastases). ^{131}I RAI dose in the range of 150–175 mCi is used to treat pulmonary metastases; higher doses may cause pulmonary fibrosis.

21.6.3.1 ^{131}I RAI Therapy in Metastatic Disease

DTC especially the papillary type can metastasize to the lymph nodes, whereas the follicular type tends to metastasize to distant organs more. Although distant metastases are detected in only less than 1 % of all differentiated thyroid cancer patients at initial presentation, they can be encountered with various frequencies during the disease course in the lungs, bones, soft tissues, and brain. The risk of distant metastases increases as the patient's age at presentation increases. Although DTC can most of the time be treated with radioiodine therapy successfully and is one of the few curable malignancies, up to one third of patients who develop recurrence and metastases during the disease course may become radioiodine refractory due to the loss of differentiation [118, 131].

The most common treatment option for lymph node metastases is surgical excision and neck dissection, whenever possible, followed by radioiodine therapy for microscopic residual disease. Surgery decreases tumor burden significantly and thus increases the effectiveness of radioiodine therapy. Gamma probe-guided surgical techniques can also facilitate nodal excision in a reoperated neck, which would be technically difficult for most of the surgeons. The dose of ^{131}I in nodal metastases is generally 150 mCi as a fixed-dose approach. In addition to the fixed-dose approach, there are some dosimetric methods, in which basically the pharmacokinetics of ^{131}I for each individual patient is studied to define a more precise therapeutic dose. However, these techniques are usually cumbersome and not easy to be standardized, hampering their routine use.

The preferred therapeutic dose for lung metastasis is about 200 mCi of ^{131}I, where it can be lowered to 175 and even to 150 mCi for patients with high risk of pulmonary fibrosis as a major complication. Lung metastases can be encountered as a macronodular (Fig. 21.3a, b) or micronodular pattern, which is seen more often in pediatric patients (Fig. 21.4a, b). Micronodular metastases present a miliary pattern mostly located in the lower part of the lungs and tend to show more diffuse and higher amount of ^{131}I uptake compared to macronodular metastases. Patients with radioiodine uptake in lung metastases are reported to have a more favorable 5-year survival rate of 60 % compared to that of 30 % in patients without radioiodine uptake [130].

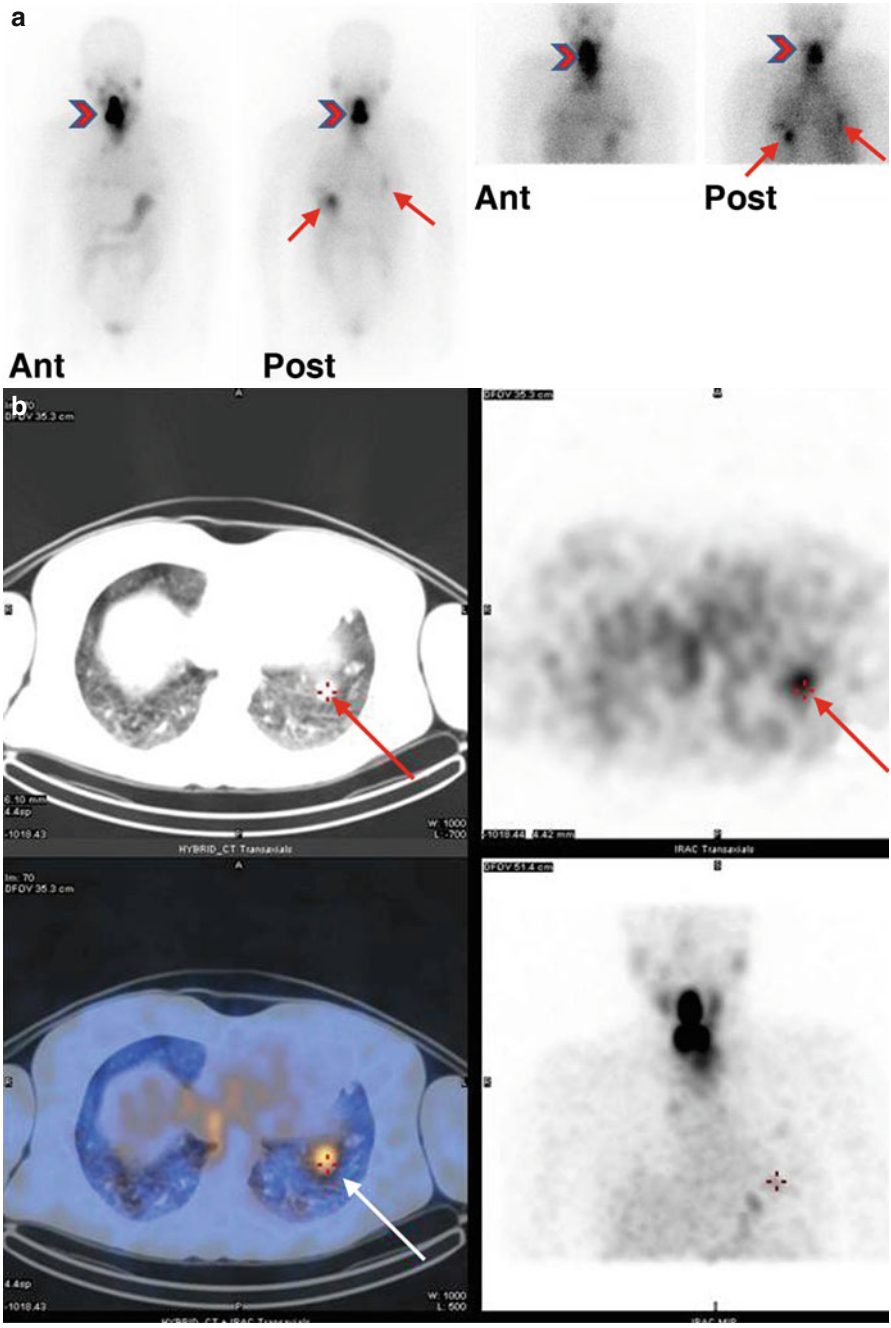


Fig. 21.3 (**a**) Post-therapy scintigraphy which was performed 4 days after the administration of 200 mCi of I^{131} in a 40-year-old woman with papillary thyroid cancer along with low differentiated thyroid cancer (TSH, 31 uIU/ml; Tg, 4508 ng/ml; anti-Tg Ab < 20 IU/ml) displays prominent radioiodine uptake around the neck region consistent with recurrent disease (*arrowhead*) as well as faint radioiodine uptake suggesting macronodular lung metastases (*arrows*). (**b**) Macronodular lung metastases are better delineated on SPECT/CT transaxial images (*arrows*)

Bone metastases are more frequently seen in follicular-type cancer compared to the papillary type, where the preferred ^{131}I dose should be about 250 mCi or at least 200 mCi as a fixed-dose approach. Bone lesions mostly show high ^{131}I uptake; however, complete resolution is achieved in only about 10 % of the patients. Surgical therapy especially to avoid vertebral collapse along with external radiation therapy is usually accompanied with radioiodine therapy in this setting.

Before the administration of high dose of ^{131}I, premedication with glucocorticoids to decrease edema and to avoid compression symptoms is strongly recommended for the treatment of brain metastases as well as soft tissue metastatic recurrences around the midline neck region, which potentially may block respiration.

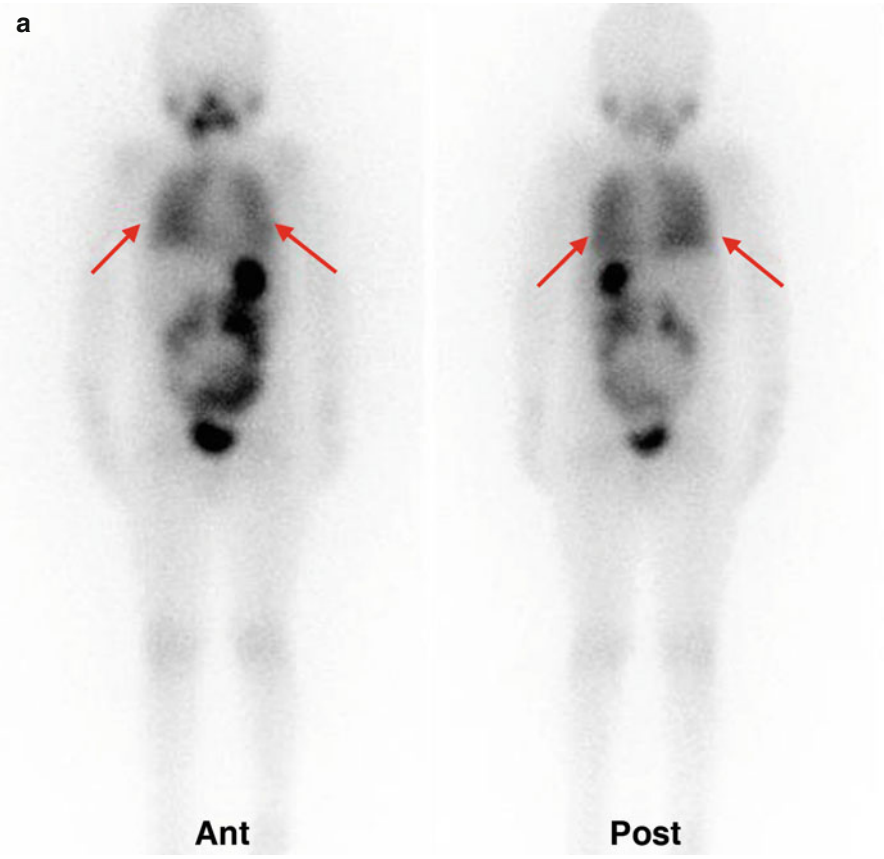


Fig. 21.4 (**a**) Diffuse lung uptake consistent with micronodular lung metastases (*arrows*) on post-therapy whole-body imaging following 200 mCi I^{131} therapy dose to a 5-year-old girl with the diagnosis of papillary thyroid carcinoma (TSH > 100 uIU/ml; Tg, 695 ng/ml; anti-Tg Ab < 20 IU/ml). (**b**) SPECT/CT I^{131} images of the same patient displays diffuse lung uptake especially in the posterobasal parts of both lungs, consistent with micronodular lung metastases

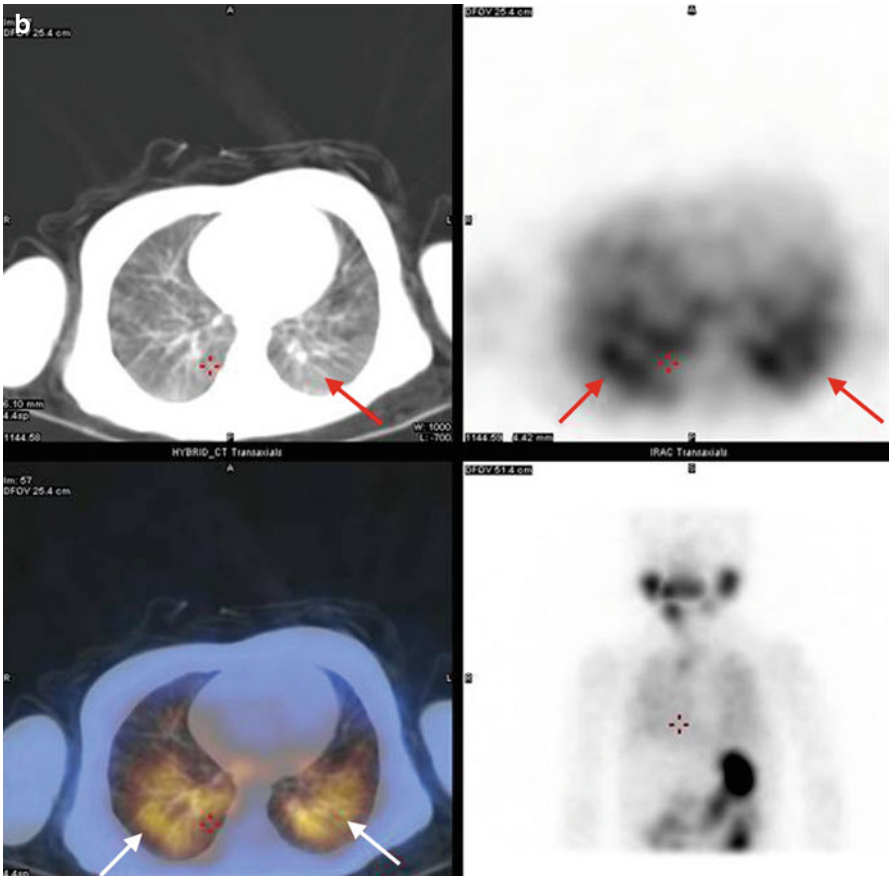


Fig. 21.4 (continued)

Radioiodine serves as a good therapeutic option also for pediatric patients with metastatic disease, where therapy doses are usually set on an individual patient basis, given that there are not much data in the literature [132].

Recombinant Human TSH

Recombinant human TSH (rhTSH) administration can alternatively be used instead of thyroid hormone medication withdrawal in order to reach high serum TSH levels which are needed for adequate stimulation of both radioiodine uptake for scintigraphic imaging and serum Tg concentration. rhTSH is mostly preferred for patients who have major risks for prolonged hypothyroidism in order to avoid serious complications and also for the patients in whom endogenous TSH level cannot be increased due to hypopituitarism or due to the presence of functioning bulky metastases. It is administered intramuscularly on two consecutive days, followed by radioiodine administration on the third day and imaging on the fifth day. Literature

data mostly conclude that rhTSH administration results with almost the same sensitivity and accuracy ratios compared to endogenous TSH stimulation in diagnostic setting regarding both ^{131}I and Tg level stimulation. However, there are still conflicting results with regard to its use in the therapeutic setting, given that the residency time of ^{131}I after rhTSH in the body is shorter than endogenous TSH stimulation at the hypothyroid state, which may potentially result in less therapeutic effectiveness, although a recent study favors its use in ablation therapy setting [133]. The other drawbacks of rhTSH are its relatively high cost and less availability, especially in developing countries which hamper its wide use.

21.6.3.2 Systemic Treatment of Metastatic Disease

Metastatic DTC can be stable and silent for many years. Because of this, only patients with symptomatic or progressive disease should be treated with systemic treatment. RAI therapy is the first option for patients with RAI uptake-positive distant metastatic disease except for local therapies such as surgery and EBRT. In RAI-refractory (^{131}I refractory) disease, clinical trials should be considered as first-line therapy for those patients. If clinical trial is not available or patient is not suitable for one, off-label use of cytotoxic chemotherapy or sorafenib should be considered.

^{131}I-refractory DTC is 5–15 % of the DTC patients [134, 135]. Different chemotherapeutic agents have been used as a single agent or in a combination regimen, with response rates of 25–38 %, mostly partial responses. These agents are doxorubicin, paclitaxel, docetaxel, cisplatin, carboplatin, and epirubicin. Response durations are very short with high toxicity [6, 7, 29, 30, 37].

A key molecular element in the biology of DTC is the tyrosine kinase *RAS*/*RAF*/*MEK MAPK*/*ERK* pathway [134]. The *PI3K*/*Akt* pathway is also involved in the pathogenesis of DTC. Targeted therapies to these pathways, tyrosine kinase and other antiangiogenic inhibitors have been studied in various phase II trials [134, 135]. Their response rates are similar to cytotoxic agents, but they have more durable responses, higher stable response rate, and less toxicity. These agents are outlined in Table 21.7. Two of them, sorafenib and lenvatinib, are currently in phase III trials in patients with I-131-refractory DTC. Sunitinib, sorafenib, and pazopanib (category 2B for pazopanib) therapies are recommended in the NCCN 2013 guideline, in the treatment of clinically progressive, symptomatic, and nonradioiodine-responsive DTC patients [42]. In this year, in the ASCO meeting, the sorafenib study (DECISION trial) results were explained [136]: The primary endpoint of the trial was progression-free survival (PFS). The DECISION trial enrolled 417 patients with locally advanced or metastatic RAI-refractory DTC. Patients were randomly assigned 1:1 to 400 mg sorafenib orally twice daily or to placebo. PFS was 10.8 months vs 5.8 months, respectively. No complete response was seen, the partial response (PR) rate was 12.2 %, and the median PR duration was 10.2 months. The most frequent adverse reactions were hand and foot skin reaction, diarrhea, alopecia, rash, fatigue, and hypertension.

Other targeted agents are lenvatinib and, sunitinib, pazopanib, vemurafenib, gefitinib, axitinib, vandetanib, everolimus, motesanib, and cabozantinib are under clinical investigations [135]. There are phase II studies with thalidomide and

Table 21.7 Targeted agents under clinical evaluation for the treatment of advanced thyroid cancer [8, 134, 135]

Compound	Class	Thyroid cancers
Axitinib	TKI[a]	DTC, MTC, ATC
Motesanib diphosphate	TKI	DTC
Pazopanib	TKI	DTC
Sorafenib	TKI	DTC (phase III)
Sunitinib	TKI	DTC, MTC, ATC
Gefitinib	TKI	Advanced thyroid cancer
Vandetanib	TKI	DTC
Lenvatinib	TKI	DTC (phase III)
Cabozantinib	TKI	MTC
Everolimus	m-TOR inhibitor	Advanced thyroid cancer
Thalidomide	Angiogenesis inhibitor	DTC, MTC
Lenalidomide	Angiogenesis inhibitor	DTC

[a]*TKI* tyrosine kinase inhibitor, *DTC* differentiated thyroid cancer, *MTC* medullary thyroid cancer, *ATC* anaplastic thyroid cancer

lenalidomide; these are angiogenesis inhibitors; in RAI-refractory DTC cases, with some partial responses (Table 21.7). Redifferentiation therapy is another treatment option for these patients (6.3.4)

21.6.3.3 Surgery, EBRT, and Supportive Therapy

Surgery is a useful treatment option for local-regional recurrences, localized painful bone lesions, spinal-cord-compressing lesions, and one or more brain metastases and localized soft tissue metastases. Bone metastases are more frequent in FTC than in PTC. Bone lesions are generally osteolytic. ^{131}I RAI therapy, EBRT, intravenous bisphosphonates therapy, and denosumab are effective treatment options for bone metastases. EBRT is a useful treatment in patients with unresectable, grossly invasive, or metastatic disease to the neck [29, 37].

21.6.3.4 Redifferentiation Therapy in RAI-Refractory DTC

Although DTC is a well-treated and even curable malignancy for most of the patients, approximately 10–15 % of them present with recurrence during the disease course, and about 30 % of thyroid cancer patients with recurrence and/or metastases show dedifferentiation of tumor cells with regard to morphology and function [118, 131]. As tumor cell dedifferentiation proceeds, sodium/iodide symporter system expression decreases and the tumor cell loses its ability to take up radioiodine, which eventually results with a radioiodine therapy-resistant thyroid cancer [137].

Redifferentiating pharmaceuticals can potentially reactivate the radioiodine uptake of tumor cells and thus make the radioiodine therapy possible. Retinoic acid derivatives, which are chemically similar to vitamin A, are the first agents used in redifferentiation therapy. Therapy includes oral administration of 1–1,5 mg/kg/day of isotretinoin or 13-cis-retinoic acid for 5–8 weeks. Some

studies in the literature using similar therapy protocols reported that retinoids could cause a slight increase in the radioiodine uptake; however, the increased rate of radioiodine uptake was not always correlated with therapy efficacy [138–140]. In a more recent study where incorporation of oral isotretinoin redifferentiation therapy with radioiodine therapy was investigated, the overall therapy response rate at 6-month follow-up was 21.3 % [141]. An antidiabetic pharmaceutical thiazolidinedione which acts a peroxisome-proliferator-activated receptor agonist was also reported to have both redifferentiating and antiproliferative effects and thus can potentially be used as a redifferentiating agent. In an open phase II study on 20 patients with radioiodine-refractory thyroid cancer, five patients had overall partial response with regard to the decrease in Tg levels and increase in radioiodine uptake on scintigraphy, even though no partial or complete response regarding the RECIST criteria was reported [142].

Due to their limited benefits and some side effects (e.g., cardiac side effects for thiazolidinedione and dermal/mucosal effects for retinoids) which may decrease patient compliance and may even cause cessation of therapy, routine use of redifferentiating pharmaceuticals have not gained wide acceptance and further studies are warranted.

21.7 Anaplastic Thyroid Carcinoma (ATC)

ATC is a rare but highly lethal form of thyroid cancer. It is both a locally and systemically aggressive undifferentiated tumor derived from follicular cells. Geographically, the prevalence of ATC ranges from 1.3 to 9.8 %, and patients have a median survival of 5 months and a 20 % 1-year survival rate [69]. More than 90 % of patients with this disease are over the age of 50 years, and a male/female ratio is 2:3. The incidence of ATC is decreasing in the last years.

The patient uniformly presents with a rapidly progressive palpable mass, frequently in a preexisting goiter. The median tumor size is 8–9 cm, with a range of 3–30 cm [29]. In 50 % of cases, this disease arises from preexisting DTC [143]. Tg immunoreactivity is absent in the pathology specimens. *TP53* (65–70 %) and *RAS* (50–55 %) mutations are frequently present in ATC [36]. *Beta-catenin* gene alterations are present in 65 % of the cases [36]. In one study, *PI3K* mutations were found in 14 % of the cases, but the *PI3K* gain of copy number was found in 39 % [144]. This is an important finding for future clinical trials.

All patients are classified by the American Joint Committee on Cancer (AJCC) TNM system as stage IV (A, B, or C) at presentation [59]. The diagnosis of ATC can often be suspected clinically. The diagnosis of thyroid pathology involves the correlation of clinical, biochemical, radiographic, and morphological features of the individual case [145].

Approximately 10 % of patients with ATC present with only an intrathyroidal tumor, whereas 40 % have extrathyroidal invasion and/or lymph node metastasis, with the remainder of patients presenting with widely metastatic disease [146, 147]. The lungs and the pleura are the most common metastatic sites (>90 %). Other

frequent metastatic sites are bones (5–15 %) and brain (5 %). Bone metastases are usually lytic.

21.7.1 Treatment of ATC

21.7.1.1 Surgery

The initial approach to patients with stage IVA or IVB disease depends on whether the tumor is resectable or unresectable at the time of diagnosis. In patients with locoregional disease, the determination of whether the tumor is resectable should be based on what structures are involved, whether a satisfactory resection can be achieved (R0/R1), and whether the resection of the involved structure results in significant morbidity or mortality. Gross tumor resection, not debulking, is the goal of surgery.

Patients with locoregional disease should be offered a resection if gross tumor resection can be achieved with minimal morbidity. This is because most studies suggest that complete resection (R0/R1) is associated with prolonged disease-free survival and/or overall survival with or without combination chemotherapy and radiotherapy [146, 148–158]. There are insufficient data to determine if there is a difference in disease-free survival rates between patients who have grossly negative margins (R1 resection) and those with microscopic negative margins (R0 resection). Patients with unresectable stage IVB disease may also respond to aggressive multimodal therapy. In patients with systemic disease, resection of locoregional disease for palliation may be considered if there is impending airway or esophageal obstruction. Patients with distant metastases (stage IVC) only rarely have responded to traditional therapies, and if an aggressive approach is desired by the patient, a clinical trial should be considered. Hospice or palliative care is also an important component of managing patients with stage IVC disease.

21.7.1.2 Chemotherapy, Radiotherapy, and Targeted Therapies

RAI therapy has no role in this tumor. Except for surgery, chemoradiotherapy with paclitaxel/carboplatin combination, paclitaxel, docetaxel, cisplatin (C), and doxorubicin (D) weekly or three-weekly can be used in local-regional disease control [145, 148–151]. D-based chemotherapy is the most appropriate therapy by many physicians and the only drug approved by the FDA in the treatment of ATC [42]. Paclitaxel is also one of the effective drugs. According to one trial, DC combination is more effective than D alone [159]. Clinical trials with chemotherapy and targeted agents (combretastatin, fosbretabulin, and crinobulin, which are vascular disrupting agents; CS-71-07 and oral *PPAR gamma* agonist; TKIs such as sorafenib, sunitinib, pazopanib, and imatinib; bevacizumab) and biologic response modifiers aimed at restoring the dedifferentiated functions of thyroid tissue in combination with chemotherapy are under clinical investigation [29, 37]. In one study, one of three patients survived more than 6 months with fosbretabulin therapy [160]. In another study, paclitaxel/carboplatin combination compared with paclitaxel/carboplatin/fosbretabulin combination. Median survival was 4 months to 8.2 months respectively without statistical significance [161]. Clinical trials are ongoing.

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Medullary Thyroid Cancer

22

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22.1 Introduction

Thyroid cancer is the most common endocrine cancer particularly frequent in women. Medullary thyroid cancer (MTC) accounts for only 5 % of all thyroid malignancies [1, 2]. MTC is a neuroendocrine tumor (NET) arising from parafollicular C cells [3–6]. The C cells originate from embryonic neural crests and constitute 1 % of thyroid cells. Initial C-cell hyperplasia progresses to microinvasive carcinoma and finally to macroscopic carcinoma with elevated calcitonin levels. Calcitonin secretion from tumor cells is the typical feature of MTC. *Rearranged during transfection* (*RET*), proto-oncogene mutations play an important role in pathogenesis [7, 8]. Clinical signs and symptoms vary, but frequently MTCs share clinical characteristics of other NETs.

22.2 Genetic Basis of MTC

The majority of the cases are sporadic [9–14]. However, 25–30 % of all cases occur as part of an autosomal dominant hereditary syndrome, multiple endocrine neoplasia type 2 (MEN-2), which is composed of three distinct variants: MEN-2A, MEN-2B, and familial MTC (Table 22.1) [3–6, 9–14]. MEN-2A constitutes majority of the hereditary cases. MEN-2A is characterized with association of MTC with pheochromocytoma and parathyroid hyperplasia. Cutaneous lichen amyloidosis and Hirschsprung disease are the other rare possible clinical manifestations of MEN-2A. Unlike MEN-2A, MEN-2B includes marfanoid habitus and mucosal

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Table 22.1 Clinical characteristics of medullary thyroid carcinoma subtypes

	Frequency (%)	Features of MTC	Associated diseases
Sporadic MTC	70	Unifocal	
MEN-2A	20	Autosomal dominant Multifocal, bilateral	Pheochromocytoma Primary parathyroid hyperplasia Cutaneous lichen planus amyloidosis (rare) Hirschsprung disease (rare)
MEN-2B	5	Autosomal dominant Multifocal, bilateral	Pheochromocytoma Multiple mucosal ganglioneuromatosis Marfanoid body habitus
FMTC	5	Autosomal dominant Multifocal, bilateral	None

MTC medullary thyroid carcinoma, *MEN-2A and MEN-2B* multiple endocrine neoplasia type 2A and 2B, *FMTC* familial medullary thyroid carcinoma

ganglioneuromatosis instead of parathyroid hyperplasia. Familial MTC does not have any other clinical manifestations of MEN syndromes.

RET gene is a proto-oncogene with 21 exons located on chromosome 10 (10q11.2) [7, 8, 15–17]. The *RET* gene encodes a transmembrane protein tyrosine kinase receptor for the glial cell line-derived neurotropic factor (GDNF) family members and their ligands such as artemin, neuturin, and persephin. The RET protein is composed of three functional domains involved in a variety of signaling pathways to regulate differentiation, proliferation, survival, and migration of the enteric neural progenitor cells and renal cells [7, 8]. Although the RET gene itself was initially discovered in 1985, its mutations were linked to inherited MTC in the early 1990s. Genetics of hereditary MTC syndromes is based on a series of missense germline mutations in different parts of the *RET* gene with autosomal dominant pattern and high penetrance. Frequently, a point mutation is the cause of malignant transformation. Several germline mutations have been found across 7 exons. Most of the mutations occur in exons 8, 10, 11, and 13–16. These mutations have different penetration rates to produce a variety of disease phenotypes. The most common mutation in MEN-2 patients is in exon 11 codon 634 (85 %) followed by mutations in codons 609, 611, 618, and 620 (10–15 %). Each of them has different importance for risk of developing MTC. C634 and M918T mutations in MEN-2A and MEN-2B, respectively, are associated with aggressive tumors and metastasis in early childhood within months from birth. Germline *RET* mutations are detected rarely in sporadic cases in 6–7 % of all patients [15].

Inherited forms of MTC occur in younger ages. Therefore, genetic counseling plays an important role in the detection of MTC risk. Genetic testing must be done in all patients with MTC. If germline mutation is detected in one case, all family members should be screened with proper genetic counseling and treatment, as necessary. Early or prophylactic thyroidectomy potentially cures this life-threatening disease in the affected kindreds [18].

On the other hand, about half of sporadic cases have acquired somatic *RET* mutation which were detected in the tumor cells [16, 17]. These mutations cannot be detected using regular genetic testing of leukocyte DNA, because these do not have

any inheritance pattern and do not affect clinical management of an individual patient, routine genetic testing of all tumor samples is not recommended outside of an academic incentive.

22.3 Clinical Presentation

Patients with sporadic MTC are usually diagnosed in the fifth or sixth decades of life [9–13]. However, hereditary forms present in younger ages. There is a trend to be more frequent in female sex. MTC is an indolent disease and overall survival of 15–20 years is possible even in disseminated disease. Most patients present with metastatic disease at initial diagnosis. Common sites of distant metastatic spread include the bones, lungs, liver, and brain [9–13].

Asymptomatic thyroid mass or enlarged lymph nodes are typically the most common initial symptoms seen in 75–95 % of the patients. Because parafollicular C cells originating from neural crest are usually located in the upper thyroid lobes, masses are usually detected there [9–13]. These tumors are unilateral in sporadic MTC. However, hereditary forms usually present with bilateral and multifocal disease. Cervical lymph node involvement is detected in about half of the cases. Multifocality is an important risk factor for the lymph node metastasis [19]. Approximately 10 % of the patients experience local invasion or compression of aerodigestive tract causing hoarseness or dysphagia. Rarely, symptoms may occur due to distant metastases such as shortness of breath, bone pain, or fractures. Several hormones such as calcitonin, serotonin, and vasoactive intestinal peptide (VIP) are released from the tumor tissue. Flushing, sweating, weight loss, and diarrhea may occur due to these hormones.

Symptoms of the other diseases such as hyperparathyroidism and pheochromocytoma may be the initial presentation of hereditary MTC.

22.4 Diagnosis

Fine-needle aspiration cytology (FNAC) is the gold standard technique for the diagnosis of MTC [20]. The pooled estimate of FNAC detection rate in MTC patients is 56 % in a recent meta-analysis. Sometimes, pathological features cannot be easily discriminated from other malignancies. In those cases, immunohistochemical staining for calcitonin, carcinoembryonic antigen (CEA), and chromogranin A might be useful. Another recent approach is to measure aspiration needle washout fluid calcitonin levels [21].

Basal serum calcitonin and CEA levels are usually elevated [22–24]. Some authors suggest that serum calcitonin has a higher sensitivity compared to FNAC in the diagnosis of MTC [22, 23]. Calcitonin levels are correlated with tumor burden and multifocality. Calcitonin levels greater than 3,000 pg/mL generally indicate metastatic disease [23]. Besides confirming diagnosis, serial measurement of calcitonin is useful for confirming treatment efficacy and monitoring disease progression or recurrence. Similarly, high CEA level above 100 ng/mL is associated with lymph

Table 22.2 Staging of medullary thyroid cancers

Stage	TNM classification	Definition
Stage I	T1,N0,M0	Tumor ≤2 cm in greatest dimension and is limited to the thyroid gland (T1)
Stage II	T2–T3,N0	Tumor >2 cm, limited to the thyroid, or any tumor with minimal extrathyroidal extension (T2–T3)
Stage III	T1–T3,N1a,M0	Any tumor limited to the thyroid, or any tumor with minimal extrathyroidal extension (T1–T3) *and* presence of nodal metastasis in level VI (N1a)
Stage IV	T4,N0–N1a,M0 T1–T4,N1b,M0 Any T, any N, M1	Tumor with gross extrathyroidal soft tissue extension (T4), or lymph node involvement outside of level VI (N1b), or distant metastases (M1)

node and distant metastasis [24]. Calcitonin and CEA levels are useful for postoperative surveillance. Preoperative high level of CEA is a prognostic factor. Serum calcitonin stimulation test is useful in familial MTC patients without RET mutation and pathological borderline cases.

After diagnosis, neck ultrasonography is used to evaluate lymph nodes. Chest and abdomen contrast-enhanced computerized tomography must be done for staging, especially in patients with lymph nodes in the neck or basal calcitonin levels >400 pg/mL. 18-fluoro-2-deoxyglucose positron emission tomography (FDG-PET/CT) is not routinely recommended for initial staging [25–27]. Its sensitivity is modest and may be recommended for a small subset of more biologically aggressive MTCs or detecting metastasis with elevated calcitonin levels >1,000 pg/mL [25, 26]. Tumor–node–metastasis (TNM) staging system suggested by the Union International Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC) is recommended for staging (Table 22.2) [28].

22.5 Treatment

Surgical resection remains the mainstay of primary treatment for MTC [29, 30]. There is limited evidence for external beam radiotherapy (EBRT) as a local therapeutic modality [31]. Treatment options for patients with advanced MTC are limited. Cytotoxic chemotherapy has negligible efficacy with poor response rates and short duration of response [29, 30]. Partial response (PR) rates of single-agent regimens such as doxorubicin, dacarbazine, capecitabine, and 5-fluorouracil have been <30 %. There is an obvious "unmet medical need" for novel therapeutic approaches in advanced MTC. Recent years have witnessed remarkable advances in the understanding of the molecular biology of MTC leading to introduction of novel agents directed to these targets.

22.5.1 Surgery

There is general consensus that the only curative treatment of MTC is meticulous total thyroidectomy with removal of any visible tumoral tissue from the cervical

region coupled with ipsilateral modified radical neck dissection provided that there is no advanced disease [32, 33]. Inherited MTC is almost always multicentric and bilateral, and about 5–6 % of patients with sporadic MTC turn out to be familial and multicentric. Furthermore, intraglandular lymphatic spread is possible in sporadic cases. Therefore, partial thyroidectomy is not recommended.

Because presence of lymphatic spread is of prognostic significance, optimal lymph node dissection is mandatory with "compartmental hierarchy" concept [34, 35]. Prognosis depends on age at diagnosis, stage of disease, and extent of initial thyroid or neck surgery [36]. Interestingly, some reports failed to show that the extent of lymphadenectomy improves the outcome in patients with MTC [37]. Lymph nodes in the central compartment between the hyoid bone and the innominate vessels, laterally to both internal jugular veins and carotid arteries, and posteriorly to the tracheal esophageal groove should be dissected. If central nodes are involved with metastasis, an ipsilateral modified neck dissection should be performed with removal of the contralateral central nodes. If central nodes are negative for metastasis, biopsy of the lateral lymph nodes is not required, but some authors recommend routine initial contralateral neck dissection. Mediastinal dissection with sternotomy may be considered in cases with gross detectable disease contained within the mediastinum. The role of salvage neck surgery remains controversial in MTC [34, 35]. Palliative re-operation may be performed for compressive symptoms or risk for imminent compression or invasion of the trachea or major vessels. Lymphadenectomy should be very restrictive in the presence of locally advanced and/or metastatic disease. Anticipated complications of surgery include vocal cord paralysis, airway obstruction, and hemoptysis.

22.5.2 Radiotherapy

EBRT may be suggested as adjuvant treatment in patients with less than optimal lymph node dissection or with extrathyroidal disease [31]. Clinical studies warrant the use of postoperative adjuvant EBRT in patients at high risk for locoregional recurrence such as locally invasive tumor, grossly positive surgical margins, extranodal tumor extension, and detectable calcitonin after surgery. In this setting, adjuvant RT may reduce the 10-year local recurrence rate by half [38]. Some MTC patients treated with EBRT might enjoy prolonged survival duration [39]. However, some authors argued against treating MTC with EBRT because they consider MTC to be relatively radioresistant [40, 41]. EBRT is best suited for palliative purposes such as symptomatic central nervous system and bone metastases or irresectable local neck recurrences [42].

22.5.3 Chemotherapy

Treatment with either single-agent or combination cytotoxic chemotherapy has been generally inadequate. Experience in this field is largely limited to small case series or phase II studies with no phase III studies available to date. Single agents

with some antitumor activity in MTC include dacarbazine, doxorubicin, DTIC, cisplatin, cyclophosphamide, streptozotocin, fluorouracil, and vincristine [43–45]. Various combinations of these chemotherapeutic drugs resulted in PR of 15–20 % with no documented complete responses. In spite of inducing short-lived PRs in <30 % of the advanced MTC patients, doxorubicin treatment is approved by the US Food and Drug Administration (FDA) for the treatment of metastatic thyroid cancer including MTC. Among other cytotoxics, DTIC is the only agent recommended by the National Comprehensive Cancer Network (NCCN) for symptomatic or progressive MTC patients despite to its modest activity in non-controlled trials [46].

Because chemotherapy in advanced MTC has an insufficient efficacy, it should be reserved for patients with progressive disease refractory to targeted agents.

22.5.4 Somatostatin Analogues

Somatostatin analogues have been used with some success in (NETs), especially in symptomatic patients for symptom palliation. Octreotide, synthetic analogue of somatostatin, has been tried in MTC patients [47]. There is no randomized controlled study, but only case series or non-controlled studies which were not able to demonstrate any consistent antitumor effect. All studies showed subjective and biological PR in about 25 % of the MTC patients with no improvement in tumor load. It seems that somatostatin analogues are only helpful in alleviating symptoms.

22.5.5 Tyrosine Kinase Inhibitors

Targeted therapies with small molecule inhibitors of tyrosine kinases (TKI) have been used in cancers with demonstrated driving mutations involved in the pathogenesis. The better defined is the target, the better is the response to treatment with its inhibitors. These molecules target several tyrosine kinases involved in a variety of steps of the signal transduction pathways regulating proliferation, survival, gene expression, angiogenesis, and migration functions. Therefore, these "multikinase" inhibitors have successfully been used in thyroid cancers including MTC directed to RET, vascular endothelial growth factor receptor (VEGFR), and the epidermal growth factor receptor (EGFR). It has been proposed that inhibition of a single kinase receptor may result in upregulation of compensatory signaling pathways to sustain cell growth. Multikinase inhibitors have been developed to bypass this potential resistance mechanism.

22.5.5.1 Cabozantinib

Cabozantinib (XL184) is an oral TKI with activity against c-MET, VEGFR-2, and RET [48, 49]. Activation of these receptors has been implicated in both development and progression of MTC. Inhibition of c-MET, which is the receptor for hepatocyte growth factor (HGF), may confer additional activity against MTC.

A phase I open-label dose-escalation study of cabozantinib was performed in patients with advanced solid tumors including 37 patients with MTC [50]. An objective PR was achieved in 10 of 35 patients with measurable disease in the MTC cohort. Stable disease (SD) of 6 months or longer was observed in 15 patients. Overall 68 % of the patients experienced SD + PR at the sixth month of assessment. Documented responses were independent of the *RET* mutation status of the tumors, suggesting that antineoplastic effects of the drug were due to inhibition of targets other than RET pathway [50]. Following the remarkable results of phase I study, a double-blind, randomized phase III EXAM study of cabozantinib 140 mg daily was conducted in 330 patients with progressive MTC [51]. Randomization was 2:1 (cabozantinib to placebo) and the primary endpoint was progression-free survival (PFS). A statistically significant improvement in estimated median PFS was observed in the cabozantinib group (11.2 vs. 4.0 months) (HR 0.28, 95 % CI 0.19–0.40; $p < 0.001$). One-year PFS was 47.3 % for cabozantinib and 7.2 % for placebo. Response rate (all PR) for cabozantinib was 28 % (vs. none for placebo) regardless of RET mutation status. There was no statistically significant difference in overall survival (OS).

Side effects were considerable. Of all patients, 79 % required dose reductions and 16 % discontinued cabozantinib permanently. Common adverse events seen in ≥10 % of the patients included diarrhea, palmar–plantar erythrodysesthesia, fatigue, nausea, weight loss, loss of appetite, hypertension, hemorrhage, hypocalcemia, and elevated liver function tests. Grade 3 or 4 adverse events were reported in 69 % of cabozantinib-treated patients. Significant electrocardiographic abnormalities (QT prolongation) were not encountered in the trial, as was observed with vandetanib [52].

In a subgroup analysis, cabozantinib markedly improved PFS in the subset of patients whose tumors contained *RET*M918T mutations (61 vs. 17 weeks; HR 0.15, 95 % CI 0.08–0.28) or whose tumors contained *RAS* mutations (47 vs. 8 weeks; HR 0.15, 95 % CI 0.02–1.10) [53]. The results of the phase III EXAM trial caused FDA to approve cabozantinib for patients with progressive metastatic MTC in November 2012.

22.5.5.2 Vandetanib

Vandetanib (ZD6474) is an oral small molecule TKI that blocks RET, VEGFR-1, VEGFR-2, and EGFR. The efficacy of vandetanib in hereditary MTC has been assessed in two phase II open-label, single-arm studies. The first phase II clinical trial enrolled 30 patients with hereditary, unresectable, or metastatic MTC to receive vandetanib at 300 mg/day [54]. A PR was achieved in six (20 %) and SD in 16 (53 %) patients. Median PFS was 27.9 months. Common adverse events were diarrhea, rash, fatigue, and nausea. The second phase II study enrolled 19 patients who receive vandetanib at 100 mg/day with escalation after progression to 300 mg/day [55]. Antitumor activity of low-dose vandetanib was verified with similar results (16 % PR and 53 % SD) and similar adverse events despite to lower dose.

In light of these results, phase III ZETA trial was carried out randomizing vandetanib 300 mg/day or placebo in 331 patients with advanced hereditary or sporadic

MTC [52]. The primary endpoint was PFS, and secondary endpoints were OS, objective response rate (ORR), disease control rate (DCR) at 24 weeks, duration of response, biochemical response, and time to worsening of pain, safety, and tolerability. Vandetanib showed efficacy in all evaluable endpoints in this trial except OS. At a median follow-up of 24 months, median PFS was not reached with vandetanib (estimated 30 months) and was 19.3 months with placebo [HR, 0.46; 95 % confidence interval (CI), 0.31–0.69; $p<0.001$]. The 6-month PFS rate was 83 % in the vandetanib group and 63 % in the placebo group. Vandetanib induced a higher ORR than placebo (45 % vs. 13 %; $p<0.001$) and higher DCR (87 % vs. 71 %; $p=0.001$). OS data were immature at the time of analysis and revealed no significant difference (HR, 0.89; 95 % CI, 0.48– 1.65). RET mutation was positive in 56.5 % of patients suggesting lack of correlation of RET mutation status with clinical outcome.

Common adverse events associated with vandetanib included diarrhea/colitis (56 %), rash (45 %), nausea (33 %), hypertension (32 %), headache (26%), and fatigue (24 %) [24]. Adverse events ≥ grade III were diarrhea (11 %), hypertension (9 %), QT prolongation (8 %), and fatigue (6 %). Because of the potential of QT prolongation, torsades de pointes, and sudden death risk, vandetanib is currently only available through the FDA Vandetanib Risk Evaluation Mitigation Strategy (REMS) Program. Electrocardiogram (ECG) and serum potassium, calcium, magnesium, and thyroid-stimulating hormone levels should be closely monitored and corrected. Vandetanib is the first multitargeted TKI approved by the FDA for advanced MTC.

22.5.5.3 Sorafenib

Sorafenib (BAY-43-9006) is another oral multikinase inhibitor that targets VEGFR-1, VEGFR-2, and VEGFR-3, RET, PDGFR, c-KIT, and BRAF [56]. Sorafenib was approved by the FDA for metastatic renal cell cancer and hepatocellular carcinoma. In vitro studies showed that it inhibits oncogenic RET V804L or RET V804M mutants that are resistant to other TKIs [57].

A phase I trial of sorafenib combined with the oral farnesyl transferase inhibitor tipifarnib to investigate combinations of drugs is synergistically effective, yielding partial response in five patients and stable disease in another five patients, with a median PFS of 15 months [58]. In a phase II trial, sorafenib (400 mg/twice daily) induced a PR in only one patient (6.2 %) and SD ≥ 15 months in 50 % of patients reaching a median PFS of 18 months [59]. Ahmed et al. showed 93 % PFS and 100 % OS rates at 1 year in metastatic MTC patients [60]. Response rates of 13 and 25 % were reported at 6 month and 12 months, respectively, suggesting that responses do occur beyond 6 months.

We reported our experience with sorafenib in thyroid cancer patients [61]. In MTC cohort, we observed a PR in 14 % of the patients. Median PFS was 14.5 months and OS was not reached. Adverse events were generally consistent with the known profile of sorafenib. It seems that sorafenib is as active as other FDA-approved TKIs, but lack of a phase III data makes it an unattractive choice.

22.5.5.4 Sunitinib

Sunitinib (SU11248) targets RET; VEGFR-1, VEGFR-2, and VEGFR-3; c-KIT; fms-related tyrosine kinase 3 (FLT3); and PDGFR. Its inhibitory effect on VEGFR and RET makes this drug a rational choice for treating MTC. Objective responses with sunitinib were reported in 6–31 % and SD in 46–80 % of patients [62–64]. In a small trial, sunitinib (37.5 mg/day continuously) induced a PR in three of seven patients with a median follow-up of 15.5 months [64]. Median PFS was 12.8 months. The most common treatment-related adverse events included neutropenia, thrombocytopenia, lymphopenia, hypertension, hand–foot syndrome, fatigue, and gastrointestinal symptoms.

22.5.5.5 Pazopanib

Pazopanib targets the VEGFR-1, VEGFR-2, and VEGFR-3, PDGFR, and c-KIT. Although the above TKIs targeting VEGF receptors also inhibit RET, pazopanib does not have any anti-RET activity [65]. Pazopanib was studied at a dose of 800 mg daily in a phase II study of 35 patients with progressive MTC; five patients (14 %) had partial response [66]. Median PFS and OS were 9.4 and 19.9 months, respectively.

22.5.5.6 Axitinib

Axitinib (AG-013736) is an orally active multikinase inhibitor of the tyrosine kinases VEGFR-1, VEGFR-2, and VEGFR-3, PDGFR, and c-KIT but not RET. A multicenter, open-label, phase II trial with axitinib (5 mg twice daily) induced a PR in 2 of 11 patients, and another three patients had stable disease lasting at least 16 weeks [67]. The most common adverse effect was hypertension.

22.5.5.7 Imatinib

Imatinib (STI571) inhibited RET Y1062 phosphorylation and induced cell cycle arrest and apoptotic cell death in MTC-derived cell lines expressing mutant RET receptors [68]. However, clinical results were disappointing with no tumor response in two trials [69].

22.5.5.8 Motesanib

Motesanib diphosphate (AMG706) targets the VEGFR-1, VEGFR-2, and VEGFR-3, PDGFR, wild-type RET, and stem cell factor receptor (c-KIT). A single-arm phase II trial using motesanib diphosphate 125 mg once daily was conducted in 91 advanced MTC patients with a dismal PR rate (2 %) [70]. However, median PFS was 48 weeks because of disease stabilization beyond 24 weeks in 48 % of the patients.

Conclusion

MTC is a rare NET derived from the calcitonin-secreting C cells of the thyroid gland. Almost 30 % of the patients have an autosomal dominant hereditary syndrome called MEN2 with three distinct subtypes: MEN-2A, MEN-2B, and familial MEN. RET proto-oncogene mutations are responsible for the

pathogenesis. Hereditary forms of MTC have germline mutations of the RET gene. Majority of the sporadic cases harbor somatic RET gene mutation, whereas about 5 % of the sporadic cases also have germline RET gene mutations. These mutations have different penetration rates causing a diversity of disease phenotypes. Identification of patients genetically at high risk is critical, because affected individuals may benefit from an early diagnosis and prophylactic thyroidectomy. The only curative treatment modality is aggressive surgical resection with inadequate impact of radiotherapy and chemotherapy. Prognosis of progressive and metastatic MTC is dismal. Better understanding of the molecular biology has led to the discovery of specific targeted inhibitors against tyrosine kinases that have changed the way metastatic disease is treated. Two of these agents are approved by the FDA in the treatment of advanced MTC.

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Diagnosis and Management of Adrenocortical Carcinomas

23

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23.1 Introduction

Adrenal tumors are becoming increasingly common with the improvement in imaging techniques. Prevalence of incidental adrenal lesions detected by computed tomography (CT) or magnetic resonance imaging (MRI) exceeds 5 % with an estimated incidence of 7.3 % from autopsy series [1–3]. Although the majority of those lesions are benign adrenocortical adenomas, some of the lesions are malignant, known as adrenocortical carcinoma (ACC). ACC is a rare and highly aggressive tumor and is usually resistant to most of the conventional chemotherapeutics [4]. Overall 5-year survival is less than 40 % [5, 6]. Significant progress has been made in delineating the pathogenesis of ACC over the past 20 years. Surgery is the preferred treatment for localized ACC with the aim to achieve complete resection with clear margins. Unfortunately current treatment options other than surgery have limited efficacy in the adjuvant and advanced setting. Given the low incidence of the disease, a number of clinical trials in patients with ACC are limited and mostly retrospective; therefore treatment recommendations are generally based on retrospective data or expert opinions. In order to understand molecular mechanisms underlying the disease and develop more effective treatment for ACC, clinical and translational studies with international collaboration are needed.

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23.2 Epidemiology

The incidence of ACC is approximately 1–2 cases per million, and ACC accounts for 0.02–0.2 % of all cancer deaths. ACC can occur at any age. The disease has a bimodal incidence peak, with a larger peak in the 4–5th decade of life and another peak in the first decade of life. The incidence is slightly higher in females [6, 7].

In the largest published series of ACC ($n = 3{,}982$), the median age at diagnosis was 55 years, median tumor size was 13 cm, and distant metastases were found on presentation in 21.6 % of patients [6, 8]. ACCs may be nonfunctional and may present with symptoms due to mass-forming effects of the tumor, such as abdominal discomfort, pain, or indigestion, or can be functional with hormone-secreting features in up to 34–62 % of the patients. Presentation and clinical symptoms are based on the type of produced hormone. Cortisol secretion and resulting Cushing's syndrome is the most common presentation followed by either virilization or a combination of both [7–10]. Feminizing syndrome due to the secretion of estradiol, prolactin, and testosterone and hyperaldosteronism are also seen. In the later phases of the disease with the growing mass in the abdomen, approximately one-third of the patients experience nonspecific abdominal discomfort. Advances in the imaging techniques may have increased the frequency of asymptomatic presentations which may also translate into the improved survival rates. Kasperlik-Zaluska et al. reported that 28.5 % of their 63 patients with ACCs presented as incidentalomas [11]. The mean duration from the onset of symptoms to diagnosis of ACC varies from 6 to 16 months and appears to be independent of whether it is functional or clinically silent [12, 13].

23.3 Pathogenesis

The etiopathogenesis of ACC is rather complex; there is no identified causative environmental agent described yet. Although ACCs are mostly sporadic, certain inherited cancer syndromes may present with ACC. Detailed description of these hereditary cancer syndromes and involved genes will be very important in the future for describing carcinogenic pathways and development of new targeted therapies. Hereditary tumor syndromes associated with ACC are Li–Fraumeni syndrome, Beckwith–Wiedemann syndrome, Carney complex, multiple endocrine neoplasia (MEN1), congenital adrenal hyperplasia, and familial adenomatous polyposis (FAP) [10].

There are some genes involved in the pathogenesis of ACC and one of them is TP53; TP53 is a tumor suppressor gene; it causes cell cycle arrest or cell death in response to DNA-damaging agents. TP53 mutations in exons 5–8 may be seen in ACCs and patients having these mutations have shortened survival. The prevalence of TP53 gene mutation in adult patients with ACC was found to be 3.9 % [14]. Genetic counseling with germline testing for TP53 is recommended to all patients with ACC, particularly if age <40 [15].

Another important gene implicated in the pathogenesis of ACC is insulin-like growth factor-2 (IGF2) gene. Rearrangements, loss of heterozygosity (LOH), and abnormal imprinting of the 11p15.5 locus are associated with elevated IGF2 mRNA expression in ACC [16, 17]. IGF2 overexpression could be used as a diagnostic tool for discriminating benign from malignant adrenal masses because IGFR 2 overexpression is a consistent finding in ACCs [18–20]. Higher IGF2 levels are associated with a malignant phenotype, and overexpression of IGF2 is associated with an increased risk of ACC recurrence [21]. Demethylation of IGF2 is also found more frequently in ACCs and correlates with IGF2 mRNA expression [17, 18]. Furthermore, LOH of the 11p15 locus has been demonstrated more frequently in ACC (67 %) compared to adrenal adenomas (13 %). It has been postulated that this leads to overexpression of IGF2 because the maternal allele is lost with duplication of the paternal allele leading to a double dose of the expressed allele [17].

Activating somatic mutation of the *CTNNB1* (beta-catenin) gene has also been identified in ACCs. Wnt/beta-catenin pathway activation was linked to decreased disease-free and overall survival in patients with resected adrenal carcinoma [22]. Other genes potentially involved in the pathogenesis of ACC are *ZNRF3*, *CDKN2A*, *RB1*, *MEN1*, CYP21B, PRKAR1A, and GNAS genes [23].

A number of studies have assessed the use of molecular markers in discriminating ACCs from adrenal adenomas. Immunohistochemistry with Ki-67/MIB1 has been found to be useful in differentiating ACCs from adrenal adenomas [24–26] with a reported sensitivity of 87.5 % and specificity of 95.5 % [25]. Combining IGF2 with Ki-67/MIB1 IHC improved sensitivity and specificity for differentiating ACCs from adrenal adenomas to 100 and 95.5 %, respectively [25]. IHC with p53 has not been found to be particularly helpful because even though it is highly specific for ACCs, its sensitivity is low, ranging from 5.4 to 73 % [24–26]. Protein expression of matrix metalloproteinase type 2 (MMP2, also known as gelatinase A) has been found to be high in ACCs but low in adrenocortical adenomas. MMP2 protein expression in ACCs was focal in two-thirds of cases and diffuse in the remainder. More diffuse expression of MMP2 in ACCs was associated with shorter overall and disease-free survival [27]. Interestingly, while MMP2 mRNA was found more frequently in ACCs compared to adrenal adenomas, mRNA was actually found in surrounding stromal tissue and not the neoplastic cell itself [28]. Serum levels of MMP2 have not been found to be useful in predicting either ACCs or adrenal adenomas [29].

None of the mentioned markers have been validated in prospective studies or tested in clinical routine.

23.4 Clinical Presentation and Diagnosis

Approximately 60–70 % of adult patients with ACC present with clinical findings of hormone excess, mostly with Cushing's syndrome. Weight gain, weakness, virilization, feminization, or hyperaldosteronism may occur depending on the hormone

secreted. Diagnosis is based on clinical, laboratory, and radiologic findings. Signs and symptoms of Cushing's syndrome, hyperandrogenism, pheochromocytoma, and hyperaldosteronism should be assessed. Laboratory evaluation should include corticotropin (ACTH), serum cortisol, 24-h free cortisol in urine, total testosterone, dehydroepiandrosterone sulfate, and estradiol. Complete blood count and serum chemistries should also be obtained [10]. The European Network for the Study of Adrenal Tumors recommends plasma aldosterone and renin measurement if hypertension or hypokalemia is present and plasma metanephrine or urinary metanephrine and catecholamine measurement in all patients to exclude pheochromocytoma [30]. Excessive amounts of adrenal steroid precursors are detected in urine in recent studies; these markers may aid in the diagnosis and follow-up to detect the presence of tumor recurrence in patients with ACC [31].

Computed tomography (CT) is widely used in the diagnosis and differentiation of benign from malignant lesions. The size of the tumor is important as increased size of the adrenal mass usually is a sign of malignancy (>5 cm tumors are usually malignant). An adrenal tumor diameter of 5 cm identifies ACC with a sensitivity of 93 % and a specificity of 64 % [32]. Second, the radiologic appearance of the suspected mass is decisive; benign adenomas usually have higher lipid content, and this results in a low attenuation on CT scans (i.e., <10 Hounsfield unit). Another feature of benign adenomas is rapid contrast enhancement and rapid washout. Irregular shape and margins, presence of hemorrhage, and heterogeneity are features suggesting malignancy [33–35].

Magnetic resonance imaging (MRI) and FDG-PET scans are helpful in evaluating adrenal carcinoma. MRI may show high lipid content of the adrenal mass as well as hemorrhage and heterogeneity of the tumor. A recent meta-analysis showed that FDG-PET had 97 % sensitivity and 91 % specificity in discriminating benign from malignant adrenal masses. Integrated PET/CT scans have even higher sensitivity and specificity. Therefore PET/CT is an important diagnostic tool for ACC, particularly for evaluation of indeterminate masses [36–38].

23.5 Pathology

The only definitive criterion for malignant adrenal tumors is the presence of local invasion or distant metastases. Fine-needle aspiration biopsy cannot discriminate between adrenal adenoma and ACC but may be useful in distinguishing adrenal metastases. On macroscopic examination, ACCs are often large, with a tan-yellow cut surface, and areas of hemorrhage and necrosis may be seen. The tumors are usually encapsulated or lobulated and can be solid or cystic [39]. On microscopic examination, the tumor usually displays sheets of atypical, eosinophilic cells with some resemblance to the cells of the normal adrenal cortex. Polygonal cells are arranged in sheets, nests, trabeculae, or ribbons and may contain anaplastic features. Other signs of malignancy are increased mitotic activity and vascular and capsular invasion. Immunohistochemistry may help to differentiate ACCs from adrenal adenomas. ACCs usually show immunostaining with Ki-67 (>10 %) and overexpression of insulin-like growth factor 2. The presence of invasion and

increased mitotic activity helps differentiate small cancers from adrenocortical adenomas [39, 40].

Differential diagnostic scores have been developed for pathological diagnosis; currently the Weiss and modified Weiss scores are the most widely accepted scoring systems and are based on histological findings. The updated Weiss score consists of five criteria including mitotic rate >5 mitoses/50 high-power fields, cytoplasm (clear cells comprising 25 % or less of the tumor), abnormal mitoses, necrosis, and capsular invasion. Each criterion is scored 0 when absent or 2 for the first two criteria and 1 for the last three when present; a total score ≥3 is highly suggestive of ACC [41, 42].

There are several relatively rare variants of ACC. These histologic variants are oncocytic adrenal cortical carcinoma, myxoid adrenal cortical carcinoma, carcinosarcoma, adenosquamous adrenocortical carcinoma, and clear cell adrenal cortical carcinoma [39].

23.6 Staging

There are several staging systems in use. Macfarlane et al. proposed a staging system in 1958 [43]. This staging system has been later modified by Sullivan et al. in 1978 [44] and later by Lee et al. in 1995 [45] (Table 23.1). Based upon the definitions of this staging system, the American Joint Committee on Cancer (AJCC)/UICC staging scheme was developed [46] (Tables 23.2, 23.3, 23.4, and 23.5). According to this scheme, the stage of ACC is determined by the size of the primary tumor, the extent of local invasion, and whether it has spread to regional lymph nodes or distant sites. Stage I–II disease is confined to the adrenal gland with a tumor size of less than or greater than 5 cm, respectively. Stage III disease is defined as invasion into adjacent organs or regional lymph nodes, while stage IV disease denotes distant metastatic disease [43, 44].

In addition to the abovementioned AJCC staging, the European Network for the Study of Adrenal Tumors (ENSAT) staging system is widely used internationally [47]. The ENSAT staging system is essentially the same as the AJCC system, but this staging proposals tend to limit stage IV patients with distant metastasis. Presence of local invasion, venous tumor thrombosis, or local lymphadenopathy is

Table 23.1 Staging of ACCs

Stage	Macfarlane [43]	Lee et al. [45]
I	T1 (<5 cm) N0M0	T1 (<5 cm) N0M0
II	T2 (>5 cm) N0M0	T2 (>5 cm) N0M0
III	T3 (local invasion without involvement of the adjacent organs) or mobile positive lymph nodes	T3/T4 (local invasion as shown by histological evidence of adjacent organ invasion, direct tumor extension to IVC, or tumor thrombosis within IVC or renal vein), and/or N1 (positive regional lymph nodes), M0
IV	T4 (invasion of the adjacent organs) or fixed positive lymph nodes or M1 (distant metastases)	T1–4, N0–1, M1 (distant metastases)

Table 23.2 Definitions of TNM

Primary tumor (T)[a]	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor ≤5 cm in greatest dimension, no extra-adrenal invasion
T2	Tumor >5 cm, no extra-adrenal invasion
T3	Tumor of any size with local invasion, but not invading adjacent organs[b]
T4	Tumor of any size with invasion of adjacent organs[b]

[a]Adopted from [90]
[b]Adjacent organs include the kidney, diaphragm, great vessels, pancreas, spleen, and liver

Table 23.3 Definitions of TNM

Regional lymph nodes (N)[a]	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastases in regional lymph node(s)

[a]Adopted from [90]

Table 23.4 Definitions of TNM

Distant metastasis (M)[a]	
M0	No distant metastasis
M1	Distant metastasis

[a]Adopted from [90]

Table 23.5 Definitions of TNM

Anatomic stage/prognostic groups[a]			
Stage	T	N	M
I	T1	N0	M0
II	T2	N0	M0
III	T1	N1	M0
	T2	N1	M0
	T3	N0	M0
IV	T3	N1	M0
	T4	N0	M0
	T4	N1	M0
	Any T		

[a]Adopted from [90]

defined as stage III. Most contemporary studies use this staging system because of superior prognostic stratification compared to AJCC scheme. According to the ENSAT scheme, 5-year disease-specific survival rates are 82 % for stage I disease, 61 % for stage II, 50 % for stage III, and 13 % for stage IV [48].

Proper staging should include CT of the abdomen and chest. Magnetic resonance imaging may increase specificity of CT evaluation. In-phase and out-of-phase T1-weighted imaging may be the most effective noninvasive method to differentiate benign from malignant adrenal masses. Extracapsular tumor invasion, extension into the vena cava, or metastases may be detected more accurately with MRI. Patency of surrounding vessels can often be demonstrated with gadolinium-enhanced sequences or flip-angle techniques [33, 35, 49].

23.7 Treatment

23.7.1 Surgical Resection

Complete surgical resection is the mainstay of treatment for ACC. Open surgical technique for adrenalectomy is recommended. Complete, en bloc, margin-negative resection should be the surgical goal [50, 51]. Lymph node dissection is recommended [52]. Resection of adjacent organs including the spleen, kidney, liver, or pancreas might be required if local invasion is present. Tumor extension into the inferior vena cava is not a contraindication to surgery, and resection can be performed by cardiopulmonary bypass. The role of laparoscopy is controversial; a number of previous studies showed that laparoscopic surgery for ACC increased the risk of local recurrence, peritoneal dissemination, and metastases [53–57], while others reported comparable results with open surgery particularly if the adrenal tumor is small [58]. Open surgery is currently the preferred approach by NCCN guidelines. Completeness of surgical resection is the most important factor that influences outcome [32, 59]. If resection is incomplete, repeated surgery for achieving clear margins should be considered.

Patients who undergo complete repeat resection of local recurrence or distant metastasis also have improved survival. One study showed that patients who had a complete second resection of local or distant recurrence had a median survival of 74 months (5-year survival, 57 %), whereas those with incomplete second resection had a median survival of 16 months (5-year survival, 0 %) [60]. Therefore surgery is still an important treatment in advanced disease when complete resection of recurrence and all metastases is feasible. Selected patients with uncontrollable symptomatic hormone excess might be candidates for debulking surgery.

The benefit of neoadjuvant systemic therapy prior to surgery for locally advanced disease is not known, and this is not considered a standard approach.

23.8 Systemic Therapy

23.8.1 Mitotane

Adrenocortical carcinoma is a rare tumor; thus there are no randomized trials of adjuvant therapy. Up to 80 % of the patients experience disease recurrence after radical resection; this forms the rationale behind adjuvant therapy. The only treatment which showed benefit in terms of disease-free and overall survival in retrospective reports is mitotane. Mitotane is a derivative of the insecticide dichlorodiphenyltrichloroethane (DDT or rothane). Mitotane is metabolized to an active metabolite which leads to necrosis of the zona fasciculata and reticularis of the adrenal cortex and inhibition of adrenocortical hormone production (blocking adrenal 11-beta-hydroxylation and cholesterol side-chain cleavage) [61]. The largest series of adjuvant mitotane use after surgery compared to surgery alone in 177 patients with ACC from 8 centers in Italy and 47 centers in Germany was published recently. All patients had radical resection with a follow-up of up to 10 years.

Forty-seven of the 177 patients received adjuvant mitotane after surgery, while the rest of the patients had surgery alone. Median recurrence-free survival was 42 months in the mitotane group, 10 months in the Italian control group ($p<0.001$), and 25 months in the German control group ($p=0.005$). Median overall survival was 110 months in the mitotane group, as compared with 52 months in the Italian control group ($p=0.01$) and 67 months in the German control group ($p=0.1$). Mitotane treatment had a significant advantage for recurrence-free survival in multivariate analysis [62]. The median duration of treatment was 29 months. Twenty-seven patients received 1–3 g/day and 20 patients received 3–5 g/day. Serum levels of mitotane were not assessed. Grade 3 gastrointestinal (nausea or vomiting or elevated serum GGT) or neurologic events (confusion, ataxia, vertigo) were observed in 15 and 20 % of the patients, and all occurred in patients who received the higher-dose mitotane regimen.

Adjuvant mitotane is indicated in patients with high risk of recurrence; however the definition of high risk is not uniform. This controversial topic was discussed in 2008 by an international panel of specialists for the treatment of patients with ACC. On these grounds, the panel unanimously stated that patients with potential residual disease (R1 or Rx resection) and/or Ki67 more than 10 % should be offered adjuvant mitotane, whereas adjuvant therapy was not considered mandatory in patients fulfilling all of the following criteria: stage I or II disease (based on the new European Network for the Study of Adrenal Tumors), histologically proven R0 resection, and Ki67 expressed in ≤10 % of neoplastic cells. The panel did not express a unanimous position with respect to whether or not patients with stage III ACC with R0 disease after surgery should receive adjuvant therapy in clinical routine [63]. Fassnacht et al. propose tumor size >8 cm and microscopic evidence of invasion of blood vessels or the tumor capsule as additional risk factors which require consideration of adjuvant therapy [4]. NCCN guidelines suggest that mitotane be “considered” (category 3 recommendation, with major disagreement that the intervention is appropriate) for all patients with resected low-grade or high-grade localized ACC regardless of stage or tumor size [52]. Intraoperative tumor spillage or fracture is also suggested by some authors as indication for adjuvant mitotane [64]. A large international randomized trial in 200 patients with low- to intermediate-risk resected disease [stage I to III, microscopically complete (R0) resection, Ki67 < 10 %] is currently ongoing (the ADIUVO trial, NCT00777244).

Response rates to single-agent mitotane in metastasized ACC are between 13 and 31 %. Responses are mostly partial and generally short-lived [65]. However these were early studies, mitotane doses were suboptimal, and response evaluations were not up to date. Median survival was less than 1 year. The main benefit of mitotane in patients with advanced disease is reduction in hypercortisolism symptoms. Mitotane is almost always administered in combination with cytotoxic chemotherapy in metastatic disease given the higher response rates.

Side effects including anorexia, nausea, vomiting, and diarrhea are common and can limit adjuvant mitotane use as a long-term treatment. Also monitoring for the blood level of mitotane is essential for effective treatment. Mitotane treatment starts 1 g twice daily orally and the dose is escalated up to 10 g/day. Patients

metabolize mitotane to different degrees. Optimum dose of the drug can be safely and accurately delivered by monitoring the blood levels. Therefore monitoring of plasma levels of mitotane is required, and a target serum level between 14 and 20 mg/L should be achieved for obtaining optimal response and decreased toxic side effects [66].

Use of mitotane routinely induces atrophy and/or steroidogenic inhibition of the normal adrenal glands, thereby necessitating replacement therapy for cortisol deficiency; the zona glomerulosa is more resistant to the adrenolytic effect of mitotane, and aldosterone deficiency may occur after several months of therapy. Monitoring of blood sodium, potassium, creatinine, and 24-h urinary free cortisol levels is mandatory to assess adrenal insufficiency. Higher doses than the routine maintenance can be needed to avoid adrenal insufficiency. If signs or symptoms of mineralocorticoid deficiency (i.e., postural hypotension, hyperkalemia, etc.) develop, fludrocortisone should be added. Patients should also be monitored for hypogonadism and hypothyroidism. The most common side effects are fatigue, nausea, vomiting, and anorexia, but skin rash, diarrhea, lethargy, sedation, confusion, dizziness, ataxia, gynecomastia, arthralgias, leukopenia, prolonged bleeding time, hematuria, and reversible growth arrest in children also occur. Mitotane has significant drug interactions and can reduce efficacy of some calcium channel antagonists, opioids, benzodiazepines, macrolide-type antibiotics, and many other drugs [67].

There is no evidence for the role of cytotoxic chemotherapy in the adjuvant treatment of ACC. However, Fassnacht et al. from the German consortium suggest 3 cycles of 90 mg/m^2 cisplatin in addition to mitotane in patients with Ki67 >30 % and a large tumor thrombus in the vena cava [68].

23.9 Chemotherapy

Unfortunately recurrences are seen very often in ACC after complete surgical resection with reported rates of 21–91 %. Recurrences are generally treated with chemotherapy–mitotane combination. Response rates of ACC to various chemotherapy agents are reported to be between 10 and 40 %. Chemotherapeutic agents should be used in combination rather than monotherapy as ACCs usually express multidrug resistance gene MDR-1 and develop resistance to chemotherapeutic agents over time.

Regarding the data in the literature, combination of mitotane with etoposide, doxorubicin, and cisplatin (m-EDP) and combination of mitotane with streptozotocin [69, 70] are the two reasonable options for the management of patients with advanced ACC [71]. These two promising combinations were compared in the FIRM-ACT trial (which is still the only published randomized trial in ACC), results of which was published in 2013. A total of 304 patients were enrolled. Response rates were 23 % in the EDP–mitotane group versus 9 % in the streptozocin–mitotane group ($p < 0.001$). The first group had a 5-month progression-free survival compared to 2 months for the streptozotocin–mitotane group

($p<0.001$), and overall survival was similar (14.8 vs 12 months, $p=0.07$) [72]. Thus, evidence-based first-line treatment of choice is m-EDP in advanced ACC. However, the following comments were made by the investigators of this trial; as median PFS was 5 months and overall survival was 14.8 months, the outcome was actually poor. M-EDP regimen had similar activity in the second-line treatment as it was in the first line in previous studies. Therefore, patients with presumably less aggressive disease (slow tumor growth, long disease-free interval after initial surgery) might receive mitotane monotherapy in the first line followed by combination chemotherapy upon progression. Second, this patient group might also be good candidates for up-front experimental therapies as efficacy of several drugs is diminished once mitotane is used due to increased drug metabolism [68].

Patients receiving mitotane with or without chemotherapy should be assessed at 2-month intervals for tumor progression. Patients who show tumor regression or stable disease should be considered for surgical resection or continuation of therapy. Patients with progressive disease should consider other chemotherapy regimens or be enrolled in a clinical trial. There are currently no established second- or third-line chemotherapy regimens for systemic disease. However, there are phase II trials of gemcitabine plus capecitabine, and this combination has shown response rates as high as 46 %. Treated patients had stable disease for more than 4 months. This combination might represent a promising second-/third-line regimen [73].

Patients should be considered for clinical trials if they have progressive disease with conventional treatment.

23.10 Radiotherapy

Radiotherapy is not routinely used in the treatment of ACC. Formerly ACC was considered radioresistant. However, recent retrospective data showed efficacy of RT both in adjuvant and advanced setting, although no prospective randomized trials exist. Older radiotherapy techniques had higher toxicity because of the proximity of the adrenal bed to radiosensitive organs such as the kidney, liver, spinal cord, and small bowel [74]. Conformal radiotherapy techniques resulted in better efficacy and less toxicity; nevertheless optimal dose delivery may not always be possible in every patient depending on anatomic extension of the tumor.

Adjuvant radiotherapy to tumor bed after surgical excision is a controversial issue in ACC. Fassnacht et al. reported outcomes of 14 patients from the German ACC registry who received adjuvant radiotherapy to the tumor bed with a matched control group of 14 patients who did not. Local recurrence was significantly lower in the radiotherapy group, 14 % compared to 79 % in the control group. Disease-free and overall survival, however, were not significantly different [75]. Sabolch et al. reported 4.7 times higher risk of local recurrence in patients with ACC who did not receive adjuvant RT, again with no difference in DFS or OS [76] compared to those who did. On the other hand, a recent report from MD Anderson Cancer Center showed no benefit of adjuvant radiotherapy compared with surgery alone

[77]; however radiotherapy indications were not uniform, and RT was applied in various community centers in this study.

Guidelines proposed by the German ACC consortium recommend adjuvant radiotherapy within 3 months of surgery for patients with microscopically involved or indeterminate resection margins and stage III disease regardless of resection status. In addition, radiotherapy should be considered for tumors greater than 8 cm, with a Ki-67 Index >10 % or invasion of adjacent vasculature [48].

In patients with locally advanced disease not amenable to surgical resection, definitive radiotherapy represents an applicable option [76]. Radiotherapy has been shown to effectively palliate symptomatic bone, brain, and inferior vena cava disease [48, 76, 78].

In conclusion, adjuvant RT in high-risk patients seems to reduce local recurrence rate without any improvement in DFS or OS. Definitive radiotherapy is an option in inoperable cases. Palliative radiotherapy may be used for symptomatic tumoral lesions.

23.11 Radiofrequency Ablation

Percutaneous image-guided radiofrequency ablation (RFA) is a minimally invasive and reasonable method for unresectable localized disease. Previous studies have shown that RFA can produce local control of primary ACC particularly for tumors less than 50 mm in size and is anatomically suitable. Among 8 patients with 15 ACC primary or recurrences, RFA resulted in decrease in tumor size or loss of enhancement on imaging in 53 % of patients. Smaller tumors had better response (<50 mm), with up to 67 % demonstrating complete ablation [79]. RFA, alone or in combination with surgical resection, may allow for better disease control in local and isolated systemic recurrences.

RFA is not an effective method of treatment in tumors near blood vessels as the vessels act as a "coolant" while RF ablation. Bleeding, infection, and injury to adjacent organs can occur. With advancing technology and growing experience, RFA has the potential to have a role in treatment options of recurrent and/or unresectable ACC in selected patients [78, 80].

23.12 Targeted Therapies

The results of studies with targeted therapies including antiangiogenic drugs, multityrosine kinase inhibitors, and epidermal growth factor receptor inhibitors are largely disappointing. Epidermal growth factor receptor (EGFR) is highly expressed in ACC; however the combination of erlotinib and gemcitabine in salvage treatment showed limited activity with low response rates [81]. Vascular endothelial growth factor (VEGF) is upregulated in ACC tumor tissue, but bevacizumab plus capecitabine also failed to show any benefit [82]. Although there are case reports of sustained clinical response with antiangiogenic drugs sunitinib or sorafenib, clinical

trials yielded disappointing results [83, 84]. Inefficacy of tyrosine kinase inhibitors in ACC may be secondary to significant interaction with mitotane, given the very long half-life of mitotane. Therefore, clinical trials using these drugs in first-line treatment (before mitotane) should be designed to overcome drug interaction.

Insulin-like growth factor 1 receptor (IGF-1R) is overexpressed commonly in ACC. A study of the oral tyrosine kinase inhibitor (OSI-906) which targets IGF-1R has shown promising results in phase I, with stabilization of disease seen in five out of 16 patients [85]. An international phase III trial of OSI-906 in patients with ACC has been recently completed and results are awaited (NCT00924989). mTOR is a downstream signaling node for a number of receptor tyrosine kinases including IGF-1R [86]; Fraenkel et al. studied an mTOR inhibitor, everolimus, as a single agent in salvage treatment of ACC, but there was no response. Inhibition of mTOR alone possibly leads to compensatory activation of other pathways, and this may limit the use of everolimus as a single agent for the treatment of ACC [87]. In a recent phase I trial, combination of everolimus with an IGF-1R antibody cixutumumab resulted in disease stabilization in 42 % of the patients for a minimum of 6 months [88]. This type of combinations warrants further clinical research.

Steroidogenic factor 1 is a nuclear receptor expressed virtually by all ACCs, and in vitro studies using inverse agonists of this receptor showed activity against ACC cell lines [89]. Heat shock protein inhibitors and proteosome inhibitors are other agents with promising in vitro study results [68].

Conclusion

In recent years, major progress has been made in understanding the pathogenesis and treatment of ACC. Molecular profiling studies in patients with ACC showed that ACC is a very heterogeneous disease. Exon-sequencing studies identified recurrent alterations in known driver genes and in genes not previously reported in ACC [23]. Chemotherapy does not seem to work but still needs further trials mototan treatment adjuvantly and in metastatic disease shows promising results. However, overall prognosis is still poor. International collaboration is needed to design translational research and prospective clinical trials. New insights into the pathogenesis of ACC and identification of potential "driver" pathways will highlight the opportunity for more personalized treatment strategies in the future.

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Pheochromocytoma and Paraganglioma 24

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24.1 The Paraganglion System

Paragangliomas (PGLs) are rare vascular, neuroendocrine tumors of paraganglia, cell clusters originating from the neural crest that have co-migrated with the autonomic nervous system [1]. PGLs derive from either sympathetic tissue in adrenal and extra-adrenal locations (the latter known as sympathetic PGLs or sPGLs) or parasympathetic tissue of the head and neck (HNPGL, formerly called glomus tumors) [1]. Approximately 70–80 % of PGLs arise from the adrenal medulla and are referred to as pheochromocytoma (PCC) [1, 2]. PGLs can be found from the skull base to the sacrum, with a predilection for the middle ear (tympanic PGL), the dome of the internal jugular vein (jugular PGL), the bifurcation of the common carotid arteries (carotid body PGL), along the vagus nerve (vagal PGL), in the mediastinum (from the aortopulmonary body or the thoracic sympathetic chain), in the adrenal medulla, and in the abdominal and pelvic para-aortic regions, including the organ of Zuckerkandl.

24.2 Pathology

PGLs are usually well demarcated and often encapsulated. PGLs are characterized by their architecture, the tumor cells being arranged in distinctive cell balls, or Zellballen. These cell balls are separated by a fibrovascular stroma. The Zellballen are composed of clusters of chief cells that are surrounded by supportive sustentacular cells at the periphery. The chief cells, which are neuroendocrine cells, express neuron-specific enolase, serotonin, and chromogranin, and the sustentacular cells

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express S-100 protein [3]. Membrane-bound electron-dense neurosecretory granules may be seen on electron microscopy [4, 5]. These neurosecretory granules vary in size, configuration, and electron density of their cores [5]. PGLs originate from chief cells. The sustentacular cells are spindled and devoid of granules. The histologic features of malignant PGLs are basically identical to those of benign tumors, but may exhibit the following features: central necrosis of Zellballen, vascular and lymphatic invasion, and the presence of mitotic spindles [6]. Because there are no accepted pathological or immunohistochemical markers that distinguish malignant from benign tumors, malignancy is only diagnosed when metastasis to non-neuroendocrine tissue is demonstrated [7, 8]. From a prognostic point of view, relative risk factors for malignancy are PGLs larger than 5 cm with necrotic areas, extra-adrenal localization, the presence of an *SDHB* mutation [9], a PASS score (pheochromocytoma of the adrenal gland scale score) >6 [10] and a Ki-67 index >3 % [11].

24.3 Clinical Presentation

PGLs are rare tumors. Their prevalence is unknown but has been estimated to lie between 1:6,500 and 1:2,500 in the United States [12]. Their prevalence is higher in autopsy series (1:2,000), suggesting that many tumors remain undiagnosed [13]. The annual incidence has been reported to be two to ten cases per million [14]. The tumors may occur in all ages but have the highest incidence between 40 and 50 years, with an approximately equal sex distribution [15, 16]. From all PGLs, PCCs have the highest relative incidence. In 340 unselected PGL patients, about 73 % of the patients had PCC, 9 % had sPGL, and 20 % had HNPGL [16].

HNPGLs usually grow slowly; the majority has a tumor doubling time of more than 10 years [17]. Because of this slow growth rate, HNPGLs may remain clinically silent for years. Although these tumors are usually benign and only a minority produces catecholamines [18], their location in the close proximity of nerves and vascular structures often results in considerable morbidity due to compression or infiltration of the adjacent structures, causing symptoms like hearing loss, tinnitus, dysphagia, and cranial nerve palsy. Carotid body tumors are the most common HNPGL, usually presenting as a painless cervical mass [19]. Large compressive tumors may result in cranial nerve paralysis. Vagal body tumors present as painless neck masses, located behind the angle of the mandible, occasionally accompanied by dysphagia and hoarseness [20]. Tympanic and jugular foramen tumors most commonly present as a vascular middle ear mass causing pulsatile tinnitus and hearing loss. Difficulties in speech, swallowing, and airway function may be the result of dysfunction of cranial nerves traversing the jugular foramen [21].

The majority of PCCs and sPGLs produce catecholamines, causing symptoms of catecholamine excess. The clinical presentation is highly variable due to different profiles of catecholamines secreted, secretion of different other vasoactive peptides (e.g., neuropeptide Y, adrenomedullin, atrial natriuretic peptide), presentation of symptoms related to tumor mass or other organ involvement in syndromic forms, and desensitization of adrenoreceptors (most likely due to long-term exposure to

high circulating catecholamine levels) [22]. Hypertension, continuous or paroxysmal, is the most common feature of PCCs and sPGLs. Hypertensive crises are frequently associated with the classic triad of severe headache, palpitations, and diaphoresis. Paroxysms can last minutes to hours, vary in interval, and occur spontaneously or be triggered by direct stimulation of the tumor (e.g., bladder localization), physical activity, diagnostic procedures, or certain drugs (e.g., metoclopramide) [23]. Other symptoms may include anxiety, nausea, vomiting, dyspnea, and weakness [24, 25]. In addition, hyperglycemia, resulting from metabolic actions of catecholamines, may be the presenting symptom, which could lead to delayed diagnosis [26]. Morbidity and mortality in PCC/sPGL patients are predominantly the result of cardiovascular complications (e.g., sudden death, myocardial infarction, heart failure, and cerebrovascular accidents). In addition, malignant PGLs may also present with systemic symptoms such as weight loss or clinical manifestations related to metastatic spread such as pain in bones.

24.4 Spectrum of Hereditary Syndromes

PGLs can occur either sporadically or hereditary, as part of a familial syndrome [27]. The major features related to predisposition for inherited disease are familial antecedents of the disease, multiple primary tumors in the same individual, and early age of onset [28]. Sporadic PGLs are usually diagnosed in patients older than 40–50 years, whereas hereditary forms are diagnosed in younger patients. Until 2000, only 10 % of PGLs were associated with hereditary syndromes: von Hippel-Lindau disease (VHL), multiple endocrine neoplasia type 2 (MEN2), and neurofibromatosis type 1 (NF1), resulting from, respectively, a germ-line mutation in tumor suppressor gene *VHL* [29], proto-oncogene *RET* [30, 31], and tumor suppressor gene *NF1* [32]. PGLs occurring in these syndromes are predominantly (bilateral) PCCs. In the last years it has been demonstrated that about 30 % of the apparently sporadic PGLs are due to a germ-line mutation in one of 10 susceptibility genes [33]. In addition to the abovementioned mutations in *VHL*, *RET*, and *NF1*, mutations have been found in one of the four subunits (A, B, C, and D) of the succinate dehydrogenase gene (*SDH*) [34–37], *SDHAF2*, which is responsible for the flavination of the SDHA subunit [38], *TMEM127* [39], and *MAX* [40]. Germ-line mutations in *SDHA*, *SDHB*, *SDHC*, *SDHD*, and *SDHAF2* genes are responsible for the occurrence of syndromes named PGL5, PGL4, PGL3, PGL1, and PGL2, respectively. *SDHA*, *C*, *D*, and *SDHAF2* mutations are associated with (multiple) HNPGL, *SDHD* and *B* with sPGLs, and *TMEM127* and *MAX* with (bilateral) PCCs [41] (Table 24.1). *SDHB* mutations are generally associated with a higher malignancy rate than mutations in the other *SDHx* genes. Recent reviews and a meta-analysis of studies involving *SDHB*-mutated patients have documented that 17–31 % of their tumors were malignant [42, 43]. In addition, also *MAX* mutations have been associated with malignant tumors [40]. Besides abovementioned hereditary syndromes, a small fraction of PCC/PGLs is associated with other syndromes, including Carney triad, Carney-Stratakis syndrome, and multiple endocrine neoplasia type

Table 24.1 Genotype-phenotype correlations in hereditary PCC/PGL

Syndrome	Gene	HNPGL	sPGL	PCC	Multiple PGL	Bilateral PCC	Malig-nancy risk	Parent of origin preference
MEN2	*RET*	Extremely rare	–	++	–	++	±	–
VHL	*VHL*	Rare	+	++	+	++	+	–
NF1	*NF1*	–	–	+	–	–	+	–
PGL1	*SDHD*	+++	++	+	+++	+	+	Paternal
PGL2	*SDHAF2*	+++	–		++	–	Not known	Paternal
PGL3	*SDHC*	++	+	±	+	–	±	–
PGL4	*SDHB*	++	+++	++	++	+	++	–
PGL5	*SDHA*	+	+	–	–	–	Not known	–
–	*TMEM127*	±	±	+++	±	++	+	–
–	*MAX*	–	–	++	–	++	+	Paternal

+, present; –, absent
HNPGL head and neck paraganglioma, *sPGL* sympathetic paraganglioma, *PCC* pheochromocytoma, *PGL* paraganglioma, *MEN2* multiple endocrine neoplasia type 2, *VHL* von Hippel-Lindau disease, *NF1* neurofibromatosis type 1, *PGL1*–5 familial paraganglioma syndrome type 1–5

1 (MEN1) [44, 45]. In addition, other tumors have been documented in *SDH* mutations, like gastrointestinal stromal tumors [46], renal cell carcinomas [47], and a growth hormone-producing pituitary adenoma [48]. Apart from *RET*, which is a proto-oncogene, all the other susceptibility genes for PCC/PGL are tumor suppressor genes. All familial PGL syndromes have an autosomal dominant mode of inheritance; interestingly, *SDHD*, *SDHAF2*, and *MAX* are characterized by paternal transmission only of the disease [41, 49–51]. This observation strongly suggests that these genes are subject to genetic imprinting, although the exact molecular mechanism remains unknown [51, 52].

24.5 Etiology

Transcriptomic studies have shown that sporadic as well as hereditary PGLs can be divided in two main clusters linked to two different signaling pathways [53]. Cluster 1 contains all *VHL* (cluster 1A)- and *SDHx* (cluster 1B)-related tumors and is characterized by activation of the hypoxia-angiogenesis pathway in normoxia [54]. Consistent with Knudson's two-hit hypothesis for tumorigenesis involving a tumor suppressor gene, a heterozygous germ-line mutation in an *SDHx* gene is usually associated with somatic loss of the nonmutant allele in the tumor, i.e., loss of heterozygosity, resulting in inactivation of SDH enzymatic activity. Inactivation of SDH has been shown to result in accumulation of succinate which acts as an inhibitor of prolyl hydroxylase (PHD) enzymatic activity. PHDs are enzymes that are

required for the degradation of hypoxia-induced factor (HIF). As a consequence, even in the presence of oxygen, HIF cannot be destroyed via proteasome-mediated degradation driven by VHL protein and is stabilized to induce angiogenesis and tumorigenesis [55–57]. The latter also happens in *VHL* mutations. Cluster 2 contains all *RET*-, *NF1*-, *TMEM127*-, and *MAX*-mutated tumors and is associated with abnormal activation of kinase-signaling pathways, such as RAS/RAF/MAPK and PI3K/AKT/mTOR, leading to abnormal cell growth and diminished apoptosis capacity [39, 40, 58–61]. Another theory interlinking the pathogenesis of different hereditary forms of PGL is based on data from Lee et al. and Schlisio et al., showing escape from apoptosis of sympathoadrenal progenitor cells at the end of the embryonic period in case of mutations in *RET*, *NF1*, *VHL*, and *SDH*, thereby bearing the potential to form PCC/PGL in later life [62, 63]. Abovementioned data have increased overall knowledge on molecular defects in PGLs and could be used for development of new effective molecular-targeted therapies.

24.6 Diagnosis

24.6.1 Biochemistry

Synthesis of catecholamines is primarily regulated by the cytoplasmic enzyme tyrosine hydroxylase (TH), which converts tyrosine to 3,4-dihydroxyphenylalanine (DOPA) (Fig. 24.1) [64]. Tissue expression of this enzyme is largely confined to dopaminergic and noradrenergic neurons of the central nervous system, sympathetic nerves, and chromaffin cells of the adrenal medulla and extramedullary paraganglia. The following step is the production of dopamine (DA) by decarboxylation of DOPA by L-amino acid decarboxylase. DA formed in cytoplasm is transported into vesicular storage granules and is converted to norepinephrine (NE) by dopamine β-hydroxylase (DBH). In adrenal medullary chromaffin cells, NE is metabolized to epinephrine (E) by the enzyme phenylethanolamine-N-methyltransferase (PNMT). E is then translocated into chromaffin granules, where the amine is stored awaiting release. Expression of PNMT in extra-adrenal PGLs is negligent, which explains the preferential production of NE by these tumors, compared to the production of both NE and E by PCCs [65].

Catechol-O-methyltransferase (COMT) is a major player in catecholamine metabolism, catalyzing O-methylation of E to metanephrine, NE to normetanephrine, and DA to methoxytyramine.

The diagnosis of PCC/sPGL depends on the demonstration of excessive amounts of catecholamines (E, NE, DA) or their *O*-methylated metabolites metanephrine, normetanephrine, and methoxytyramine [66]. Since metanephrines have a longer half life and are produced continuously within tumor cells, whereas catecholamines are converted to metanephrines by the high methyltransferase activity of chromaffin tissue, measurement of plasma-free metanephrines and/or 24 h urinary excretion of fractionated metanephrines provides the best test with an excellent sensitivity of >96 % for detecting PCC/sPGL [67]. Since dietary constituents (especially caffeine and

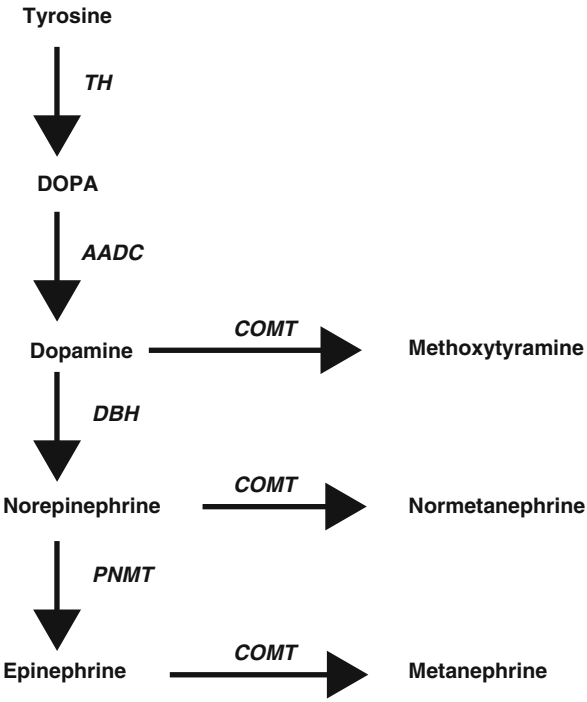

Fig. 24.1 Pathways of catecholamine synthesis and O-methylation. *TH* tyrosine hydroxylase, *DOPA* 3,4-dihydroxyphenylalanine, *AADC* aromatic amino acid decarboxylase, *COMT* catechol-O-methyltransferase, *DBH* dopamine β-hydroxylase, *PNMT* phenylethanolamine-N-methyltransferase

nicotine) or medication (especially acetaminophen, antihypertensive, and tricyclic antidepressant medication) can interfere with the analysis of or impact on levels of catecholamines or its metabolites, urine and plasma samples can best be collected while abstaining from these substances and/or timely stopping or changing medication (calcium entry blockers are relatively safe), with plasma being sampled in the supine position, to minimize sympathoadrenal activation [23, 68, 69]. PGLs exhibit different biochemical properties as PCCs mainly produce E and NE, sPGLs NE, and malignant PCCs NE or DA due to a dedifferentiation of the enzymatic machinery [70, 71]. Chromogranin A (CgA) is secreted from neurosecretory vesicles, along with catecholamines [72]. Plasma CgA level is often elevated in functioning and nonfunctioning PGLs [73]. The levels correlate with tumor mass, making CgA a useful tumor marker [74]. Sensitivity for identifying PGLs is 83–89 %, false-positive results however occur often due to liver or kidney failure or use of proton pump inhibitors [75]. Plasma CgA levels are particularly elevated in patients with malignant PCC [76].

24.6.2 Anatomical and Functional Imaging

Once a biochemical diagnosis of sPGL has been established, the source of catecholamine excess is subsequently localized by anatomical and functional imaging studies. Anatomical imaging such as computed tomography (CT) or magnetic resonance imaging (MRI) has an excellent sensitivity (77–98 % and 90–100 %,

resp.) but lacks specificity (29–92 % and 50–100 %, resp.) for detecting PCC/sPGL [77, 78]. On CT, PCCs usually present as homogenous tumors with soft tissue density of more than 10 Hounsfield units (HU) and uniform enhancement with contrast; however, larger PCCs may undergo hemorrhage and necrosis resulting in areas of low density [79]. On MRI, PCCs present as a mass absent of fat on chemical shift, with a high signal on T2 sequences as a result of their hypervascularity [80]. MR imaging with pre- and post-contrast-enhanced 3D time-of-flight (TOF) MR angiography sequence represents the most important imaging technique for evaluation and characterization of HNPGLs [81]. MR imaging of HNPGL provides more diagnostic information than does CT scanning, because of the better soft tissue contrasts as compared to CT [82]. MRI enables multiplanar imaging of tumor extension and vessel encasement. Ultrasound has a limited diagnostic yield but can be of use in imaging HNPGL [83].

Tumors detected by anatomical imaging can subsequently be identified as PGL by functional imaging agents that specifically target the catecholamine synthesis, storage, and secretion pathway of chromaffin cells. ^{123}I- or ^{131}I-metaiodobenzylguanidine (MIBG) scintigraphy is the most widely used nuclear technique in first-line functional imaging of PGL (Fig. 24.2). MIBG has chemical similarities to NE and is taken up by the human NE transporter (hNET), which is expressed in most chromaffin cells and is normally responsible for catecholamine uptake [84]. The sensitivity (83–100 % for ^{123}I – and 77–90 % for ^{131}I-MIBG, resp.) and specificity (95–100 % for both ^{123}I – and ^{131}I-MIBG) are high for primary tumors; however, sensitivity of MIBG-scintigraphy for metastasis is relatively poor [77, 85, 86]. In patients with negative MIBG-scintigraphy, other tracers may be used.

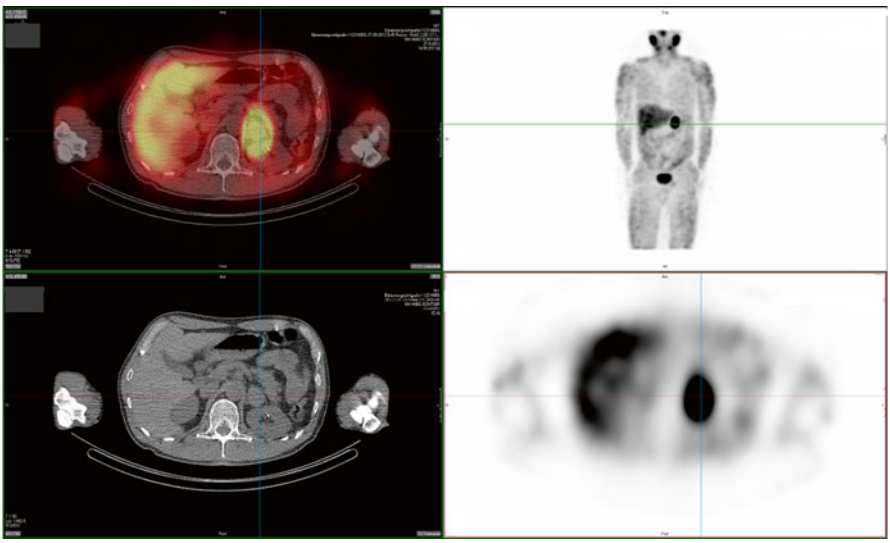

Fig. 24.2 ^{123}I-MIBG scan of a patient with a left-adrenal pheochromocytoma

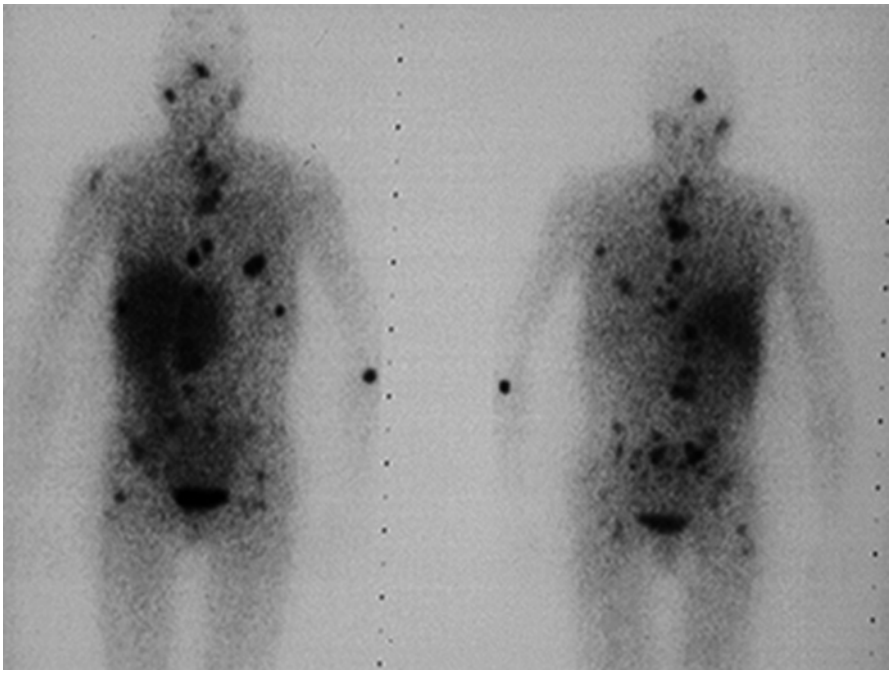

Fig. 24.3 ^{111}In-Octreoscan of a patient with diffuse metastatic sPGL of the bladder

PGLs have been found to express somatostatin receptor type 2, 3, and 5 on their cell surface. ^{111}In-pentetreotide scintigraphy is of limited value in detecting PCC but may be useful in detecting sPGL and MIBG-negative metastases (Fig. 24.3) [77]. In addition, it was shown to have an excellent sensitivity of 97 % and a specificity of 83 % to detect HNPGL [87–89]. Radiolabeled dopamine (DA) or dihydroxyphenylalanine (DOPA) may be used as tracers in positron emission tomography (PET) imaging. ^{18}F-DOPA PET has been confirmed to be useful in the evaluation of sPGL and HNPGL (Fig. 24.4) [90]. For patients suspected for metastatic PGL, ^{18}F-FDG or ^{18}F-FDA PET is recommended in *SDHB* mutation carriers and ^{18}F-DOPA or $^{18\text{F-FDA}}$ PET in non-*SDHB* mutation carriers [91, 92].

24.7 Treatment

The treatment of choice for PCCs and sPGLs is surgical resection, preferably laparoscopically [93], but in cases of large tumors with a high risk of malignancy, conventional laparotomy should be considered. In order to avoid surgical complications (hypertensive crisis, arrhythmias), all patients should be adequately pretreated with α-blockade (phenoxybenzamine, doxazosin), if necessary followed by β-blockade (propranolol, atenolol) and volume expansion as these patients are in a constant status of volume depletion [27]. This has been shown to reduce operative mortality

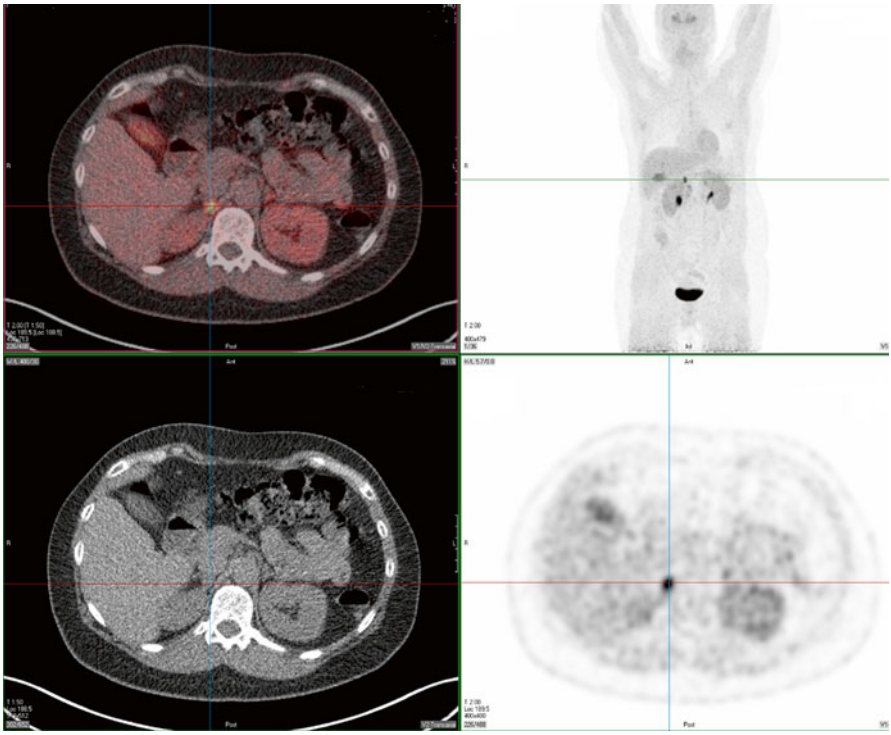

Fig. 24.4 ^{18}F-DOPA PET scan of a patient with a right-adrenal pheochromocytoma

to below 1 % [94]. In patients with bilateral PCC, laparoscopic cortical sparing adrenalectomies must be considered to avoid chronic glucocorticoid deficiency [95]. Preoperative injection of ^{123}I –MIBG in combination with intraoperative use of a gamma probe may localize small lesions that are difficult to find [96, 97]. After resection of ^{123}I –MIBG positive malignant PGL, postoperative ^{131}I –MIBG treatment for consolidation is recommended [98]. Postoperative follow-up with biannual measurement of blood pressure and annual plasma or urinary metanephrines and anatomical imaging should be performed.

Treatment for HNPGL must be considered in relation to tumor growth velocity, biological activity of the tumor, patient age and medical condition, tumor size and site, and potential for treatment-related morbidity [17]. Because most tumors grow slowly, a wait-and-scan policy is often advised [17]. However, although HNPGLs are indolent tumors, tumor growth may lead to serious morbidity and cranial nerve impairment due to their location in close proximity to important neurovascular structures. The main treatment modalities for HNPGL are surgery and radiotherapy. A multidisciplinary team approach is recommended for the choice of treatment of most HNPGL except for the very small and easy to resect tumors. With surgery, it is possible to remove the tumor without recurrence. Preoperatively, HNPGL should be tested for catecholamine excretion, and pharmacological preparation should be

started in positive cases [99]. The rate of surgical complications rises with the size of the tumor [100]. The most common complications are cranial nerve damage and vascular complications [101–104]. External beam radiotherapy and radiosurgery are alternative treatment modalities for HNPGL patients, resulting in local tumor control and sometimes regression by producing fibrosis and vascular sclerosis. These may be options in patients with large growing tumors, in which resection may result in considerable morbidity, or after incomplete resection of tumor with intracranial or skull base invasion [105].

24.8 Malignant Paragangliomas

Because there is no definite histological substrate for malignant PGL [106, 107], malignancy is defined by the presence of metastases: tumor spread in sites where chromaffin tissue is normally absent [7, 8]. Nearly 10 % of PCCs and 10–20 % of sPGLs are malignant [108], whereas HNPGLs are usually benign [109]. Malignant HNPGLs are confirmed by the presence of local metastases in cervical lymph nodes or systemic metastases, usually to bones, lung, and liver [106, 107, 109]. Malignant HNPGLs occur most frequently in patients with *SDHB* gene mutations [110–112]. In patients with *SDHD* gene mutations, malignant HNPGLs are rare [113, 114]. The primary management of patients with malignant HNPGLs should be directed toward complete surgical resection of the primary tumor and regional lymph nodes. Postoperative radiation may be beneficial in slowing the progression of residual disease [109]. Malignant PCC/sPGLs are especially associated with *SDHB* and *MAX* mutations [40, 43]. Treatment for malignant PCCs/sPGLs includes surgical debulking, pharmacological control of catecholamine-induced symptoms, external radiation, and systemic antineoplastic therapy. There is no effective treatment for malignant sPGLs. Although the usefulness of debulking of tumor has not been established, it may reduce catecholamine-related problems and improve response to further treatment. Up to 60 % of malignant PCC shows a positive MIBG uptake [115]. Treatment with therapeutic doses of ^{131}I-MIBG resulted in an objective tumor response in 30 % of patients, stabilization of disease in 57 % of patients, and progression of disease in 13 % of patients [98, 116]. A reduction in catecholamine secretion has been seen in up to 45 % of patients. In MIBG-negative cases, patients can be treated with combination chemotherapy, of which combination of cyclophosphamide, vincristine, and dacarbazine, the so-called CVD protocol, is the most effective regimen, producing partial remissions and in single cases complete remissions, however no significant change in survival [117, 118]. In the last years, treatment with radiolabeled somatostatin analogues like ^{90}Y-DOTATOC and ^{177}Lu-DOTATOC has found its way in the battle against malignant PCC/PGL. Forrer et al. reported 13 stable diseases, 2 mixed responses, and 6 patients which remained progressive after treatment of 28 somatostatin receptor-positive patients with metastatic PCC/PGL with DOTATOC, without severe toxicity [119]. In addition, targeted therapies have been employed in patients with malignant PCC/PGL, of which especially sunitinib was promising in small series [120]. Conventional radiotherapy

may provide effective palliation in patients with painful bone metastases; arterial embolization, chemoembolization, or radiofrequency ablation may be useful in case of liver metastases [121, 122]. Catecholamine-induced signs and symptoms can be treated with catecholamine synthesis inhibitors (alpha-methyl-para-tyrosine) or α-receptor-blocking medication. Prognosis in confirmed malignant PGL is difficult to predict but is known to be poor secondary to local recurrence or widespread metastases. Five-year survival rates are only 20–50 %; the outcome is related to tumor size [123, 124].

24.9 Genetic Testing

To date, 10 susceptibility PGL genes have been identified, so that the initial 10 % of cases classified as genetically determined has increased to 30 %. The frequency is likely to increase, as there are still young patients (with a higher likelihood of a mutation) being classified as sporadic and patients from PGL families where no mutation is found in one of the 10 susceptibility genes. Genetic testing can be of great importance for patients and their relatives because it gives the opportunity for early diagnosis through periodical imaging studies in family members of the affected individual [125]. Early detection of a familial PGL allows early detection of potentially malignant PGLs, especially in *SDHB* and *MAX* mutation carriers, and early surgical treatment, reducing the complication rate of this operation [126, 127]. Genetic testing algorithms can be made to reduce time and costs. The clinical and biochemical phenotype and *SDHB* and *SDHA* immunohistochemistry of the tumor tissue [128, 129] may be valuable tools to guide the order of genetic testing [43]. Mutation-positive carriers need regular clinical follow-up [28, 113], except for the children of female mutation carriers of *SDHD*, *SDHAF2*, and *MAX*, because of maternal imprinting. Although there is currently no international consensus regarding follow-up programs, annual biochemical testing, e.g., plasma-free metanephrines and/or urine fractionated metanephrines, and every 1–2 years radiological imaging of neck and/or thorax/abdomen/pelvis, depending on the mutation, with additional functional imaging in selected cases can be recommended as a surveillance program [113, 130].

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Surgical Approach in the Treatment of Neuroendocrine Tumours

25

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25.1 Classification

The European Neuroendocrine Tumor Society (ENETS) in 2006 provided a TNM staging for classification of foregut NETs (stomach, duodenum and pancreas), together with a histopathological grading system based on the Ki-67 proliferation index and mitotic counts (MC), which was followed by similar classifications for other NETs (see Chap. 6) [1–3]. Grade 1 were tumours with Ki-67 index ≤2 %, or mitotic counts (MC) <2/10 high-power fields (HPF); grade 2 were tumours with Ki-67 index of 3–20 %, or 2–20 MC/10 HPF; and grade 3 were tumours with Ki-67 index >20 %, or >20 MC/10 HPF).

The WHO 2010 (UICC/AJCC) classification used the ENETS Ki-67- and MC-based grading to distinguish low grade 1 (G1) and intermediate grade 2 (G2) well-differentiated NETs (WDNET) and poorly differentiated grade 3 (G3) neuroendocrine carcinoma (PDNEC), with cutoff Ki-67 index between G1 and G2 changed to <3 % [4, 5]. The ENETS TNM staging, later modified for clinical data, subsequently proved to be superior to the AJCC/WHO staging system by accurately predicting survival [4–7]. Further revision of the Ki-67 cutoff values to 5 % has been suggested to better separate G1 and G2 tumours and to correlate more accurately with prognosis for pancreatic NETs and Si-NETs [6, 8–10]. Distant metastases should be expected also in grade 1 WDNET with the lowest Ki-67 index, but the progression has been less aggressive with the lower grade. Positive effects of surgery can be expected in WDNET of low to intermediate grade (GI and G2), but has been generally considered of little value for PDNEC with expected short survival.

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25.2 Diagnosis

Ultrasound (US)-guided fine-needle aspiration biopsy (FNAB) has been commonly used for cytologic diagnosis of primary and metastatic NETs. Formalin-fixed paraffin-embedded, US-guided semi-fine (1.2 mm)-needle tissue biopsies are now routinely used for final diagnosis (together with surgical tumour specimens) [11, 12]. This allows appropriate immunohistochemistry for diagnostic stainings and evaluation of proliferation by the Ki-67 index, which has become the important parameter for grading [12]. Positive staining for either of the general neuroendocrine markers chromogranin A (CgA) or synaptophysin confirms the NET diagnosis [12]. Positive staining for serotonin (5HT) indicates a primary tumour in the small intestine. Immunoreactivity for the vesicular monoamine transporter type 2 (VMAT2) is used to diagnose ECL (histamine) cell differentiation in gastric carcinoids [12]. Neuron-specific enolase (NSE) has been less specific, but of value for some duodenal and rectal NETs. For poorly differentiated lesions, CgA staining may be variable and involve only subsets of cells, and synaptophysin reactivity is more intense [12].

25.3 Biochemical Markers

25.3.1 Plasma Chromogranin A (CgA)

Plasma CgA levels are sensitive and early markers for NETs with relation to tumour load and have been used to monitor effects of treatment [10, 12, 13]. However, CgA levels are unspecific as they are raised with all types of NETs and some non-endocrine malignancies (as prostate carcinoma). CgA values are also raised due to endocrine cell hyperplasia in chronic atrophic gastritis (CAG) (high levels) and in patients with renal failure, liver failure, heart failure, stress and inflammatory bowel disease and as a result of chronic proton-pump inhibitor (PPI) therapy (moderately raised values). CgA decrease of $\geq$80 % has predicted resolution of symptoms and disease stabilisation [14].

25.3.2 Urinary 5-Hydroxyindole-Acetic Acid (5-HIAA)

Raised levels of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in 24-h urinary samples are 85 % specific markers for serotonin-producing NETs, but are only seen at advanced disease stage and generally signify presence of liver metastases [12]. Rise may be caused by tryptophan-/serotonin-rich foods (bananas, avocados, plums, eggplant, chocolate, figs, tomatoes, pineapples, pecan nuts, walnuts, and wine), which should be avoided prior to urine collection for the 5-HIAA measurements [10, 12]. Reduction of urinary 5-HIAA concentrations of 80 % or more (or normalisation) has been predictive of symptomatic relief but not of disease stabilisation [15].

25.4 Localisation and Radiology

25.4.1 Endoscopy

Endoscopy is used to detect NETs in the oesophagus, stomach, duodenum and colorectum. It may be complemented by *endoscopic ultrasonography (EUS)* to allow determination of submucosal invasion and presence of regional metastases for staging. EUS-guided biopsy can be performed. Percutaneous US with dynamic i.v. contrast enhancement has been sensitive for detection of liver metastases and commonly used to guide semi-fine- or fine-needle biopsy for tissue sampling [12].

25.4.2 Computed Tomography (CT)

Computed tomography (CT), with i.v. contrast enhancement, is the common method to detect NETs. Primary Si-NETs tumours are often too small to be visualised, though associated mesenteric metastases often show typical features. The presence of a circumscribed mesenteric mass with radiating densities due to fibrotic strands is highly suspicious of Si-NET mesenteric metastasis [12]. For detection of liver metastases, 3-phase CT scans for arterial and venous portal phase studies are needed.

25.4.3 Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) may be more sensitive than CT for the detection of small liver metastases, with use of contrast, arterial phase and T-2 weighted images [12]. MRI during follow-up may reduce radiation exposure.

25.4.4 Somatostatin Receptor Scintigraphy (SRS)

Somatostatin receptor scintigraphy (SRS), OctreoScan (with ^{111}indium pentetreotide), has been a routine during primary investigations and follow-up of patients with NETs [10]. SRS may detect primary tumours, regional metastases (less often tiny primary tumours of Si-NETs) and liver metastases and has been efficient for detection of extra-abdominal metastases (with more than 80 % sensitivity) [10, 12]. SRS is often routinely done with SPECT and combined with high-contrast diagnostic CT. For detection of liver metastases, SRS has been less sensitive than both CT and MRI. Small tumours or tumours with low somatostatin receptor density may not be visualised, and small liver metastases may be concealed by high background liver uptake [10, 12]. Somatostatin receptors may be expressed in tissues with inflammation, infections and non-endocrine malignancies and then provide false-positive indications of NETs. The SRS, however, gives information about somatostatin receptor expression, which can be used to predict response to peptide receptor radionuclide therapy (PRRT) with somatostatin analogues [10, 12].

25.4.5 Functional PET Imaging

Positron emission tomography (PET) has been developed with radiotracers for functional imaging, including ^{11}C-5-hydroxytryptophan [^{11}C-5-HTP], ^{18}F-L-dihydroxy-phenyl-alanine [^{18}F-DOPA] and ^{68}Ga-DOTATOC/PET/CT [10, 12]. These PET studies provide higher resolution and improved sensitivity compared to conventional SRS and have proven to be of considerable value in patients with NETs and negative or inconclusive results on SRS [10, 12, 16]. ^{18}F-DOPA-PET has been efficient for visualisation of Si-NETs [12]. The recently evolved ^{68}Ga-DOTATOC/PET/CT has shown the highest sensitivity and commonly been able to detect additional metastases, especially within the skeletal system (in >30 %) [17] and established localisation of unknown primary NETs in up to 39 % of cases [10, 18]. In a recent study ^{68}Ga-DOTATOC/PET/CT revealed additional hepatic or extrahepatic metastases in ~50 % of patients planned for liver resection or liver transplantation, which strongly impacted treatment decisions based on CT and/or MRI investigations that had failed to reveal these additional metastases [19]. ^{18}F-fluorodeoxyglucose (FDG)-PET imaging may be used to detect intermediate and high-grade WDNET or poorly differentiated neuroendocrine carcinoma (PDNEC) (sensitivity of 92 % with Ki-67 >15 %), but will not visualise low-grade, well-differentiated lesions [20].

25.5 Specifics for NETs with Different Location

25.5.1 Oesophageal NETs

Oesophageal NETs are rare (<1 % of all NETs) and reported to occur with male predominance at around 60 years of age [12, 21]. Symptoms are usually similar to adenocarcinoma with dysphagia and retrosternal pain. Most tumours have been located in the lower third of the oesophagus or in the gastro-oesophageal junction. Most have been small cell PDNEC, often with components of adenocarcinoma and with lymph node spread reported in 50 % of patients. The tumours should only be operated if radical resection is possible, and preoperative chemo-radiation has been suggested to improve outcome. Survival depends on disease stage and has been overall poor [12, 21].

25.5.2 Gastric NETs

The WHO guidelines depict three groups of enterochromaffin-like (ECL) cell-derived gastric NETs: type 1, type 2 and type 3 [12]. The majority of gastric NETs, type 1 (70–80 %), occurs in patients with hypergastrinaemia due to chronic atrophic gastritis (CAG). Type 2 patients (5–8 %) also have hypergastrinaemia, but due to a Zollinger-Ellison syndrome (ZES) as part of a familial multiple endocrine neoplasia type 1 syndrome (MEN1). Type 3 gastric NETs (15–20 %) occur as sporadic,

solitary lesions in patients without hypergastrinaemia; rare tumours may be of non-ECL type. The important predictors of gastric NETs are size, type and histology. Rare PDNECs have been named type 4.

25.5.2.1 Type 1 Gastric NETs

Type 1 NETs occur with female predominance (3:1 vs. males) at a mean age of around 65 years [10, 12]. The tumours arise in patients with chronic atrophic gastritis (CAG) with hypo- or achlorhydria due to destruction of acid-secreting parietal cells. CAG may be autoimmune or nonimmune [22]. Hypergastrinaemia occurs because gastrin-secreting g-cells of the antrum are not inhibited in the absence of gastric acid. The excessive gastrin secretion will in combination with other predisposing factors, such as diet or possibly bacteria, further stimulate the ECL cell hyperplasia and development of the ECL cell tumours. Hypergastrinaemia alone does not appear to cause gastric NETs, as tumours occur in a minority of CAG patients and not due to chronic PPI treatment or vagotomy [12, 22–24]. More than half of the patients have vitamin B12 malabsorption and pernicious anaemia, and in this population the NETs have often been accidentally revealed by endoscopy, as the patients have generally been without symptoms. Although CAG is common in elderly individuals, only few patients (1 %) with long duration of markedly raised serum gastrin values develop gastric NETs. The NETs will typically occur as multiple (60 %), small polyps in the gastric body and fundus or in the transitional zone to the antrum, developing in a surrounding atrophic mucosa where ECL cell hyperplasia and dysplasia can be detected. Individual tumours can present as broad-based, round polypoid lesions; some can be flat and broad or appear as discoloured spots or slight mucosal protrusions. The number and size vary from uncountable pinpoint-size lesions to few prominent lesions measuring up to 1–1.5 cm and uncommonly up to 2 cm or more [12, 22–24]. Some are solitary and may be difficult to distinguish from adenopolyps, which also occur in CAG patients. Only rare larger lesions may be ulcerated or bleeding. Small type 1 gastric NETs are benign with low proliferation (grade 1) and low risk of invasion beyond the submucosa. Larger lesions (>1 cm) are also predominantly benign, but may occasionally invade the muscularis propria (<10 %) or have higher grade. The incidence of regional lymph node metastases is low (<2–5 %), distant liver metastases are exceptional (<2 %) and disease related death is rare, though earlier reports with greater proportion of larger lesions described more frequent metastases [12, 23, 24]. Exceptional CAG patients have had large invasive tumours, representing poorly differentiated NEC or composite endocrine tumours/and adenocarcinoma with unfavourable prognosis.

25.5.2.2 Type 2 Gastric NETs

ECL cell hyperplasia can be detected in 80 % of MEN1 patients with ZES, and 5–30 % of MEN1-ZES patients develop type 2 gastric NETs [12, 22–24]. The patients have high gastric acid secretion and present with typical increased mucosa thickness in contrast to the distinct atrophy of type 1 lesions. Patients with sporadic ZES may also often exhibit ECL cell hyperplasia, but rarely (<1 %) develop gastric NETs. Type 2 gastric NETs occur in the gastric body and fundus and occasionally

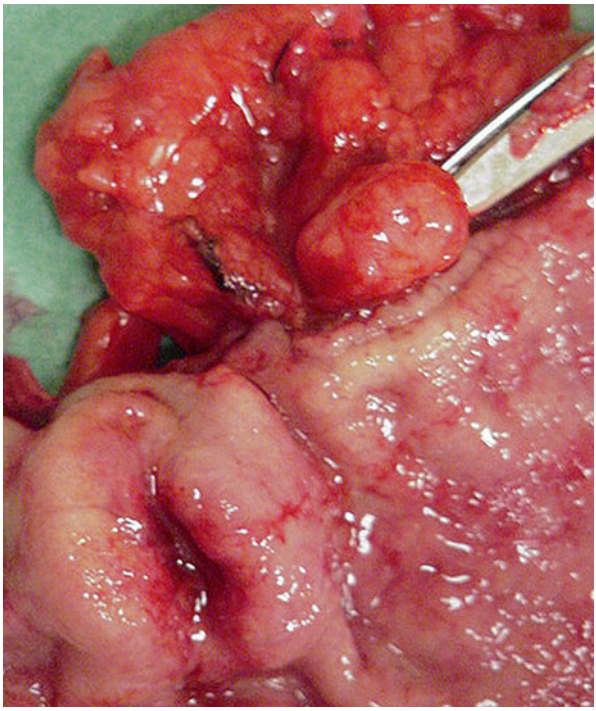

Fig. 25.1 Sporadic, solitary gastric NET with lymph node metastasis removed by gastric resection (From Åkerstrom et al. [12])

in the antrum [11, 20, 22]. They are also often multiple and usually small (<1–2 cm), but generally larger than type 1 tumours, and occasionally markedly larger in size of up to 4–5 cm or more. The biopsies from the surrounding gastric mucosa show hypertrophy rather than atrophy. The tumour grade may be 1 or 2, and the malignant potential is intermediate between CAG-associated and sporadic gastric NETs. Lymph node metastases occur in ~30 % of patients and liver metastases in 10–20 % [12, 24]. More aggressive tumours with liver metastases and higher grade have been more frequent with long-standing ZES. Poorly differentiated gastric NECs with local invasion, angioinvasion and high proliferation rate have occasionally been associated with MEN1.

25.5.2.3 Type 3 Sporadic Gastric NETs

Type 3 sporadic gastric NETs occur in patients with normal serum gastrin. The tumours are most common in males, with a mean age around 50 years [12, 22–24]. Determination of serum calcium and the family history helps exclude MEN1. A majority of patients have symptoms as with gastric adenocarcinoma with pain, weight loss and gastrointestinal bleeding.

Majority of type 3 NETs occur in the gastric body and fundus as solitary and often large tumours, usually >2 cm (Fig. 25.1); occasional tumours occur in the antral or pre-pyloric region [12, 22–24]. The tumours develop in non-atrophic gastric mucosa, without ECL cell proliferation; most tumours originate in ECL cells. Uncommon forms generally occurring in the antrum may originate from G (gastrin) cells or

constitute a mixture of EC (serotonin) cells and other cell types and can then be associated with worse prognosis. Most G cell tumours have sparse staining for gastrin. Tumours with intense gastrin reactivity are rare in the stomach, but can occur in the pre-pyloric mucosa and represent an exceptional cause of hypergastrinaemia and ZES.

Two thirds of type 3 tumours have infiltrated the muscularis layer and 50 % have invaded all layers of the gastric wall. Regional lymph node metastases may be present in 20–50 %, also with small tumours, and liver metastases eventually develop in two thirds of the patients [12, 24]. The sporadic gastric NETs can have atypical histology, with pleomorphism, high mitosis rate and high Ki-67 index. The atypical tumours are larger, with mean size around 5 cm, more frequently invasive and have unfavourable survival [12].

25.5.2.4 Atypical Carcinoid Syndrome

The ECL cells have the ability to secrete histamine and disseminated lesions of type 3 gastric NETs may in 5–10 % be associated with an "atypical carcinoid syndrome". The syndrome can occur also in exceptional patients with disseminated type 1 and 2 tumours [12]. The syndrome has a patchy "geographic" flush, urticarial rash, cutaneous oedema, mucous membrane swelling, bronchospasm, salivary gland swelling and lacrimation. Since the syndrome is caused by histamine release from tumour cells, urinary estimates of the histamine metabolite methylmidazole-acetic acid (MelmAA) serve as tumour marker. Most gastric NETs lack the enzyme L-amino acid decarboxylase and few patients have elevated serotonin levels. Due to this decarboxylation defect, the patients will have only minimal elevation of urinary 5-HIAA values [12].

25.5.2.5 Diagnosis

Diagnosis of CAG-related type 1 NETs is based on demonstration of high serum gastrin levels, lack of gastric acid secretion with high pH in gastric aspirate (pH >3 in the gastric aspirate excludes ZES) and confirmation of mucosal atrophy together with ECL cell hyperplasia in endoscopic biopsies from the antrum (two biopsies) and fundus (four biopsies) [12]. Basal and pentagastrin-stimulated gastric acid secretion studies are recommended. Endoscopy is used to evaluate multiplicity and size of the gastric NETs. Tumour biopsies should be stained with endocrine tumour markers (CgA, synaptophysin and VMAT2) and proliferation markers (Ki-67 index) [12]. Multiple NETs in the gastric fundus in an elderly are most likely to represent CAG-associated NETs.

Diagnosis of MEN1-associated type 2 gastric NETs and ZES is based on demonstration of low gastric pH and raised basal acid output in gastric acid secretion studies, generally together with markedly raised gastrin levels [12]. Serum gastrin >1,000 pg/m is diagnostic of ZES if gastric pH is <2. In other patients a secretin test may be required and is diagnostic with paradoxical rise in gastrin, 200 pg/mL over baseline, but 15 % of patients have negative secretin test. The important differential diagnosis is CAG, where patients have high gastrin, without gastric acid (with high gastric pH, pH >3 excludes ZES). Biochemical screening for other MEN1 endocrinopathies includes measurements of serum calcium and parathyroid hormone, pituitary hormones (prolactin and

insulin-like growth factor 1, IGF-1), pancreatic hormones and CgA [12]. MEN1 genetic testing is done if the familial disease is not previously recognised.

Diagnosis of the type 3 gastric NETs is essentially settled by the features and by verifying absence of hypergastrinaemia.

Radiology is generally not needed in type 1 gastric NETs without signs of spread. CT with contrast enhancement (3-phase), SRS and PET studies (increasingly ^{68}Ga-DOTATOC/PET/CT) are used to determine metastatic spread in type 2 and 3 NETs [12]. For type 3 lesions with higher proliferation, FDG PET may be of value. EUS should be added to the endoscopic examination in type 1 and 2 lesions larger than 1 cm and all type 3 lesions to provide information about invasion and the depth and may reveal regional lymph node metastases (and possibly pancreaticoduodenal lesions in MEN1 patients) [12, 22].

25.5.2.6 Surgical Treatment

Type 1 gastric NETs <1 cm are generally indolent and can be followed with yearly endoscopic surveillance [10, 12, 22–24]. Tumours >1 cm or multiple lesions without invasion can be treated with endoscopic mucosal resection or multiple band mucosectomy [10, 25]. Few large invasive tumours require local surgical excision, and only rare larger, multifocal lesions need gastric resection [11, 23, 24]. Antrectomy combined with tumour excision may be considered in type 1 cases with invasion and repeated recurrence, but the effect on remaining clinically significant lesions may be limited [23]. Treatment with somatostatin analogues has been associated with time-limited regression of type 1 lesions, but disease progression has been noted at follow-up 5 years after termination of therapy [10, 26–29].

Surgical treatment in type 2 gastric NETs is focused on removing the source of hypergastrinaemia, generally by excision of duodenal gastrinomas via duodenotomy, together with clearance of lymph node metastases (see below) [22, 24, 26]. The type 2 gastric NETs per se require endoscopic or surgical excision that should be radical. Gastric resection or gastrectomy combined with regional lymph node clearance is recommended for larger and multifocal tumours or those with deep gastric wall invasion or angioinvasion [22, 24]. If hypergastrinaemia has not been reversed by surgery for gastrinoma, somatostatin analogue treatment may be used to reduce tumour growth, especially in cases with multiple gastric NETs, though proton-pump inhibitors are the most often suggested therapy [26, 30–32].

Type 3 sporadic gastric carcinoids should be radically treated with gastric resection and regional lymph node clearance. Tumours larger than 2 cm and those with atypical histology or gastric wall invasion are most appropriately treated with gastrectomy [12, 23, 24]. Endoscopic resection is only occasionally performed for small non-metastasised tumours [10, 12]. The overall 5-year survival for patients with type 3 lesions has been ~50 %, but only 10 % in cases with distant metastases [10, 12].

25.5.2.7 Gastrinoma

Tumours with sparse staining for gastrin may occur in association with CAG. Tumours with intense staining for gastrin are rare in the stomach and have been located in the pre-pyloric mucosa close to the duodenum. Few gastric NETs have caused hypergastrinaemia and peptic ulcer disease, but they still represent an exceptional but possible origin of gastrin excess and ZES [12]. Rare tumours may present with ectopic Cushing's syndrome due to ACTH secretion [12].

25.5.3 Duodenal NETs

NETs of the duodenum are rare and comprise less than 2 % of all GEP-NETs [12, 22]. Duodenal adenomas or adenocarcinomas are more frequent. Duodenal NETs comprise gastrinomas (48 %) (not described here; see Chap. 6), somatostatinomas (44 %), non-functioning tumours, staining for serotonin (28 %) or calcitonin (9 %) and rare gangliocytic paragangliomas or carcinomas [22, 33, 34]. The duodenal NETs generally present in the sixth decade of life. The majority (>90 %) occurs in the first and second part of the duodenum and 20 % in the periampullary region. The tumours are characteristically small (<1.5 cm); most are T1, but lymph node metastases has been reported to occur in around 50 % and liver metastases in <10 %.

25.5.3.1 Somatostatin-Rich NETs

NETs with somatostatin reactivity occur in the ampullary or periampullary region, almost exclusively in the ampulla of Vater, causing obstructive jaundice, pancreatitis or bleeding [22, 34, 35]. A clinical somatostatinoma syndrome is very rare. The tumours appear as 1–2 cm homogeneous ampulla nodules, which only occasionally are polypoid, larger or ulcerated. Regional lymph node or liver metastases are present in nearly 50 % of patients. Unlike conventional NETs, the tumours have a glandular growth pattern and may characteristically contain special laminated psammoma bodies. The lesions are often associated with von Recklinghausen's neurofibromatosis (neurofibromatosis type 1, NF1) and occasionally with pheochromocytoma [22, 34, 35]. The ampullary somatostatin-rich NETs may be locally excised, together with clearance of regional lymph glands, but since there is no established correlation between tumour size and metastases, pancreaticoduodenectomy has been recommended in younger patients [32].

25.5.3.2 Gangliocytic Paragangliomas

Gangliocytic paragangliomas are rare tumours, occurring almost exclusively in the second portion of the duodenum, and are sometimes associated with NF1 [33]. The tumours consist of a mixture of paraganglioma, ganglioneuroma and NET tissue, with reactivity for somatostatin and pancreatic polypeptide (PP). The tumours may be recognised incidentally or because of bleeding. They may be large but are generally benign and often have an excellent prognosis following surgical excision [33].

25.5.3.3 Other Duodenal NETs

More unusual WDNETs have reactivity for other hormones, such as calcitonin, PP and serotonin [12, 22, 33, 36, 37]. Most of these tumours occur as small polyps (<2 cm) in the proximal duodenum. Multiple tumours should raise suspicion of an associated MEN 1 syndrome. The majority are low-grade malignant and often suitable for endoscopic mucosal resection or local surgical excision, only rare large tumours require pancreaticoduodenectomy [33, 36, 37].

A distinct group of duodenal NETs without release or staining for hormones is also recognised. These tumours have a somewhat different biology and have less often metastasised than the gastrinomas and somatostatinomas [22, 33, 36, 37]. Some are asymptomatic and incidentally found at endoscopy, others present with non-specific abdominal symptoms, gastrointestinal bleeding and sometimes vomiting or weight loss. Most tumours are located in the first portion of the duodenum, occasionally in the second part and rarely in the third portion (horizontal duodenum). The majority stains for CgA and some for synaptophysin and/or NSE [36, 37]. Up to one-third of the patients have had other primary malignancies as well, including adenocarcinomas of the gastrointestinal tract, prostate or other organs [36, 37]. More than half of the tumours are smaller than 2 cm and generally have a good prognosis after resection. Lesions smaller than 1 cm can be endoscopically excised, but re-examination with follow-up endoscopy is required to ensure complete removal. Size >2 cm, invasion beyond the submucosa and presence of mitotic figures are independent risk factors for metastases (grade 2 tumours). Tumours with these risk factors are likely to recur also after apparently curative surgery, even if no lymph node metastases have been detected, whereas lesions smaller than 2 cm rarely metastasise [36, 37]. Rare duodenal tumours may cause an ectopic Cushing's syndrome due to ACTH secretion [33].

The treatment suggested for larger tumours is segmental duodenal resection or pancreaticoduodenectomy in order to decrease the risk of recurrence [36, 37]. Periampullary tumours behave in malignant fashion and need more radical surgery. Patients with metastasising duodenal NETs may survive for decades, substantiating that NETs are less aggressive than adenocarcinomas.

25.5.3.4 Poorly Differentiated Duodenal Neuroendocrine Carcinomas (PDNECs)

PDNECs (large or small cell) in the duodenum are exceptionally rare, occurring most often in the ampulla of Vater, and the patients present with obstructive jaundice and invariably with a rapidly fatal course [36, 37].

(For excellent review including endoscopic management of gastroduodenal NETs, see [22]).

25.5.4 Pancreatic NETs

(Pancreatic islet cell tumours are reported in Chap. X). Exceptional PNET stain intensely for serotonin (and may also contain other biogenic amines) [12, 38]. They appear histologically as classical GEP-NETs, but few have been associated with the

carcinoid syndrome. The tumours are managed surgically according to guidelines similar to those for non-functioning malignant pancreatic NETs. Hepatic metastases and a carcinoid syndrome may be treated with somatostatin analogues and interferon or with chemotherapy in the presence of higher proliferation rate [38].

25.5.5 Jejunoileal Small Intestinal (Si-NETs)

The jejunoileal small intestinal NETs (Si-NETs), also named "classical" midgut carcinoids, originate from intestinal enterochromaffin (EC) cells in intestinal crypts and can be recognised by typical tumour cell serotonin immunoreactivity. Carcinoids may be detected in up to 1/150 of routine autopsies, indicating that the tumours may remain silent throughout life [39, 40]. The Si-NETs account for 25 % of small bowel neoplasms and have been diagnosed with slight male predominance at a mean age of 65 years.

The primary Si-NETs occur most often in the distal ileum, within 60–80 cm of the ileocaecal valve, less often in the proximal ileum or the jejunum. Occasional tumours in the caecum with serotonin immunoreactivity are classified as Si-NETs [21, 41, 42]. The primary tumour is typically a small, flat and fibrotic submucosal lesion; occasional tumours may be polypoid and fungating [43]. The tumours may be difficult to detect at surgery, often appearing only as a localised fibrosis or thickening of the intestinal wall (Fig. 25.2a), but may even with minimal fibrosis cause intestinal obstruction (Fig. 25.2b) [43]. In an own series more than 30 % of patients subjected to surgery were found to have multiple, submucosal tumour nodules in the small intestinal wall close to the primary tumour, likely to represent local lymphatic spread [43, 44]. Rare cases have had additional larger polyps in the proximal intestine, possibly representing additional primary tumours [43, 44]. Mesenteric metastases occur in high frequency, irrespective of tumour size, and microscopic spread has been almost invariably present [43]. When growing close to the intestinal wall, the metastases may be easily mistaken to represent primary tumours, and some

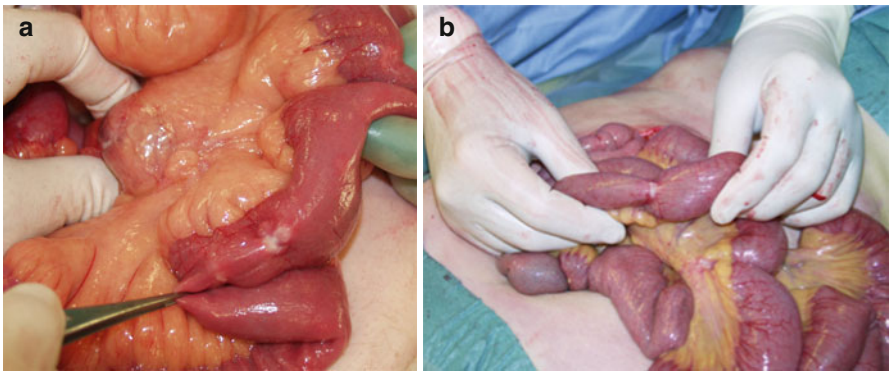

Fig. 25.2 (**a**) Si-NET primary tumour visible as a localised fibrotic thickening of the intestinal wall; (**b**) Si-NET primary tumour with minimal fibrosis causing partial intestinal obstruction

primaries have extended directly into node metastases [43, 45]. The mesenteric metastases have often grown larger than the primary tumour and evoked a desmoplastic reaction with a typical pronounced mesenteric fibrosis (Fig. 25.3). The fibrosis may be due to local effects of serotonin, growth factors and other substances released from the Si-NET metastases [12, 45–47].

Larger metastases and more extensive fibrosis have sometimes caused mesentery contraction and tethered the mesenteric root to the retroperitoneum, with fibrous binding to the horizontal duodenum [43, 45]. The mesenteric tumours may extend high in the mesenteric root and may sometimes grow into the duodenal wall or into the pancreas or spread to the hepatoduodenal ligament or to the retroperitoneum/and para-aortal spaces [42, 43, 45, 48]. The fibrotic adhesions may kink and entrap the intestines and cause partial or complete small bowel obstruction and at advanced stage occasionally duodenal obstruction [43, 45, 48]. With extended mesenteric tumour growth and fibrosis, mesenteric vessels may be encased, and intestinal segments of variable length will appear blue-reddish due to incipient venous ischaemia (Fig. 25.4). The patient may experience diarrhoea or functional obstruction and occasionally abdominal angina [12, 43, 45, 48, 49]. A specific angiopathy, named vascular elastosis, with elastic tissue proliferation in the adventitia and marked thickening of mesenteric vessel walls has been reported with advanced Si-NETs [50], though in our experience tumour compression and fibrosis has been the obvious cause of intestinal ischaemia [43, 45].

Cases with higher mesenteric root tumours and central mesenteric vein or artery occlusion may have longer involved intestinal segments with blue pale cyanosis or occasionally absent arterial circulation (Fig. 25.5). Rare patients with occlusion of a larger mesenteric vein branch may experience a WDHA syndrome-like condition, severe watery diarrhoea, malnutrition and emaciation [51, 52]. If the tumour and fibrosis have remained for longer time, bowel adhesions and kinking have often created a conglomerate of intestinal loops fixed to the abdominal walls, which sometimes need to be removed en bloc [12, 43, 45]. The incidence

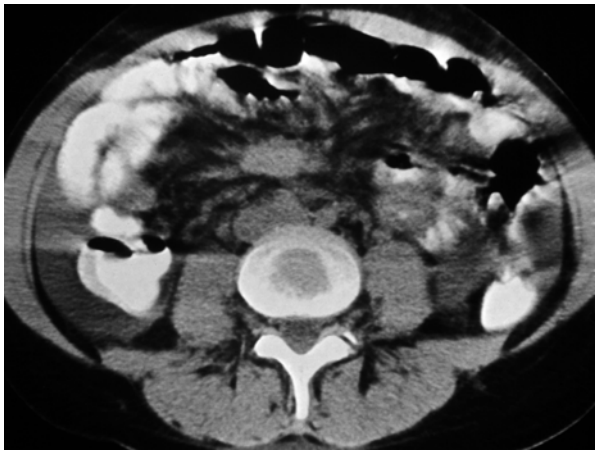

Fig. 25.3 CT image of a mesenteric metastasis from a Si-NET surrounded by fibrosis (appearing like a "hurricane centre") (From Åkerström et al. [45])

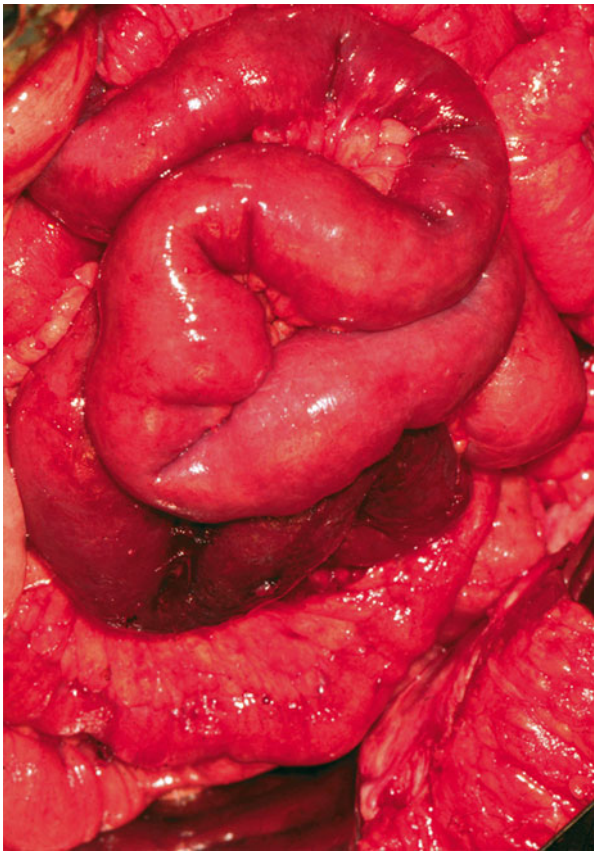

Fig. 25.4 Limited intestinal segment with venous ischaemia due to Si-NET mesenteric tumour (From Åkerström et al. [45])

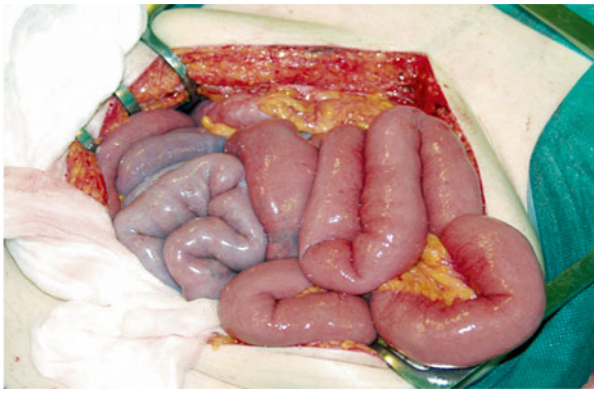

Fig. 25.5 Longer ischaemic intestinal segment with compromised mesenteric vein and artery circulation, due to a high Si-NET mesenteric tumour

of mesenteric metastases at time of diagnosis has been 36–39 % in reports based on imaging studies [53].

In the recent own large series of Si-NET patients, 93 % of operated patients had mesenteric metastases at operation [42]. Distant metastases in retroperitoneal/para-aortal location or more occasionally in the hepatoduodenal ligament were identified in 18 % of patients. Liver metastases were present at diagnosis or discovered at operation in 61 % of patients and occurred during follow-up in an additional 16 % [42]. The majority of patients had bilateral and multiple liver metastases; around 14 % had fewer than five metastases in one liver lobe; occasional metastases were dominant with conspicuous growth [42]. Around 10 % of patients presented with liver metastases without identified mesenteric lesions. Peritoneal carcinomatosis was revealed in 20 % of operated patients, ovarian metastases in 4 % and pancreatic or splenic metastases each occurred in 0.5 % [42]. Extra-abdominal spread to lymph nodes occurred at diagnosis in 4.0 % and to other sites in 6.1 % including bone, breast and skin [42]. Many patients had multiple sites of extra-abdominal spread, most often to mediastinal and peripheral lymph nodes (including neck lymph glands, which occasionally was the first presentation) and bone [42]. During follow-up of our patient series, another 12 % developed extra-abdominal spread [42]. Especially bone metastases have been previously associated with poor prognosis [41, 54].

25.5.5.1 Clinical Symptoms

Many patients with Si-NETs have experienced long periods of unspecific episodic abdominal pain and diarrhoea, often misinterpreted as irritable bowel syndrome or food allergy before the disease has been clinically diagnosed [10, 43, 45, 55]. Others have had unrecognised features of the carcinoid syndrome with discrete flush misinterpreted as menopausal complaints or palpitations and intolerance for specific food or alcohol [10, 43, 45, 56]. Intestinal bleeding has generally been rare with Si-NETs due to the submucosal location of the primary tumour and has been more likely to occur with more rare, larger, polypoid, fungating primary tumours [43, 45]. Bleeding may also occur when mesenteric metastases have grown into the horizontal duodenum or in the presence of intestinal venous stasis and incipient ischaemia [12, 43, 45]. Intermittent pain attacks have been common and often increased in frequency until the patient has developed subacute or acute intestinal obstruction requiring surgery. In around 50 % of patients, the Si-NET diagnosis has become evident after needle biopsy of liver metastases [12, 43, 45].

25.5.5.2 Carcinoid Syndrome

The carcinoid syndrome, described in 1954 by Thorson, has been reported to occur in approximately 20 % of patients with jejunoileal NETs, though the frequency may be higher at referral centres [12, 40–43, 53]. The syndrome includes flushing, diarrhoea, right-sided heart valve disease and bronchoconstriction. Serotonin (5-hydroxytryptamine), derived from the amino acid tryptophan, has been identified as the major cause of the syndrome [10, 41, 45]. Serotonin is inactivated by monoamine oxidase in the liver and in the kidney converted to 5-HIAA, which is excreted

in the urine. Presence of the carcinoid syndrome generally implies that the patient has liver metastases, with secretion directly into the systemic circulation [12, 41, 45]. Occasionally the syndrome may occur in patients with large retroperitoneal or ovarian lesions, where the secretion exceeds the capacity of the monoamine detoxification or can bypass the liver and drain directly into the systemic circulation [12, 41, 45]. The syndrome is related to release also of other vasoactive factors tachykinins (substance P, neuropeptide K), prostaglandins and occasionally norepinephrine [12, 45, 57].

Secretory diarrhoea is the most common feature of the syndrome (reported in around 70 %), but may initially be mild and unspecific. Diarrhoea tends to be most prevalent in the morning and is often meal related. The diarrhoea may have other causes in NET patients, especially when previously operated upon or having advanced disease [12, 40, 45]. The commonly performed distal small intestinal resection leads to reduced bile salt absorption; treatment with bile acid-binding agents (cholestyramine) may provide relief. Somatostatin analogues in high doses may cause malabsorption diarrhoea, with foamy, floating diarrhoea after meals, which can be treated with pancreatic enzymes (Creon). Patients with long-standing poorly controlled carcinoid syndrome may develop niacin deficiency and pellagra (a syndrome with diarrhoea, dementia and dermatitis), which can be prevented by niacin supplementation. Other causes are a short bowel after surgery and partial intestinal obstruction. In patients with the large mesenteric tumours, intestinal venous stasis or ischaemia may contribute significantly to stool frequency. Occlusion of main mesenteric vein branches may cause severe watery diarrhoea and malnutrition, due to severely oedematous, fluid-leaking intestinal segments [12, 41, 45, 51, 52].

Cutaneous flushing (reported in around 65 %) affects the face, neck and upper chest and is the most characteristic feature of the carcinoid syndrome [12, 41, 45, 56]. Flushing may nevertheless easily be overlooked, especially in females at menopause. The flush may be provoked by stress, alcohol, certain food, aged cheese or coffee. It is often of short duration, lasting for 1–5 min, but may occasionally be prolonged for hours or even days. Flushing may occasionally be severe and long-standing, and with chronic flush, the patients may develop telangiectasies and persistent blue-cyanotic discolouration of the skin on the nose and chins.

Heart valve fibrosis, with plaque-like fibrotic endocardial thickening, affects the tricuspid and pulmonary valves, as a late consequence of a severe and long-standing carcinoid syndrome and high serotonin levels [12, 41, 45, 58–60]. This will cause retraction and fixation of the heart valves and subsequently regurgitation and stenosis, leading to tricuspid regurgitation and pulmonary valve stenosis. Tricuspid valve abnormalities was in one series reported in 65 % of patients with the carcinoid syndrome, and 19 % had pulmonary valve stenosis [60]. In less than 10 % the fibrosis may involve also the left-sided heart valves, in case of a patent oval foramen or when the pulmonary monoamine oxidase activity is exceeded, and then possibly contribute to bronchoconstriction.

The carcinoid heart disease may lead to progressive right-sided heart failure and severe lethargy and used to be the cause of death in 50 % of patients with the carcinoid syndrome [12, 40, 48, 58–60]. Nowadays, after introduction of somatostatin

analogues, patients more often die from progressive tumour disease. Affected patients may require heart surgery and prostheses replacement of fibrotic heart valves [59, 60]. The operation may lead to substantial improvement, but can especially in older persons be associated with complications [60]. The heart disease is efficiently diagnosed by echocardiography, which should invariably be performed prior to major abdominal surgery.

Bronchial constriction as part of the carcinoid syndrome caused by small intestinal NETs is relatively rare, but may occur during anaesthesia.

25.5.5.3 Imaging

The primary Si- NETs generally escape detection by conventional *bowel contrast studies* because of limited size [10, 12, 21, 61]. Typical arcading of entrapped intestines, with segments of partial obstruction, may be revealed with advanced disease and occasionally the signs of chronic obstruction, with dilatation, and oedematous thickened bowel wall. In the presence of large bowel symptoms, entrapment of the sigmoid or transverse colon may be important to identify prior to surgery. Concomitant colorectal adenocarcinoma has been reported, and it may be necessary to exclude coexisting recto-sigmoidal adenocarcinoma by endoscopy, prior to surgery or during follow-up of Si-NETs.

CT can rarely visualise a primary Si-NET, but will often reveal mesenteric lymph node metastases, retroperitoneal extension of such masses and liver metastases. The presence of a circumscribed mesenteric mass with radiating densities is highly suspicious for an Si-NET mesenteric metastasis. Dynamic CT with contrast enhancement is important prior to operation, by possibility to reveal relation between the mesenteric metastases and the main mesenteric vessels [12, 45]. In cases with intestinal ischaemia, CT may show a characteristic image of dilated peripheral veins (Fig. 25.6) and oedematous loops of intestine. CT with contrast enhancement is often the primary method for visualisation of liver metastases, but fails to identify the smallest lesions. *MRI* can sometimes be

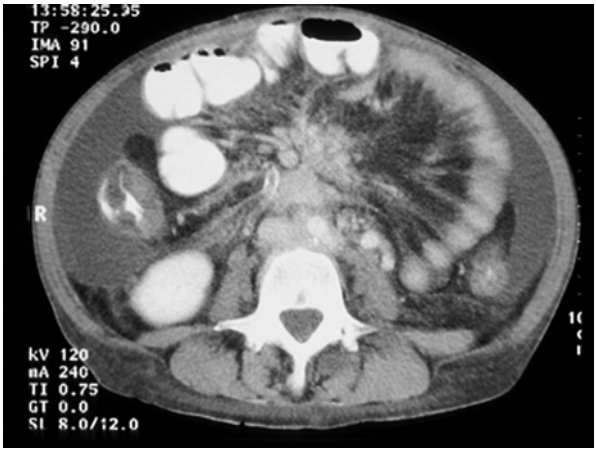

Fig. 25.6 CT image of Si-NET case with intestinal ischaemia showing mesenteric tumour surrounded by dilated peripheral mesenteric veins

more efficient than CT for demonstration of liver metastases. *Percutaneous US* with power Doppler enhancement, and use of US contrast, may increase sensitivity for the detection of liver metastases and is mainly used to visualise liver metastases and to guide fine or semi-fine-needle biopsy of the liver or mesenteric metastases for histological diagnosis and grading.

OctreoScan®

In nearly 90 % of cases, Si-NETs express somatostatin receptor types 2 and 5, for which the somatostatin analogue octreotide has high affinity [12, 21, 45]. Somatostatin receptor scintigraphy, SRS (OctreoScan®), has detected Si-NETs with a sensitivity of 90 % and has been commonly utilised to determine metastatic spread. OctreoScan® is especially efficient for detection of extra-abdominal spread and can visualise bone metastases better than isotope bone scan, which may miss osteolytic metastases.

Positron Emission Tomography (PET)

PET with the serotonin precursor 5-hydroxytryptophan, labelled with ^{11}C (5HTP-PET), or gallium-68 (^{68}Ga) PET can identify the Si-NETs with the highest sensitivity and has been increasingly used to diagnose stage and monitor effects of therapy [12, 17–19, 45, 62]. ^{68}Ga-DOTATOC/PET/CT has revealed additional hepatic or extrahepatic metastases in more than 60 % of patient planned for liver resection or liver transplantation, which strongly impacted treatment decisions based on CT and/or MRI investigations that had failed to reveal these additional metastases [19]. FDG-PET imaging may be used to detect intermediate and high-grade poorly differentiated neuroendocrine carcinomas (PDNECs) (sensitivity of 92 % with Ki-67 >15 %), but will not visualise low-grade well-differentiated lesions [20].

25.5.5.4 Diagnosis

Needle biopsy specimens from metastases are often used for diagnosis. The NET cells stain immunocytochemically with neuroendocrine tumour markers, mainly CgA and synaptophysin, and reactivity with serotonin-specific antisera implies that a primary tumour should be searched for in the midgut [12, 21, 45]. Most jejunoileal NETs show a mixed insular and glandular growth pattern; occasional tumours have a pure insular and trabecular pattern and have been reported to have slightly less favourable prognosis [12, 21]. Proliferation rate determined with the Ki-67/MIB antibody has often been very low <2 % in classical Si-NETs. Occasional tumours have higher proliferation rate (grade 2) or may change to higher grade during the disease course [12, 21]. Very rare lesions present as PDNEC with poorly differentiated pattern and high proliferation rate with poor prognosis and sparse effects of surgery.

25.5.5.5 Surgery

Since Si-NETs patients may be subjected to acute laparotomy due to abdominal pain or intestinal obstruction with unknown diagnosis, it is important that surgeons in general learn that the Si-NETs are common small bowel neoplasms and also

come to recognise the typical features of a small ileal tumour and conspicuously larger mesenteric metastases with surrounding fibrosis [12, 43, 45, 48, 49, 63, 64]. Since an intestinal bypass may complicate more radical surgery, the locoregional tumour should if possible be removed by mesenteric wedge resection and intestinal resection, with clearance of lymph node metastases around the mesenteric artery and vein branches [12, 49]. The procedure is generally justified also in patients with moderately spread liver metastases. If the surgery has been inadequately performed, reoperation for removal of a remaining mesenteric tumour may be considered, as the patient may otherwise risk future abdominal complications [12, 43, 45, 48, 49, 63]. If grossly radical locoregional tumour removal has been accomplished, the Si-NETs patients often remain symptom-free for long periods from treatment with somatostatin analogues and interferon (IFN-α). However, the Si-NETs are markedly tenacious and recurrence with liver metastases should be expected in the vast majority of patients (60–80 %) if follow-up is long enough [43, 45, 48, 49, 63]. Since the Si-NETs are slow-growing tumours, clinically overt recurrence may occur after long duration of up to 10–25 years [45, 49, 63]. Earlier recurrence may be diagnosed by serum CgA estimates, rather than the urinary 5-HIAA measurements.

Many Si-NETs patients present with liver metastases and sometimes also features of the carcinoid syndrome. Life expectancy used to be poor in patients with liver metastases and the carcinoid syndrome; now symptoms may be efficiently controlled with somatostatin analogues and IFN-α, which together with other new treatment modalities have markedly increased life expectancy and life quality [21, 45, 49, 63]. The carcinoid heart disease used to be a principal death cause in Si-NETs patients, together with abdominal complications, which played a significant role in 40 % of patients [12, 63]. Possibly due to treatment with efficient biotherapy, the heart disease has become less common, and a majority of patients now die with spread malignancy or liver failure after an extended disease course [41, 61, 63]. Improved survival by somatostatin analogue treatment documented by the PROMID study has widened indications for locoregional tumour removal in patients with advanced Si-NETs [41, 63–65]. Because partial intestinal obstruction or incipient intestinal ischemia may cause similar symptoms of feeding-related abdominal pain, laparotomy can be needed to distinguish these causes.

Abdominal pain is unlikely to be caused merely by the carcinoid syndrome, and complications of the mesenteric tumour are important to recognise since considerable long-term palliation may be achieved by appropriate surgery [63, 64]. Not all mesenteric metastases will cause extensive fibrosis, and the course of the mesenterico-intestinal disease can be variable, but elective surgery at an early stage may allow removal of the mesenteric tumours with more limited intestinal resection before involvement of major mesenteric vessels becomes too extensive [12, 63, 64]. The authors advocate removal of the mesenterico-intestinal tumour also in asymptomatic patients and suggest liberal surgical consultations when abdominal symptoms occur during periods of medical treatment [63]. Too late surgical consultation may allow the disease to be increasingly difficult or impossible to manage surgically.

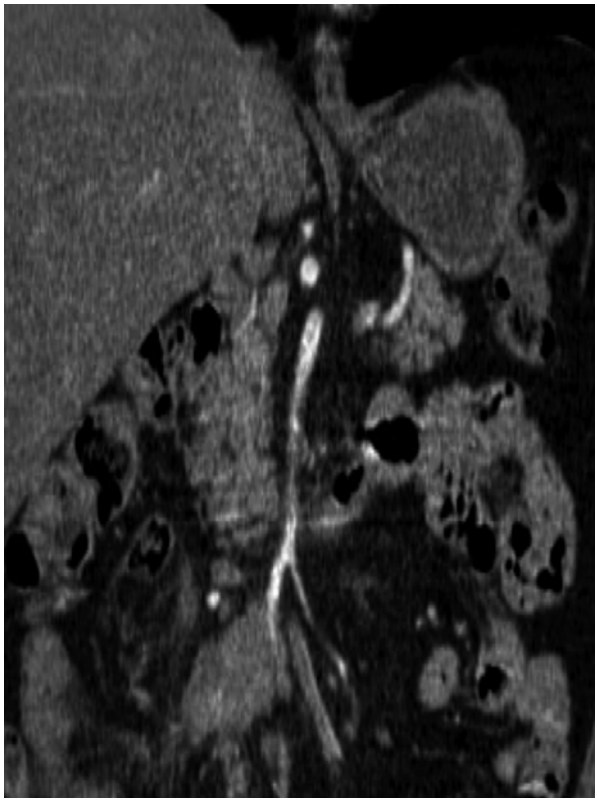

Fig. 25.7 The extension below or above the horizontal duodenum (depicted with coronal CT) is crucial for evaluation of operability of an Si-NET mesenteric metastasis, as this can determine if metastases are regional or located high in the mesenteric root

25.5.5.6 Surgical Technique for Removal of Mesenteric Metastases

The extension below or above the horizontal duodenum (as can be depicted with coronal CT) is crucial for preoperative evaluation, as this can determine if metastases are regional or located high in the mesenteric root (Fig. 25.7). At operation the advanced Si-NETs may appear inoperable, if high mesenteric metastases with surrounding fibrosis appear to encase the mesenteric root and the major intestinal vascular supply [12, 43, 45, 49, 63]. Incautious wedge resection in the fibrotic and contracted mesentery may risk to seriously compromise the intestinal circulation. It is recommended to begin the operation with palpation of the small bowel from the Treitz ligament to the caecum, to identify the primary tumour, exclude the presence of multiple intestinal tumours and allow judgement of the length of the small intestine that needs to be removed. Ischaemic changes and venous stasis can be found to comprise limited intestinal segments, and the locoregional tumour can then be safely removed by a distal intestinal resection. This is preferably combined with caecal resection or right hemicolectomy, with proximal vascular ligature, and extended wedge resection for improved clearance of regional metastases [49]. For tumours originating in the proximal intestine, segmental small intestinal resection is performed.

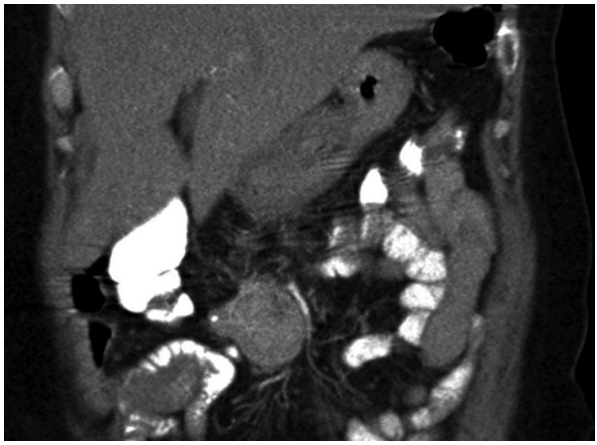

Fig. 25.8 CT image with contrast enhancement in a patient with Si-NET, where a large and high mesenteric tumour has caused intestinal ischaemia, with oedema in the terminal ileum

With advanced growth of bulky mesenteric tumours with higher location in the mesenteric root, above the horizontal duodenum, the management can be more complex (Fig. 25.8) [12, 45, 49]. The majority of these metastases originate from distal intestinal tumours and tend to be deposited onto the right side of the main mesenteric vessels. A method has been proposed (Fig. 25.9), where the right colon and the mesenteric root are mobilised from adhesions to the retroperitoneum to the level of the horizontal duodenum and the pancreas [12, 45, 49]. Staple transection of the right colon then allows visualisation of the mesenteric artery and vein from the right side and possibility for vascular control above the mesenteric tumour during excision. With posterior view in the lifted mesenteric root, fibrotic bands can be transected and the tumour dissected from the serosa of the horizontal duodenum, occasionally, with duodenal wall excision or short duodenal resection in case of tumour invasion. It may then be possible to free-dissect the mesenteric tumour from the major vessels and carefully preserve the arcades of collateral circulation along the intestine and limit the required intestinal resection [12, 45, 49] (Fig. 25.10).

An own arbitrary staging of the mesenteric metastases has been suggested (Fig. 25.11) to describe the extension in the mesenteric root. Mesenteric tumours with very high extension, encircling major vessels with severe fibrosis, or with growth into the pancreas or the retroperitoneal/para-aortal spaces have in our experience been unresectable. The median colic artery circulation should be carefully preserved [12, 45, 49]. Some of the cases with circumferential involvement have originated from jejunal tumours.

Repeated surgery may sometimes be required in Si-NET patients with chronic or intermittent abdominal main due to partial or complete intestinal obstruction or segmental intestinal ischaemia [12, 45, 49]. These operations may be difficult due to fibrosis and carcinoidosis between loops of intestine. Reoperations, and indeed any surgery in patients with Si-NETs, should be done with caution, since mistakes may cause intestinal fistulation or creation of a short-bowel syndrome [12, 45, 49]. It is recommended that the surgery for the difficult Si-NETs is referred to colleagues with experience.

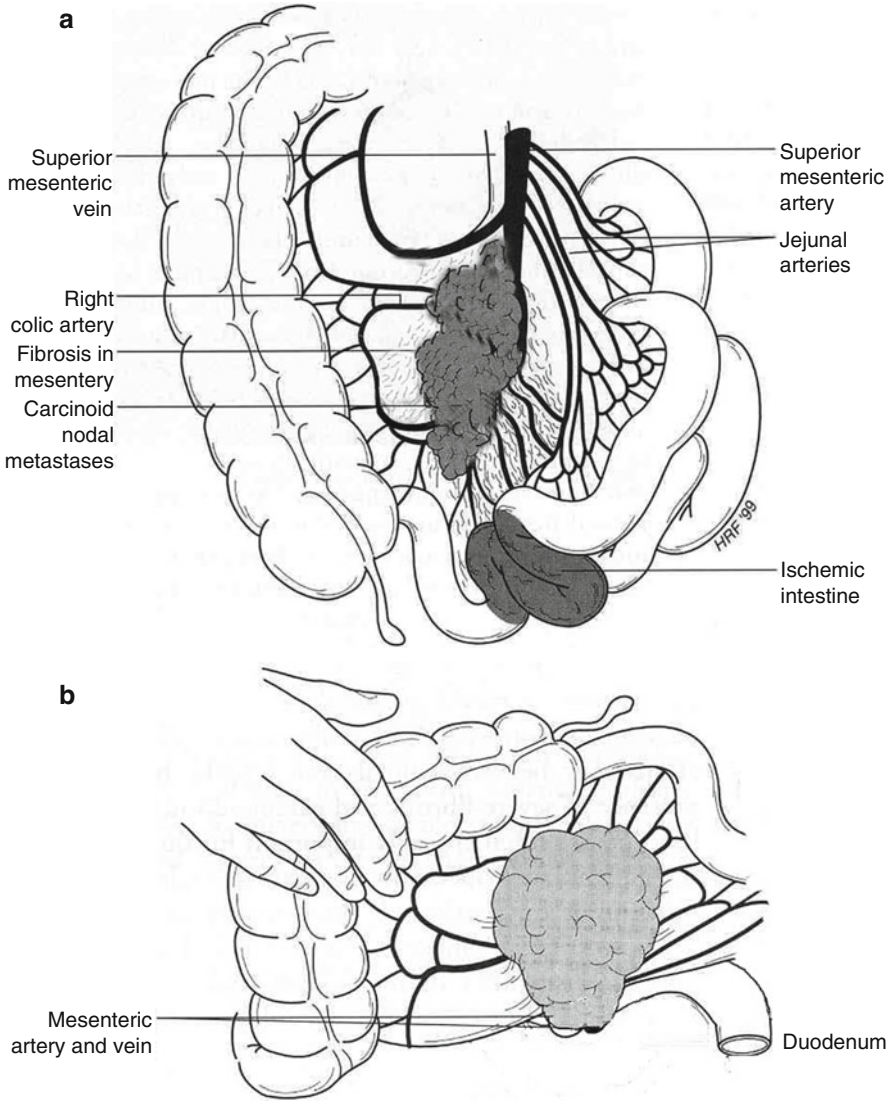

Fig. 25.9 Method for resection of the Si-NET primary tumour and mesenteric metastases. (**a**) The mesenteric tumour may extensively involve the mesenteric root and appear impossible to remove; (**b**) mobilisation of caecum, terminal ileum and the mesenteric root by separation of retroperitoneal attachments allows the tumour to be lifted, approached from a posterior angle and separated from the duodenum and main mesenteric vessels; and (**c**) tumour removed with preservation of collateral circulation along the intestine can maintain the intestinal length; the bowel can then be anastomosed and the mesenteric defect repaired (Redrawn from Åkerström et al. [45])

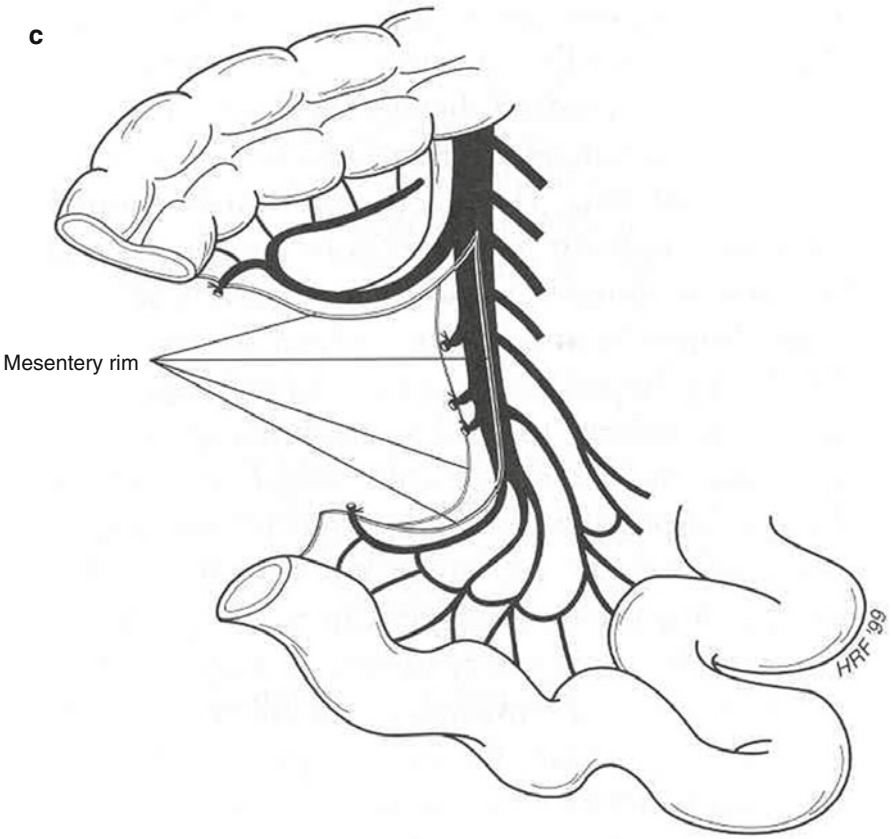

Fig. 25.9 (continued)

25.5.5.7 Peritoneal Carcinomatosis

NET peritoneal carcinomatosis (PC) has been most commonly encountered in the Si-NET patients (reported to occur in 10–30 %) and has often been incidentally detected at operation (or by histopathology), as a few millimetre large tumour nodules on peritoneal surfaces [42, 66, 67]. In some patients CT may have revealed larger tumour nodules within the omentum or between the intestines, which occasionally have caused intestinal obstruction. Lateral deposits have rarely caused ureter block and hydronephrosis (the right ureter may also be blocked by a mesenteric lymph node metastasis with fibrosis). Larger deposits may also occur in the recto-uterine pouch (Douglas pouch), on the uterus and on the rectum and may then threaten to obstruct the rectum and both ureters. Occasional Si-NET patients have been operated in Uppsala for pelvic NET deposits with hystero-salpingo-oophorectomy and anterior resection for pain relief and palliation. Ovarian deposits have been frequently noted in some series [41, 54].

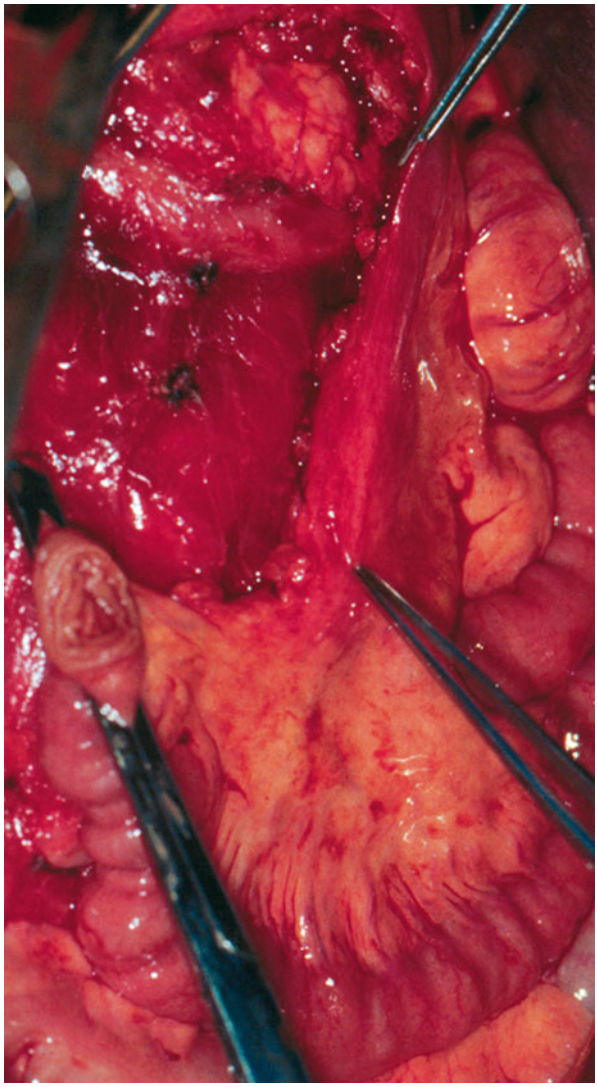

Fig. 25.10 Image from operation in Si-NET patient with alleviation of intestinal ischaemia after removal of a high mesenteric tumour, which was possible to free-dissect from the mesenteric root, the pancreas and the duodenum. Maintenance of collateral circulation along the intestine allowed a limited resection of the ischaemic part of the small bowel (From Öhrvall et al. [49])

A common Gilly classification of PC is based on nodule size (>5 to >2 cm) and whether extent of involvement has been localized or diffuse [66]. The ENETS has proposed an abdominal gravity PC scoring system (GPS), which incorporates the Gilly classification, together with the extent of lymph node and liver metastases [66]. Scoring may then be done at the first laparotomy, by digital and visual exploration of subdiaphragmatic spaces, the large and small omentum and lateral abdominal spaces, and can be combined with intraoperative US and palpation of the liver. PC was revealed as a strong prognostic factor, with markedly negative effect on survival in the Uppsala series of Si-NET patients [42], and it is obviously important to have this spread diagnosed concomitant with the primary surgery.

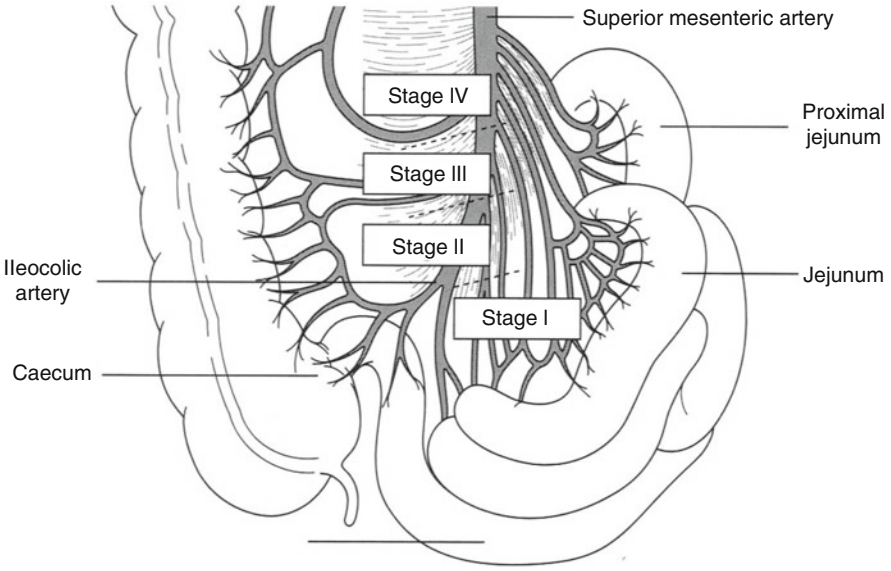

Fig. 25.11 Surgical/anatomical stages of Si-NET mesenteric metastases. Stage I tumours located close to the intestine; stage II tumours higher in the mesentery; stage III tumours extending along, but without encircling the mesenteric trunk; stage IV tumours growing around the mesenteric trunk, involving origins of the proximal jejunal arteries and median colic artery or extending to the retroperitoneal/para-aortal spaces (have been inoperable) (From Öhrvall et al. [49]. Springer Science and Business Media)

The ENETS guidelines have provided a low-grade recommendation that peritoneal cytoreductive surgery may be considered in Si-NETs patients, if they can be judged to be fit enough to undergo this somewhat extensive procedure [66]. Study evidence of the long-term outcome of peritoneal cytoreductive surgery for NET is still lacking, and there is a need to emphasise a risk for high morbidity, especially if treating peritoneal metastases and liver metastases at the same operation [66, 67]. A multistep procedure may be recommended if either operation is expected to be extensive [66]. The peritoneal cytoreductive surgery implies cautious dissection of all abdominal quadrants (right and left diaphragmatic peritoneum, small and great omentum, posterior face of stomach, mesenteric axes and the pelvis) [66]. It has been recommended to remove both ovaries in females older than 55 years [66]. The PC resection should be reported as completeness of cytoreduction (CCR) [66]. Perioperative hyperthermic intraperitoneal chemotherapy (HIPEC) has been performed for other malignancies, but remains experimental for Si-NETs, where most systemic chemotherapy has been inefficient [66].

25.5.6 Liver Metastases

Presence of liver metastases is crucial for the prognosis of NETs [10, 12, 68]. The most common origin of NET liver metastases is the small intestine, and liver spread

is also often seen with pancreatic and colonic NETs and less often with appendiceal, gastric and rectal NETs [12, 68]. Metastases are found at diagnosis or develop during follow-up in about 40–90 % of Si-NETs, pancreatic NETs and colonic NETs, in contrast to gastric, rectal and appendiceal NETs, which in 85–90 % remain as local tumours (these latter tumours have often been incidentally discovered) [68]. The Ki-67 index is prognostic for progression of pancreatic NETs, whereas jejunoileal NETs have high probability of developing liver metastases despite the low grade and low Ki-67 index (<3 %) [10, 42, 68–70].

Biopsy for grading with Ki-67 index should exclude presence of PDNEC, where liver surgery should not be considered due to poor survival. In patients with low- to intermediate-grade (G1 and G2) WDNETs, surgical treatment of liver metastases should be considered in the absence of unresectable extrahepatic disease [12, 68]. The benefit would be to achieve grossly complete (R0 resection) or efficient debulking leading to reduction of hormone levels and palliation of symptoms and thereby reduce the risk to develop long-term complications such as the carcinoid heart disease. Long-term palliation of the carcinoid syndrome has been reported after removal of unilateral, large or dominant liver metastases and if 70–90 % of the tumour burden has been removed. There has not been consensus on how extensive the surgical debulking must be to achieve symptomatic or survival benefit [71]. Liver surgery has proved to be safe and postresection 5-year survival has at specialised centres reached 85–94 %, but early recurrence should be expected, with 5-year disease free survival of <50 % reported in most series [72–76]. A recent "thin slice" pathology examination could reveal that numerous small metastases often accompanied the larger resected liver metastases, and only half of these additional metastases had been detected by preoperative imaging [77]. Liver resections combined with radiofrequency ablation (RFA) has been increasingly used in patients with multiple, bilateral liver metastases [72], but could in a propensity score-matched study not be demonstrated to have positive effects on survival [78].

Alternatives to surgical debulking of liver metastases are hepatic artery embolisation/chemoembolisation, peptide receptor radionuclide therapy (PRRT) with ^{177}Lu-labelled DOTA-Tyr3-octreotate, the use of selective intrahepatic radiotherapy (SIRT) or medical treatment with long-acting somatostatin analogues (and IFN-α) [79–82]. Due to high incidence of multifocal and bilateral liver metastases, radical liver resection is possible in less than 10–20%, and the majority of patients with advanced NETs are therefore mainly considered for other treatment modalities [79–82].

Before liver surgery it has often appeared wise to "stabilise" the tumour situation with somatostatin analogue treatment, which can control hormone release and time to progression (PROMID and CLARINET studies) [65, 83]. In cases with large colon or rectal NET metastases, PRRTs with Lu-labelled somatostatin analogues have occasionally provided successful preoperative treatment [81, 84].

25.5.6.1 Liver Surgery

A classification of NET liver metastases based on morphological features has been proposed to facilitate patient selection: type I (simple) depicts single or multiple metastases confined to one lobe or adjacent segments; type II (complex), one major focus but with contralateral smaller satellites; and type III, disseminated spread with both lobes involved [72]. In cases with solitary, unilateral or grossly dominant liver

metastases, formal hepatic lobectomy or parenchyma-saving liver resections should be undertaken, and this may be combined with RFA, wedge resections or simple enucleations of superficially located and even bilateral lesions [75, 78, 85].

Two-stage surgical resection may be performed to allow regeneration of the contralateral lobe to avoid liver insufficiency, and preoperative portal embolisation may be used to induce regeneration of a hepatic lobe that is planned to remain after hepatic lobectomy for removal of metastatic tumour. The two-step surgery has allowed safe resection of bilateral tumours, though high recurrence has been evident [2, 12, 71, 74]. Substantial amount (up to 75 %) of the liver parenchyma may be resected if the remaining liver parenchyma is healthy.

Outcome of liver surgery is difficult to assess on a larger cohort basis, but individual benefit occurs, which has sometimes been difficult to demonstrate in prospective studies. However, retrospective analyses with statistical enhancement methods have shown benefit on symptoms and increased the time to progression in G1 and G2 tumours, if the total liver involvement has been less than 50 % [78, 86]. A number of reports have described series of patients who have undergone successful liver surgery with long survival without evidence of disease and with significant long-term symptom alleviation [87–90]. On the other hand, virtually every patient will recur with new metastases after liver resection or RFA or microwave ablation, if follow-up time is long enough. Five-year survival has been around 75–80 % whether liver surgery was performed or not. Thus, although survival has not proved to be increased in G1 or G2 tumours after treatment of liver metastases with surgery or RFA, symptomatic improvement and palliation of the carcinoid syndrome and reduction of tumour markers are regularly seen [78, 86].

25.5.6.2 Radiofrequency or Microwave Ablation

RFA or microwave ablations are safe and efficient methods for moderately large NET liver metastases (<4 cm) [91, 92]. An ultrasound-guided insertion of the ablation needle and application of RFA or microwave energy lead to ablation of an area surrounding the needle tip. This may be performed either percutaneously or intraoperatively (open or laparoscopically). Somewhat larger tumours may be coagulated by overlapping treatment or by applying a Pringle's manoeuvre to reduce the hepatic circulation during the procedure. While RFA can treat somewhat larger tumours than microwave, the latter is theoretically more efficient if the metastasis is situated close to larger vessels, due to less negative heat-sink effects, and microwave has like RFA been proven to provide symptom relief [93].

The use of RFA, microwave and surgery in combinations has allowed treatment of more or bilateral metastases. Although there has been no absolute maximum, we have advocated a highest number for this treatment of approximately 7 metastases, before choosing alternative methods (liver embolisation, PRRT, etc.) [94–97]. The optimal method of assessing the number of metastases appears to be ^{68}Ga-DOTATOC/PET/CT [17–19]. However, also this investigation may underestimate the number of metastases that can be detected intraoperatively and by intraoperative ultrasound. Therefore, the proper treatment for each patient may have to be decided during surgery. Since large vessels reduce the efficiency and bile ducts seem to be the most

vulnerable structures, RFA and microwave should be avoided for tumours located in the hepatic hilum. Also, patients who have undergone hepatico-jejunostomy or papillotomy are more prone to develop cholangitis or hepatic abscesses afterwards and should be covered with proper antibiotic prophylaxis during the procedure and be carefully monitored afterwards.

25.5.6.3 Liver Transplantation

Liver transplantation may be considered for few selected patients with liver metastases from Si-NETs, with slow disease progression and threatening liver involvement [12, 98–101]. Meta-analysis of patients with neuroendocrine tumours subjected to liver transplantation revealed a nearly 50 % 1-year survival, but varying 5-year survival (24–48 %), possibly due to different selection procedures between centres [101]. Si-NETs have been reported with more favourable survival (69 % at 5 years) than pancreatic NETs. Recent results indicate reduced operative risks and improved results after transplantation in NETs, with up to 77 % 1-year tumour-free survival and 90 % overall 5-year survival, but only around 20 % 5-year tumour-free survival [98–101]. A recent study demonstrated favourable survival of non-transplanted patients with Si-NETs and liver metastases subjected to conventional oncologic therapy that overall theoretically appeared to exceed the expected survival of patients of the same age groups subjected to liver transplantation [102].

Important for selection of patients for transplantation have been a low Ki-67 proliferation index (<5–10 %) and absence of other markers for aggressive tumours [12]. Patients should have extra-abdominal metastases excluded by sensitive imaging (^{68}Ga-DOTATOC/PET/CT), but even with this investigation, spread disease may not always be detected. The new liver will often become the site for new metastases, emphasising a possible need to combine with other treatments.

25.5.6.4 Prophylaxis Against Carcinoid Crisis

A carcinoid crisis with hyperthermia, shock, arrhythmia, excessive flush or bronchial obstruction is always a risk during treatment of patients with Si-NETs [103]. Initiation of somatostatin analogue treatment is recommended before surgery (patient's regular dose or daily 100 μg × 3 s.c.), and prophylaxis has to be given during operation, or any other intervention, preferably with octreotide (i.v. octreotide 500 μg in 500 mL saline, 50 μg/h), with possibility to increase the dose (to 100 μg/h) if carcinoid syndrome should not resolve [103]. Adrenergic drugs should be avoided if hypotension should occur during surgery. Patients with foregut NETs and an atypical carcinoid syndrome may be treated with octreotide, histamine blockade and cortisone, with recommendation to avoid histamine-releasing agents during anaesthesia (morphine and tubocurarine).

25.5.6.5 Survival

Survival data from the Uppsala series of 603 Si-NET patients admitted to various operations and treatments in Uppsala, between 1985 and 2010, were recently reported [42]. Median survival was related to stage and grade and was overall 8.4 years, with 67 % 5-year survival. With adjustments for co-morbidity by

multivariate analyses, survival advantage could be demonstrated in patients subjected to mesenteric tumour removal, with low mortality for the primary surgery (<1 %) [42]. Previously recognised prognostic factors were carcinoid heart disease, old age at diagnosis, liver tumour load and extra-abdominal metastases; new prognostic factors were remaining mesenteric metastases, distant abdominal metastases (para-aortal/retroperitoneal) and peritoneal carcinomatosis. A recent US series of 691 Si-NET patients also showed strong relation to tumour stage and grade and also substantiated higher survival in patients with resectable compared to unresectable mesenteric tumours [104].

The ENET Ki-67 grading appears increasingly important for long-term outcome and for results of surgery. Suggestions have been forwarded for further improvement of grading for pancreatic NETs, by change of Ki-67 to 5 % to separate grade 1 and grade 2, and this has been suggested to be valid also for Si-NETs [6, 8, 9]. A further proposal for pancreatic as well as Si-NETs has been to change the Ki-67 index for separation of G2 and G3 WDNET to 15 %, because lesion with Ki-67 >15 % may respond to platinum-based chemotherapy [105]. The PDNECs are rare among Si-NETs (1 %). It has been emphasised that different metastases sites in the same patient may express different Ki-67 levels [9, 42]. Upgrading may occur in Si-NET metastases, and if carefully examined, there may be variable grading within metastases [9].

25.5.7 Appendiceal NETs

Appendiceal NETs constitute ~5 % of GEP-NET and have a recently estimated annual incidence of 0.5/100,000, which is increasing. However, all NETs have rising incidence, and the share of appendiceal NETs is probably decreasing. It is found in approximately 1/300 appendectomies and constitutes up to 80 % of all types of appendix tumours [106, 107]. The majority of appendiceal NETs originate from serotonin-producing EC cells, but have a more benign course than other GI-NETs [108].

Appendiceal NETs are usually an incidental finding at surgery [104]. The majority is located in the tip of the appendix, and only few cases have had appendicitis due to an occluded appendix lumen [12, 108]. The mean age for discovery of appendiceal NETs is between 38 and 41 years, somewhat younger than for other NETs. Also children are affected, in some series with higher incidence, which has lead to the assumption that some appendiceal NETs undergo involution with age. The overall rate of metastases has been 3.8 %, with distant metastases in 0.7 % [53].

Appendiceal NETs are usually small, <1 cm, and only few metastasise with a risk increasing with size. Therefore, small NETs in the tip of the appendix may be treated with simple appendectomy [12, 109]. According to ENETs guidelines, tumours <1 cm, with a maximum invasion of 3 mm into the mesoappendix and with clear surgical margins, have no risk of recurrence and appendectomy is sufficient [109]. Invasion of the serosa is not correlated with lymph node metastases and is not correlated to survival. Lesions >2 cm, as well as cases with residual tumour at

resection margins or with lymph gland metastases, should be treated with right hemicolectomy and lymph node clearance. Tumours between 1 and 2 cm in size are treated according to associated risk for metastases, where the pathology report about vascular invasion is crucial [109]. Vascular invasion is associated with a 30 % risk of metastases and negatively influences survival [107]. Tumours in the base of the appendix require hemicolectomy since they may represent colon- rather than appendix-derived tumours.

The prognosis for appendiceal NETs is good, with a 5-year survival rate of 100 % for the majority without metastases, 84 % for patients with regional metastases and 28 % for the few patients with distant metastases (which presumably include patients with adenocarcinoid appearance) (see below) [107–109].

After surgery, regular CT scanning, somatostatin receptor scintigraphy or ^{68}Ga-DOTATOC/PET/CT PET may reveal residual disease, since somatostatin receptors may be expressed. The only tumour marker is CgA, which has low sensitivity. The carcinoid syndrome is absent, as well as hormone secretion, and there is no indication for urinary 5-HIAA measurements.

25.5.7.1 Atypical Goblet-Cell NET/Adenocarcinoid/MANEC

A more malignant variant of appendiceal NETs is the goblet-cell variant, sometimes included in other terms as adenocarcinoid or recently as MANEC (mixed adenocarcinoma neuroendocrine carcinoma) [110, 111]. These tumours are rare (annual incidence 0.01–0.05/100,000). Generally, they should be treated as adenocarcinomas and have similar survival rates. The mean patient age is the sixth decade, i.e. 10 years later than classical appendiceal NETs. Recent 5-year survival has varied between 40 and 75 %, and subtypes appear to determine the outcome. Although classical appendiceal NETs may have low expression of CgA as well as somatostatin receptors, the atypical goblet-cell variant does not express these receptors and cannot be visualised by somatostatin receptor scintigraphy or PET. Goblet-cell NETs may spread to the mesentery or the peritoneal surface and may, besides standard hemicolectomy with lymph node clearance, also benefit from aggressive surgery including peritonectomy and heated intraperitoneal chemotherapy (HIPEC) in analogy with colorectal cancers [112, 113]. However, although several treatment strategies have been reported in recent reports, no systematic study exists concerning either treatment (surgery, RFA, extent of surgery, chemotherapy, timing of different treatments, etc.). The overall 10-year survival is around 60 %.

25.5.8 Colon NETs

Colon NETs are rare and constitute only about 7 % of GEP-NETs and 1–5 % of colorectal neoplasms [12, 53, 114]. The incidence varies highly between different registers (0.06/100,000 in Europe and 0.2/100,000 in the USA). The 5-year survival rate for colon NETs has averaged around 40–50 %, slightly better than the survival for colon adenocarcinoma [115]. The colon NETs are more common in elderly age groups compared to other NETs and have been most often located in

the proximal colon. A fraction of colon NETs originating in the caecum appear with serotonin immune-reactivity and should be regarded and treated as Si-NETs, as they have similar behaviour. The carcinoid syndrome is rare, and secretion of hormones measurable in serum or urine is absent. The majority of colon NETs are non-serotonin reactive and generally have more aggressive behaviour, but are still slow growing, and the primary tumour may reach conspicuous size and lead to obstruction, bleeding or even a palpable mass as initial symptoms. With these tumours metastases are common at diagnosis. The lower differentiation state is noted by higher Ki-67 index and risk for presenting with spread disease at diagnosis [115]. Diagnosis may be difficult, but synaptophysin immunostaining is usually positive, supporting the neuroendocrine origin. Left colon NETs may mimic rectal NETs (see below) with an accompanying L cell expression and other histopathological profiles.

Colon NETs are divided according to Ki-67 proliferation index – G1 < 2 %, G2 = 3–20 % and G3 > 20 % – and should be staged according to TNM. Treatment is usually similar as for colon adenocarcinoma, due to the presence of very few G1 tumours. G3 tumours are denoted PDNEC and should be treated according to special protocols. Due to their generally slow growth rate, palliative tumour debulking, before or after down-staging treatment, may also be undertaken.

25.5.9 Rectal NETs

Rectal NETs have previously been regarded as uncommon, but the incidence is increasing, according to SEERS 0.86/100,000/year. They constitute 25 % of all GEP-NETs, but there is a similar difference between Europe and the USA as for colon NETs, with higher incidence in the USA [116]. The generally increased incidence compared with earlier reports may be due to improved awareness of the diagnosis and the increased use of new diagnostic methods including endosonography. The overall prognosis is favourable, with 5-year survival varying in different series between 45 and 88 %.

25.5.9.1 Presentation and Diagnosis

Rectal NETs usually occur in the sixth decade, about 10 years earlier than non-NET rectal tumours. The rectal NETs are generally small and polypoid, located between 4 and 20 cm above the dentate line (75 % within 8 cm, reachable for palpation by a finger) [12, 114, 115], and may be discovered after presenting symptoms such as perianal pain or discomfort or haematochezia that may lead to the endoscopic investigation. However, the small rectal NETs are generally not the cause of symptoms and therefore more often an incidental finding. Diagnosis is made after histopathological evaluation of biopsies or after removal of the whole nodule. The most sensitive histological tumour markers are NSE (being positive in 87 %) and prostate-specific acid phosphatase (positive in 80–100 %), which can make differentiation from prostatic carcinoma difficult [117]. Other markers are glicentin and glucagon. Classical tumour markers like CA19-9, CA50 and alpha-fetoprotein are poorly expressed or

absent, while carcino-embryonic antigen (CEA) has been demonstrated in up to 25 % of cells. The CEA expression is possibly related to the occasional rectal NET with histopathological signs of both adenocarcinoma and NET.

Rectal NETs generally show focal and patchy multi-hormonal expression, where glucagon, somatostatin, pancreatic polypeptide, substance P and β-endorphins may be detected. A small and limited fraction of cells generally are positive for CgA, synaptophysin or serotonin, but measurable release of these hormones in the circulation (or as urinary 5-HIAA) is extremely rare, as is the carcinoid syndrome. These tumours have rarely metastasised at diagnosis.

Some patients have more aggressive disease with larger tumours and more local symptoms, such as obstruction or bleeding. With these larger tumours hormonal release or the carcinoid syndrome is also extremely rare. Occasional large tumours can be fixed to perirectal tissues and may initially be difficult to distinguish from rectal adenocarcinoma. Metastases are common, and the larger the tumour, the higher the risk for presentation of metastases. As with appendiceal NETs, tumours smaller than 1 cm rarely metastasise (<2 %), while those >2 cm frequently do (60–80 %). Patients with 1–2 cm large tumours have an intermediate risk for metastatic spread (10–15 %). The main sites for tumour spread are regional lymph nodes and the liver and less commonly lung and bone.

25.5.9.2 Surgical Treatment

The size separation of tumours is followed in the treatment recommendations. Patients with tumours <1 cm seldom have symptoms, have favourable outcome and are generally cured by local excision. Thus, the smaller (<1 cm) and the larger (>2 cm) tumours have predictable outcome, whereas the intermediate tumours measuring 1–2 cm in diameter are unpredictable. These latter tumours need to be carefully followed and examined. Transrectal endosonography should be used for more precise assessment of tumour extension (possible infiltration into the muscularis propria layer) and to reveal regional lymph node metastases. CT or MRI can clarify local tumour growth in the pelvis and also the presence of lymph node and liver metastases. Due to a general lack, or presence, of few somatostatin receptors, scintigraphy or PET with somatostatin analogues is insensitive. Only occasional cases may express high somatostatin receptor density leading to additional treatment options and imaging possibilities. Presence of local invasion or regional metastases favours aggressive approach with abdominoperineal or anterior resection with total mesorectal excision (TME). Incompletely removed polyps that are found to be NETs after removal may be re-excised using the same techniques.

However, results have documented more favourable survival rates than previously depicted, and local tumour removal may be advocated also in the presence of metastases. Five-year survival for rectal NETs with distant metastases is about 20–32 %, which favours an aggressive approach for this group of patients, even in the presence of local pelvic invasion or distant lymph node, liver, lung or skeletal metastases.

Our experience of individual patients with large tumours and distant metastases supports this view. Thus, we suggest surgery in these patients, often in combination with preoperative down-staging by chemotherapy, or in cases with scintigraphically

documented somatostatin receptor expression PRRT with ^{177}lutetium-labelled somatostatin analogues, which can be highly effective in some patients. However, patients with atypical histopathological features are less likely to benefit from the aggressive surgical treatment.

IFN-α and paclitaxel have in some patients resulted in reduced disease progression and also regression of metastases. Occasional patients with liver metastases may benefit from hepatic artery chemoembolisation. In carefully selected cases liver resection may be indicated, with the understanding, however, that these patients generally have poor outcome and short survival.

25.5.9.3 Recommendations

Patients with rectal NETs measuring 1.0–1.9 cm and those >2 cm should be thoroughly investigated (MRT, CT, transrectal endosonography) for evidence of spread disease, e.g. local or distant metastases [12]. These patients should generally undergo surgery to achieve regional tumour clearance, and the specimens should be carefully investigated for signs of atypical histopathology. The larger the tumour, the higher will the risk be for metastatic disease. No studies have demonstrated benefit of preoperative medical or irradiation treatment. Patients with rectal NETs >1 cm, including those with infiltrative or disseminated disease, need follow-up and if not removed may require palliation against pelvic pain. Selected individuals may be offered more aggressive medical (and surgical) treatment for distant metastases.

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Liver-Directed Therapies in Neuroendocrine Tumors

26

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26.1 Introduction

Liver metastases (LM) represent the most crucial prognostic factor for gastroenteropancreatic neuroendocrine tumors (GEP NET), altering both quality of life and prognosis regardless the primary site [1]. Liver is the predominant site for NET metastases besides regional lymph nodes [1]. At diagnosis, about 65–95 % of GEP NET (excluding appendiceal, gastric, and rectal NET) are associated with liver metastases [1]. Studies based on histological cohorts of untreated patients with NET have shown a dismal prognosis in patients with LM compared to patients without (0–40 % vs. 75–99 %) [2–4]. Surgery of LM is the standard of care and the sole curative treatment. Surgery is recommended when complete resection or debulking more than 90 % seems feasible [5]. This option justifies aggressive surgical approach which could require either 2-step surgery in synchronous bilobar LM or patient preparation

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to surgery as portal vein ligation or embolization [6]. Complete resection of LM is definitely the goal to achieve with a 5-year survival of 80 % [7]. Yet, overall survival is still satisfactory in R1 resection with a 5-year survival of 70 % [7].

In a large retrospective series of patients with LM from NET of the jejunum/ileum and pancreatic origin who were treated with best supportive care or hepatic arterial embolization or liver resection, the only significant factor on multivariate analysis was curative intent to treat [8]. Furthermore, the 5-year survival rates for patients treated with medical therapy, hepatic arterial embolization, and operation were <25, 50 and 76 %, respectively [8].

However, surgery cannot be proposed to all patients, especially to those with diffuse LM. In Chamberlain's paper, only 34 patients out of 85 had surgical resection [8]. Hence, for unresectable lesions, optimal selection of palliative nonsurgical treatments is crucial. Liver-directed treatments have a place of choice and are discussed in tumor boards for many of these patients.

26.2 Thermal Ablative Techniques

Thermal ablative ablation is based on the cytotoxic effects of nonphysiologic temperature that are locally administrated by probes placed within the liver. Radiofrequency ablation (RFA) has been the most widely used and studied, but recent studies using microwave ablation (MWA) seem to report similar results.

26.2.1 Radiofrequency Ablation

With RFA, high-frequency current is transmitted to the liver through one or several electrode needles (uni- or multipolar technique). The ionic vibrations generated by the high-frequency current induce heat which denatures intracellular proteins and leads to apoptosis and cell death. Pathologically, the destroyed tumor is replaced by coagulation necrosis. Thermal ablation zone should include the tumor and sufficient margins to prevent from local recurrence (Fig. 26.1).

RFA can be performed percutaneously under CT or US guidance or intraoperatively mostly in combination with liver resection using either laparoscopic or open approach.

Classical indications of RFA are LM fewer than five lesions and tumor size less than 5 cm [5]. Yet, two other issues should be discussed in LM from NET: the role of RFA in tumor debulking and in controlling functional syndromes due to specific hormones excess. This explains that most series of patients with LM from NET had more than five ablated tumors with intraoperative RFA during one session [9, 10].

In Elias's series, 16 patients had combined liver surgery and RFA [9]. A mean of 15 and 12 LM per patient were surgically removed and RF ablated, respectively. Morbidity was observed in 69 % of the cases. The 3-year overall survival and disease-free survival were similar to their previous experience of liver resection alone of LM from NET.

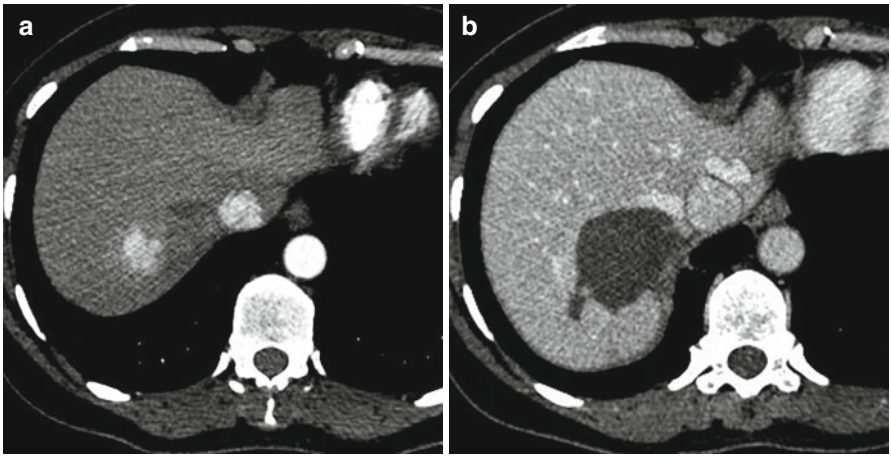

Fig. 26.1 A 45-year-old man with small bowel neuroendocrine tumor, with left hepatectomy for liver metastasis. (**a**) Axial CT scan (arterial phase) shows new metastatic liver lesion in the remnant right liver. (**b**) Axial CT scan (portal venous phase) obtained after radiofrequency ablation of the lesion shows hypoattenuation with no residual lesion

In Akyildiz's series, 119 laparoscopic RFA without liver resection were performed in 89 patients with LM from NET. The mean tumor size was 3.6 cm, and the mean number of tumors was 6 (range 1–16) [10]. Perioperative morbidity was 6 %, and 30-day morbidity was 1 %. Forty-four patients had hormonal symptoms prior the procedure. One week after RFA, 97 % of these patients reported at least partial symptoms relief, and 73 % had significant or complete relief. The symptomatic response lasted for a median of 14 ± 5 months [10]. Median disease-free survival was 1.3 year, and overall survival was 6 years after RFA.

Follow-up by imaging (CT and or MR imaging) is essential to assess complete tumor necrosis. One of the major problems is the recurrence of metastases within the liver as new tumors are reported up to 63 % in the largest series of patients treated with RFA [10]. Conversely, local liver recurrence was observed from 3.3 to 7.9 % per lesion [10, 11]. Interestingly, in a meta-analysis including 5.224 ablated tumors of various origin, the rate of local recurrence was lower in neuroendocrine LM than in others [11]. This might be due to tumor characteristics such as well-circumscribed margins or to natural history of these tumors [11]. As in other liver malignancies, factors predictive of tumor recurrence are tumor size, ablation margin, and blood vessel proximity [12]. In a multivariate analysis, statistically significant determinants of survival were only gender (with males having the worse prognosis) and size of the dominant liver metastasis (a tumor size exceeding 3 cm was associated with a greater mortality) [13].

Complications observed after RFA are not related to tumor type. They include pain, bile leakage, liver abscess, intra-abdominal hemorrhage, bowel perforation, and pulmonary complications [5, 10, 12, 13].

In patients with LM who had previous Whipple procedure and bilioenteric anastomosis, we have to keep in mind that RFA dramatically increases the risk of liver abscess formation (40 % vs. 0.4 %) [12].

In summary, RFA of LM from NETs differs from other liver metastases due to the large number of lesions per patient. Then RFA is mostly palliative aiming at debulking and controlling hormonal symptoms. This explains why intraoperative approach with or without combined liver resection is preferred rather than percutaneous approach.

26.2.2 Microwave Ablation

MWA uses electromagnetic devices with frequencies ≥900 MHz. The principle of this technique is similar to RFA but has several theoretical advantages. First, the intratumoral temperatures are consistently higher than can be achieved with RFA. Second, MWA is overcoming the "heat-sink" effect observed in RFA due to the cooling effect of blood flow in large vessels close to the tumor, both resulting in a better tumor control.

MWA has not been extensively evaluated in LM from NET. Only one series reported 11 patients with LM from NET out of 100 patients [14]. As with RFA, most procedures were performed intraoperatively either with concomitant hepatic resection (7/11) or concomitant extrahepatic tumor resection (6/11). The median number of ablated LM was 4 ranging from 1 to 13 tumors. Complications were observed in three patients. No local liver recurrence was noticed [14].

26.2.3 Cryotherapy

Cryotherapy is based on the decreased cell viability at low temperatures. The obtained tissue temperature should be −50 °C to achieve necrosis in neoplastic tissue.

To our knowledge, only three series have evaluated cryotherapy in LM from NETs (the largest with 19 patients) [15–17]. As with other thermal ablative techniques, hormonal symptom relief was observed in the vast majority of patients. Notably, postprocedural coagulopathy has been found in all patients of the two main series requiring transfusion of either platelets or fresh frozen plasma [15, 16]. In one of these series, two patients required intra-abdominal packing and transfusion of clotting factors [16]. The authors have not observed similar complications in any other liver malignancies and speculated that the necrosing carcinoid tumors were releasing substances that may disrupt the coagulation cascade [16].

Despite the efficacy on hormonal symptoms, cryotherapy has been gradually replaced by RFA, mainly for safety reasons.

26.3 Transarterial Chemoembolization and Bland Embolization

26.3.1 Rationale and Results

The rationale for transarterial hepatic embolization (TAE) is based on the fact that most LMs from NET are hypervascular and derive their blood supply from hepatic artery. The goal of TAE is to induce ischemia of tumor cells thereby reducing hormone output and causing necrosis. Various particles have been used including gelfoam, polyvinyl alcohol particles, and more recently, microspheres.

In the 1990s, transarterial chemoembolization (TACE) has been developed based on the principle that ischemia of the tumor cells increases sensitivity to chemotherapeutic substances [18]. Another advantage of TACE over TAE is the higher drug concentration obtained by regional delivery of chemotherapy. In TACE, embolization is performed immediately after intra-arterial injection of cytotoxic agents.

Despite the large number of TACE or TAE studies performed in patients with LM from NET, there are no randomized trials. Most of studies have evaluated clinical, biological, and morphological responses. Partial or complete symptom relief was observed in 42–100 % which lasts between 9 and 24 months [19, 20]. Significant decrease in tumor markers occurred in 13–100 % [19, 21]. Morphological response (either complete or partial) was seen in 8–94 % [21, 22]. Yet, imaging criteria for assessing tumor response have not been detailed in all published articles. When evaluated, overall survival since TAE or TACE initiation ranges from 15 to 80 months [23, 24].

Predictive factors of tumor response after TAE or TACE have been identified. Some of them depend on LM characteristics such as tumor liver involvement <30 % and tumor enhancement on arterial phase CT images [25, 26]. Primary tumor of the jejunum/ileum is associated with a better tumor response of the LM than pancreatic tumor [23, 25–28].

26.3.2 Technical Issues

Careful analysis of the literature highlights many disagreements on technical issues.

Such as choice between TAE and TACE is not clear. Several studies have retrospectively compared TAE and TACE in patients with LM from NETs. In all studies but one, treated patients had NET from the jejunum/ileum and NET from pancreatic origin, and no subgroup analysis has been performed. In two studies, no differences have been shown in terms of patient survival and tumor response [29, 30]. In one study, chemoembolization demonstrated trends toward improvement, in time to progression, symptom control, and survival (although not significant) [31]. Furthermore, these authors, as others, have shown that chemoembolization was not associated with a higher degree of toxicity than bland embolization [31, 32]. Gupta et al. have separately analyzed their results in small intestinal tumors and pancreatic

tumors. They have shown that the addition of intra-arterial chemotherapy to embolization did not improve the overall survival nor progression-free survival in patients with small intestinal tumors. Moreover, it had a deleterious effect on the morphologic response rate [27]. In contrast, a tendency toward prolonged survival and improved response rate was noted in patients with pancreatic tumors treated with TACE compared to TAE [27]. A prospective comparison between TAE and TACE in neuroendocrine LM from the midgut has been published recently [29]. Primary endpoint was progression-free survival. The expected number of enrolled patients was not achieved explaining that this study may suffer from a lack of power. Yet, no difference was seen in the two groups [29]. The 1st year progression-free survival rates were 91.6 and 90 % in the TAE and TACE arms, respectively. The median PFS was 24 and 19 months in the TAE and TACE arms, respectively. There results confirm that the addition of intra-arterial chemotherapy to embolization does not prolong PFS. In summary, TACE has not been proved superior to TAE in LM from the jejunum/ileum. The question is still open in LM from neuroendocrine tumors of the pancreas.

It is known that embolization stimulates release of VEGF into the circulation. Authors have speculated that sunitinib, an oral VEGFR inhibitor, could be administered following embolization [33]. They observed high rates of PFS (15.2 months) and OS (95 and 59 % at 1 and 4 years, respectively) associated with this sequence of therapies.

Most cytotoxic drugs that have been injected during TACE procedure are drugs that are currently used with systemic chemotherapy. Various drugs have been used: doxorubicin and streptozotocin being the most common injected and, alone or in combination, mitomycin C, cisplatin, and gemcitabine. Even some teams have injected a mixture of doxorubicin, mitomycin, and cisplatin. Most teams recommend doxorubicin in small intestinal tumors and streptozotocin in pancreatic tumors [24, 25]. As drug assignment was not controlled nor randomized, it is not possible to determine which drug is more efficient. However, authors see potential advantage in using streptozotocin, especially in LM from the pancreas, which may save doxorubicin for subsequent use and chemotherapy [25] (Fig. 26.2). As injection of streptozotocin has been reported to be painful, the procedure is then performed under general anesthesia [34].

No comparison between absorbable and nonabsorbable particles has been made in LM from NETs. Moreover, most studies have included patients treated with absorbable and nonabsorbable particles [27, 30, 35, 36] (Fig. 26.3). Only one study has focused on TAE with trisacryl gelatin microspheres (Embosphere®) (Fig. 26.4). Hepatic embolization was performed using either particles sized 300–500 μm, 500–700 μm, and/or 700–900 μm. Absence of disease progression was seen in 91 % of the cases, and 35 % of the patients had partial response on imaging using RECIST criteria despite the fact that some patients had extensive tumor necrosis [19]. No major complications occurred in this series. Notably, all patients with bilobar involvement were treated sequentially [19].

Studies have compared the conventional TACE technique and the drug-eluting beads technique and have shown a more prolonged retention of drug within

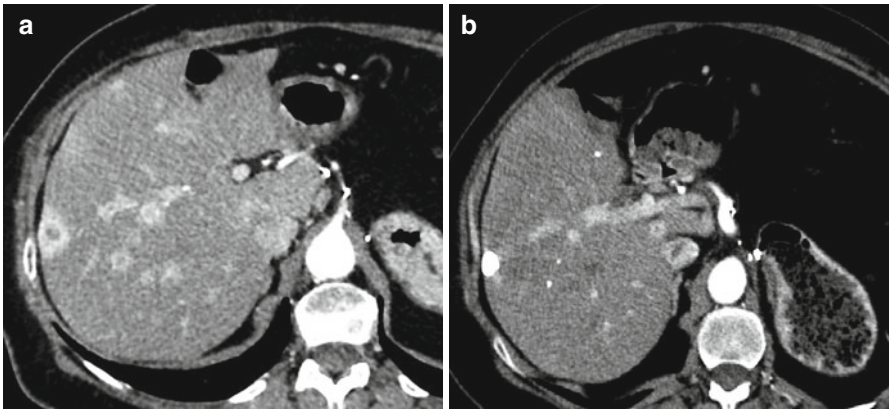

Fig. 26.2 A 76-year-old man with pancreatic neuroendocrine tumor and multiple liver metastases. (**a**) Axial CT scan shows hypervascular lesions at the arterial phase. (**b**) Axial CT scan after chemoembolization using streptozotocin shows a major lipiodol uptake of lesions with no residual hyperarterial tumor suggesting complete response

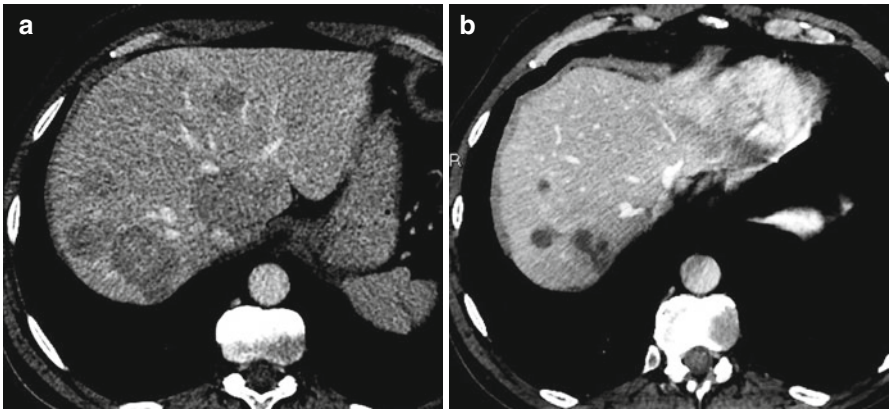

Fig. 26.3 A 56-year-old man with resected small bowel neuroendocrine tumor. (**a**) Axial CT scan shows hypoattenuating liver metastases (portal venous phase). (**b**) Axial CT scan at the portal phase after bland embolization of the right liver with sponge shows partial response with important necrosis and decrease in size of all lesions

hepatocellular carcinoma in the latter [37]. Drug-eluting beads are particles which are preloaded with any chemotherapeutic agent. The principle is to deliver high dose and more sustained release of drug into the tumor compared to systemic chemotherapy [38, 39]. Recently, three trials have evaluated drug-eluting beads with doxorubicin in LM from NETs. Preloaded in LM from neuroendocrine tumors is doxorubicin (DC Bead, Terumo, Japan) [38, 39]. Stabilization or partial response on imaging was observed in 95 and 100 % of cases. The mean PFS was 14 and 15 months, respectively [38, 39]. Again, no comparison has been made with conventional TACE in those patients. Yet, the PFS rates were in the range of the others.

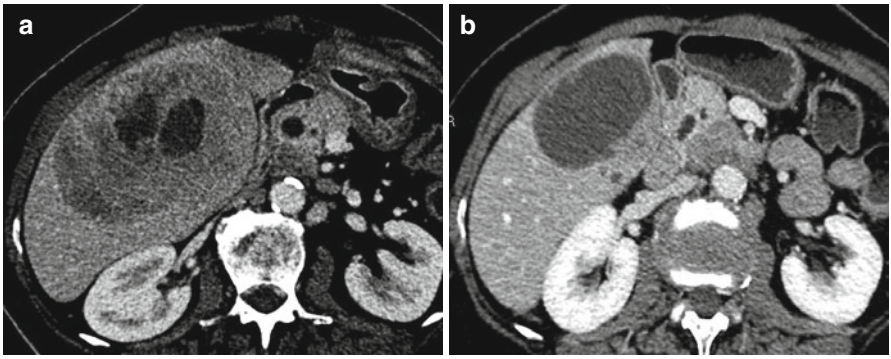

Fig. 26.4 A 67-year-old woman with resected small bowel neuroendocrine tumor. (**a**) Axial CT scan shows big hypoattenuating liver metastases with little area of necrosis (portal venous phase). (**b**) Axial CT scan at the portal venous phase after bland-selective embolization of this lesion with microspheres shows a possible complete response with major necrosis and decrease in size of the lesion

Interestingly, biliary and liver injuries such as dilated bile ducts, portal vein narrowing, portal venous thrombosis, and biloma/liver infarcts have been reported in patients with LM from neuroendocrine tumors and are more often observed than in patients with hepatocellular developed on cirrhosis [40]. This first observation was largely confirmed by a study which showed that 7/13 (54 %) patients with LM from neuroendocrine tumors developed bilomas which forced interruption of the trial. Notably, all of these patients had multiple small LM [41]. It is hypothesized that hypertrophied peribiliary plexus observed in cirrhosis could protect against the ischemic/chemical insult of bile ducts suggesting caution when using drug-eluting beads in noncirrhotic liver [40].

26.3.3 Complications, Toxicity, and Exclusion Criteria

In a retrospective series of 72 patients with neuroendocrine LM, the median length of stay was 4 days [42]. The most common and classical complication is the postembolization syndrome which is seen in up to 80–90 % of the patients [27, 43]. It includes fever, leukocytosis, abdominal pain, nausea, and a transient increase in liver enzymes. Some of the severe complications are also observed in other liver malignancies such as liver failure, cholecystitis, gastric ulcers and bleeding, whereas some others such as carcinoid crisis are specific of LM from neuroendocrine origin [5].

TAE and TACE can be repeated safely in patients with LM from neuroendocrine tumors and especially in patients with disease progression [44]. The complication rate after repeat TACE is lower than after the first TACE [44]. As in other indications, TACE used to be performed at fixed delays whatever the tumor response. The trend is now to adapt the number of sessions and the interval between sessions to the tumor response.

Portal vein thrombosis and hepatic insufficiency are considered exclusion criteria for both TAE and TACE [1]. As the odds ratio of developing abscess in patients with bilioenteric anastomosis is very high (894), TACE should be avoided in those patients [45]. In a retrospective series of 489 TACE performed in various tumors, the three patients who developed abscess formation had a neuroendocrine tumor and a bilioenteric anastomosis [46]; in another retrospective series, 48 % of patients (12/25) with bilioenteric anastomosis developed an abscess, and two of them died [47]. If it must be performed, very broad-spectrum prophylactic antibiotics and bowel preparation before the procedure should be considered [45].

Tumoral liver involvement is an important issue for both efficacy and toxicity. Best morphological responses are obtained in patients with limited liver involvement (<30 or <50 %) [22, 26]. On the other hand, toxicity is increased in major liver involvement (>70 or 75 %) [22, 26, 27, 48]. This threshold has been first used as an exclusion criterion by many teams. However, Gupta et al. have been able to treat many patients with >75 % liver involvement successfully and safely by treating only a small portion of the liver in each embolization session [27].

26.4 Radioembolization

Radioembolization is defined as the injection of micron-sized embolic particles loaded with radioisotope by use of percutaneous transarterial techniques. Radioembolization with yttrium-90 microspheres involves infusion of embolic microparticles of glass or resin impregnated with the isotope yttrium-90 through a catheter directly into the hepatic arteries. Yttrium-90 is a pure β-emitter and decays to stable Zr-90 with a physical half-life of 64.1 h.

The efficacy of this radioembolization technique, as for the chemoembolization, is based on the fact that intrahepatic malignancies derive their blood supply almost entirely from the hepatic artery, as opposed to the normal liver, which mainly depends on the portal vein. The microspheres are injected selectively into the proper hepatic artery and subsequently become lodged in the microvasculature surrounding the tumor. Very high irradiation doses are delivered to the tumors, whereas the surrounding liver parenchyma is largely spared. Sommer et al. have shown that the only baseline imaging parameter statistically associated with the progression-free survival (PFS) was the hypervascular pattern [49].

The use of yttrium-90 for the treatment of primary and secondary liver malignancies is no longer investigational or experimental, and both devices have got FDA and European approval.

The technique comprises two steps: The first step is patient eligibility and conditioning. Selective mesenteric and hepatic angiography and scintigraphy are performed to isolate the hepatic circulation by occluding extrahepatic vessels with prophylactic embolization of extrahepatic arteries (e.g., right gastric, gastroduodenal artery). The second step is the radioembolization therapy itself. Several days after patient eligibility and conditioning, treatment is performed with microsphere

infusion proceeding at flow rates similar to that of the native hepatic artery. Treatment of the contralateral lobe, if needed, is usually performed 30–60 days.

The largest series of selective interval radiation therapy (SIRT) of LM from NET is a retrospective review of 148 patients from ten institutions. Complete and partial tumor response were seen in 2.7 and 60.5 % of the cases according to RECIST criteria, respectively [50]. Stable disease was observed in 22.7 % of the cases, and progressive disease occurred in only 4.9 % of the cases [50]. Similar results were reported in the other series including a prospective one [51–56]. Paprottka et al. have observed that 97.5 % of liver metastases become necrotic or hypovascular explaining the high rate of overall response when using imaging criteria which aim to depict tumor changes such as EASL or mRECIST criteria [54]. For Ceelen et al., the post-procedural MR parameters associated with a longer PFS were a both decrease in sum of diameters and arterial enhancement and an increase in necrosis [57].

Symptomatic responses were observed in 55–100 % [53, 58, 59]. Disease control rate was 93 %, much better than for other types of malignancy (59 % for colorectal primaries and 63 % for other primaries) [60].

Low toxicity is another advantage of radioembolization. Side effects are mainly represented by fatigue, nausea or vomiting, and abdominal pain; no Grade 4 toxicities but one were seen in articles which detail complications after the procedure. Moreover, no radiation-induced liver failure was described in those patients [50, 54, 58].

26.4.1 Embolization and Chemoembolization vs. Radioembolization

To date, there has been no randomized trial but two review papers and a multicenter, prospective treatment registry with radioembolization which have evaluated the efficacy of radioembolization and TAE/TACE in neuroendocrine LM [61–63]. Treatment efficacy seems similar. Time to progression (TTP) was not different from the groups [61]. TAE/TACE seems more appropriate in patients with bulky and large tumors which require a segmental targeted approach whereas radioembolization could be more advantageous in patients with small LM that have a miliary bilobar distribution.

26.5 Indications of Liver-Directed Treatments

Presence of liver metastases largely influences prognosis in all types of neuroendocrine tumors [64]. Prognosis has improved with significant overall survival increasing in both patients with LM from the jejunum/ileum and the pancreas undergoing multidisciplinary treatment [64]. This includes hepatobiliary surgery, locoregional, and/or medical therapies.

Patient management depends on LM characteristics (tumor pattern and tumor burden), tumor differentiation and proliferative activity, and natural history of LM.

LM may be defined according to three different macroscopic patterns: (1) a *simple* pattern corresponding to LM confined to one liver lobe or limited to two adjacent segments, (2) a *complex* pattern assessed when LM primarily affect one lobe but with smaller satellites contralaterally, and (3) a *diffuse* pattern corresponding to diffuse, multifocal liver metastases [64].

Tumor differentiation and proliferative activity are also important factors for patient management. In this article, we will only consider liver-targeted therapy in Grade 1 and 2 neuroendocrine LM as systemic chemotherapy is the recommended treatment in Grade 3 tumors.

Last, natural history course is also a key factor. The "watch-and-wait" attitude is recommended in nonprogressive and nonsymptomatic liver metastases in patients with limited tumor burden (30–50 %) [65].

In single pattern LM, the standard of care is surgical resection if possible. Local ablative therapies (mainly RF ablation) play a significant role and are performed when surgery is contraindicated.

In complex pattern of LM, local ablative treatments (mainly intraoperatively) may be used in combination to surgical resection in order to extent the number of patients amenable to complete resection.

In diffuse LM, surgery and local ablative therapies are no longer indicated. The role of transarterial treatment (TAE, TACE, radioembolization) is crucial and particularly in LM from the jejunum/ileum because efficacy of systemic chemotherapy has not been proved in these tumors. In LM secondary to neuroendocrine tumors of the pancreas, transarterial treatments are competing with systemic therapy including targeted therapy.

In conclusion, liver-directed therapies are widely performed in LM from neuroendocrine tumors. As these tumors largely differ from the other LMs (number, imaging findings, prognosis, treatment, etc.), tumor boards dedicated to neuroendocrine tumors are advisable. Interventional radiologists should also be aware of the indications and specific contraindications of liver-directed therapies in these tumors.

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Peptide Receptor Radionuclide Therapy

27

Murat Fani Bozkurt

27.1 Introduction

The theoretical basis of PRRT depends on delivering therapeutic dose of radiation inside the tumour cells, owing to the internalization of the somatostatin receptor and radiolabelled somatostatin analogue complex. Therefore, PRRT is actually an internal radiation therapy. Since indium-111-labelled octreotide (^{111}In-octreotide) was approved by the FDA as a diagnostic imaging agent for neuroendocrine tumours in 1994, studies have begun to assess whether it would be possible to apply radiolabelled somatostatin receptor analogues for therapy on the basis of the same molecular mechanism. The first trial to perform PRRT with radiolabelled octreotide dates back to the early 1990s in a multicentre study in which high doses of diagnostic ^{111}In-octreotide were administered to patients with neuroendocrine tumours, in whom somatostatin receptor overexpression was confirmed by a previous ^{111}In-octreotide scintigraphy [1]. Although ^{111}In is only a gamma emitter radionuclide and does not emit any beta or alpha particles which are necessary for therapy, owing to its relatively high amount of Auger and conversion electrons which act like beta particles, it was expected to exert therapeutic effect if it is administered at high doses. This was the first approach to PRRT. However, ^{111}In-octreotide therapy did not result with promising results, and even partial remissions were exceptional. These disappointing results have given rise to use higher-energy and longer-tissue range radionuclides instead of ^{111}In, such as yttrium-90 (^{90}Y). ^{90}Y is a pure beta emitter radionuclide with a high maximum energy (Emax:2.27 MeV), long tissue range (Rmax:11 mm) and a

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relatively long physical half-life (T1/2:64 h) which make this radionuclide a quite suitable agent to be used as a therapeutic radiopharmaceutical. Along with ^{90}Y, another radionuclide lutetium-177 (^{177}Lu) has also been introduced as having suitable therapeutic characteristics (Emax:0.49 MeV, Rmax:2 mm, T1/2:6.7 days). The main difference of ^{177}Lu from ^{90}Y is that ^{177}Lu is not a pure beta emitter and it emits both beta radiation for therapy and gamma radiation for imaging. Both radionuclides are tagged to somatostatin analogues with bridging macrocyclic chelator 1,4,7,10-tetraazacyclododecane-n, N', N", N"'-tetraacetic acid (DOTA) to form different forms of therapeutic radiopharmaceuticals according to their N-terminal amino acids such as ^{90}Y/^{177}Lu-DOTATOC/DOTATATE/DOTANOC. These radiopharmaceuticals have been studied in different trials since the early 2000s to recent years [2, 3].

This chapter focuses on the use of therapeutic radiopharmaceuticals, indications, practical aspects and monitorization of PRRT for neuroendocrine tumours.

27.2 Rationale of PRRT for Neuroendocrine Tumours

Neuroendocrine tumour cells are characteristically regulated by particular hormones that exert their actions by binding some specific transmembrane domain G protein-coupled membrane surface receptors. The most abundant and most studied receptor-ligand system in neuroendocrine tumours is somatostatin receptor system. PRRT basically depends on the presence of overexpression of somatostatin receptors on cell membranes of neuroendocrine tumour cells, and after radiolabelled somatostatin analogue binds to specific receptor; the radiopharmaceutical-receptor complex is internalized into the cell, where therapeutic irradiation from the radiopharmaceutical is emitted. There are a number of somatostatin receptor subtypes, out of which subtype 2 (sstr2) is most frequently expressed on many kinds of neuroendocrine tumour cells. Therefore, radiolabelled somatostatin analogues which have high affinity to sstr2 are preferred as therapeutic use, based on the same rationale for diagnostic imaging [2, 3].

27.3 Indications of PRRT

1. Gastroenteropancreatic and bronchial tract neuroendocrine tumours, which mainly overexpress sstr2.
2. Pheochromocytomas and paragangliomas, which show uptake on diagnostic somatostatin receptor imaging.
3. Medullary thyroid carcinomas which are positive on diagnostic somatostatin receptor imaging.
4. Any tumour is to be potentially treated if sstr2 overexpression is confirmed and documented on diagnostic somatostatin receptor imaging and/or confirmed histopathologically.

27.4 Patient Selection and Eligibility for PRRT

Ideally, a tumour board with a minimum number of different specialists including surgeon, pathologist, oncologist, radiologist-interventional radiologist, nuclear medicine physician, radiation oncologist, endocrinologist, gastroenterologist and others who are interested in neuroendocrine tumour management should be established on the first referral of neuroendocrine tumour patients. Decision for PRRT should be made by the tumour board after evaluation of all other therapeutic options starting from surgery, proceeding to systemic and local therapies. A complete clinical history and informed consent should be obtained from all patients referred to PRRT. For patient eligibility, these following information is mandatory:

1. Patient has to have histopathologically proven and documented neuroendocrine tumour.
2. High somatostatin receptor expression has to be documented by diagnostic somatostatin receptor imaging study and/or by immunohistochemistry.

The density of receptors on tumour tissue versus healthy organs should also be considered. In routine practice, the receptor density can easily be evaluated by diagnostic somatostatin receptor imaging, according to a kind of visual scale known as "Rotterdam scale" [4]. Rotterdam scale is graded as follows:

Grade 1: Tumours that have an uptake on planar images lower than that of normal liver uptake
Grade 2: Tumours with uptake equal to the liver uptake
Grade 3: Tumours which show higher uptake than that of normal liver
Grade 4: Tumours which show very high uptake, higher than kidneys and spleen (the hottest organs in ^{111}In-octreotide scintigraphy)

Generally, tumours with Rotterdam grades 2–4 are candidates for PRRT.
Along with Rotterdam scale, the following criteria should also be met [2–4]:

1. Karnofsky performance status >60 or ECOG <2.
2. Tumour differentiation, preferably in G1–G2.
3. Tumour proliferation rate, preferably Ki-67 mitotic index ≤20 %. The rate of tumour growth which is detected by CT and/or MRI may also be helpful. Generally, less differentiated tumours with high proliferation are more eligible to chemotherapy compared to PRRT.

27.4.1 Pretherapy Renal Function Evaluation

For PRRT with ^{90}Y-labelled somatostatin analogues, a normal renal function is essential, since this radiopharmaceutical has a potential to deteriorate renal parameters. However, patients with compromised renal function may still be

candidates for PRRT with ^{177}Lu-labelled somatostatin analogues, since this radiopharmaceutical has much less toxicity on renal function. For ^{177}Lu-labelled PRRT, a mild to moderate grade of renal impairment (creatinine ≤1.7 mg/dl) can be tolerated [2, 3].

Patients' renal function should be assessed by blood biochemistry (creatinine and blood urea nitrogen) and GFR calculation.

27.4.2 Haematological Evaluation

1. For PRRT with ^{177}Lu-labelled somatostatin analogues, white blood cell (WBC) should be >3,000/μl, and platelet (Plt) count should be >75,000/ μl.
2. For ^{90}Y-labelled somatostatin analogues, WBC > 3,000/μl, Plt > 90,000/μl and red blood cell count >3,000,000/μl.

27.5 Precautions for PRRT

1. Before PRRT, obstructive renal disease should always be ruled, out and if there is, it should be corrected before PRRT.
2. Previous myelotoxic chemotherapy and radiation therapy which were given recently (especially in a couple of weeks before PRRT) can constitute a risk for bone marrow failure and serious bone marrow suppression, particularly in repeated therapies or subsequent cycles. Platelet counts after each cycle of PRRT can determine whether an interruption would be needed.
3. Before PRRT, hepatic failure should be regarded with caution.

27.6 Contraindications for PRRT

1. Pregnancy and breastfeeding (if not quitted)
2. Acute or severe concomitant diseases
3. Severe psychiatric disorder

27.7 Therapeutic Dose Assessment for PRRT

Ideally PRRT should be given on dosimetry-based regimens. However, this approach is rarely preferred in routine practice since it is cumbersome and needs complex calculations. Therefore, fixed-activity treatment cycles or individualized dosing which is adjusted on the basis of some clinical parameters such as body surface

area, clinical status and haematological and renal function are the most preferred dose calculation methods.

For ^{90}Y-labelled somatostatin analogues, cumulative activities up to 19 GBq (~500 mCi) can be administered in 2–4 cycles, providing that renal absorbed dose threshold is not exceeded. Reported cumulative activities for ^{177}Lu-labelled peptides are between 22 and 30 GBq (~600–800 mCi) which can be administered in 3–6 cycles, according to different protocols [2, 3].

27.8 Patient Preparation for PRRT

27.8.1 Withdrawal of Somatostatin Analogues

Somatostatin analogues (cold somatostatin) should be discontinued for a certain period before PRRT. The duration of interruption depends on whether the drug is long or short acting. For long-acting drugs, it is 3–4 weeks; for short-acting ones, 1 day withdrawal is advised, although there is still debate on this subject.

27.8.2 Premedication for PRRT

1. Just before dose administration, serotonin 5-HT3 receptor antagonists such as ondansetron, granisetron or tropisetron should be given and can be repeated when necessary.
2. Corticosteroids such as dexamethasone 4 mg or more may be given short before PRRT and can be repeated if necessary.

27.8.3 Pretherapy Renal Protection for PRRT

The renal absorbed dose is higher for ^{90}Y-labelled peptides compared to ^{177}Lu-labelled peptides, due to different beta radiation features. Positively charged amino acids such as L-lysine and/or L-arginine are commonly used to competitively inhibit the proximal tubular reabsorption of radiopharmaceutical to reduce renal absorbed radiation dose. At least 25 g of positively charged amino acid solution should be infused before PRRT, in order to exert a protective effect on kidneys. Four amino acid protection methods have been introduced so far as follows [5, 6]:

1. Single-day 50 g amino acid protection
2. Three-day 25 g amino acid protection
3. Three-day 50 g amino acid protection
4. Single-day 50 g amino acid plus Gelofusine protocol

Table 27.1 Different treatment regimens for PRRT

Radiopharmaceutical	Administered activity	Number of cycles	Time interval between cycles (weeks)
^{90}Y-DOTATATE/^{90}Y-DOTATOC	3.7 GBq (100 mCi)/m^2	2	10–12
^{90}Y-DOTATATE/^{90}Y-DOTATOC (another protocol)	2.78–4.44 GBq (75–120 mCi)	2–4	10–12
^{177}Lu-DOTATATE/^{177}Lu DOTATOC	5.55–7.4 GBq (150–200 mCi)	3–5	10–12
^{177}Lu + ^{90}Y Combination-Cocktail protocol	5.55–7.4 GBq (150–200 mCi) (for ^{177}Lu) 2.5–5 GBq (68–135 mCi) (for ^{90}Y)	2–6	10–16

27.9 Treatment Regimens

Treatment regimens according to different radiopeptides for patients with normal bone marrow and renal function are given at Table 27.1.

Treatment doses should be reduced and individualized for compromised patients according to clinical and biochemical parameters.

27.9.1 PRRT for Paediatric Patients

In children, ^{90}Y-DOTATOC can be given in 1.5–1.85 GBq/m^2 activities per cycle in up to 3 cycles. There is no widespread use of 177Lu-DOTATATE in paediatric patients, and activities should be adjusted per body surface area [7].

27.9.2 Retreatment with PRRT

Retreatment should be considered for patients with well-documented disease progression, who previously responded. The decision of retreatment should be taken within tumour board, and the same eligibility criteria apply like the first treatment. Retreatment can be done with the same or a different radiopeptide.

27.10 Imaging After PRRT

Since ^{177}Lu emits gamma radiation in addition to therapeutic beta particles, scintigraphic imaging using gamma rays should always be done following each treatment cycle to confirm targeting of radiopharmaceutical and to assess functional response. Planar and SPECT and/or SPECT/CT tomographic images are acquired usually on days 1, 4 and 7 after PRRT with ^{177}Lu-DOTATATE (Fig. 27.1a, b).

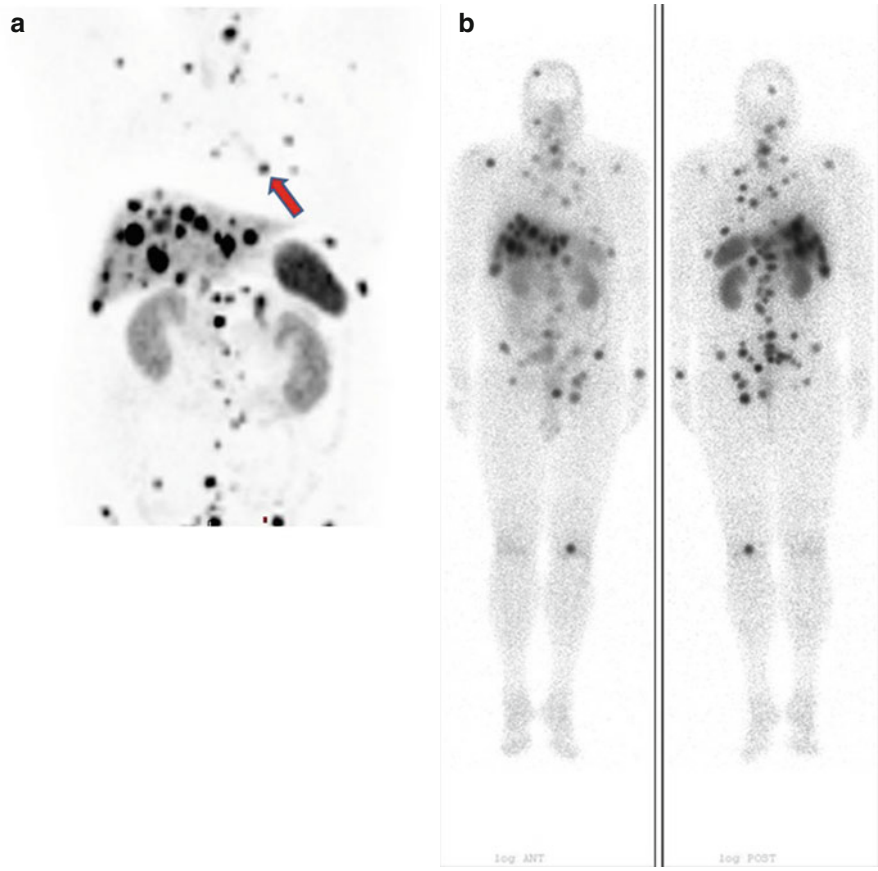

Fig. 27.1 (**a**) Whole-body maximum intensity projection (MIP) ^{68Ga}-DOTATATE image of a 56-year-old male patient with the diagnosis of multiple hepatic neuroendocrine tumour metastases from an endobronchial carcinoid tumour primary (*arrow*) shows multiple foci of uptake in the liver, in the bones and in the thorax consistent with extensive metastatic disease and referred to PRRT. (**b**) Whole-body anterior (*left*) and posterior (*right*) 177Lu-DOTATATE posttherapy images show more number of lesions compared to diagnostic imaging

Since ^{90}Y is a pure beta emitter, it does not permit direct scintigraphic imaging, but even if it is poor in quality, Bremsstrahlung (braking radiation) rays can be used to obtain a posttherapy imaging. It is usually done in 24 h following PRRT with ^{90}Y-DOTATOC.

27.11 Outcomes of PRRT for Neuroendocrine Tumours

27.11.1 Response

Response evaluation for PRRT includes functional and anatomic images to assess functional and morphological response as well as biochemical and symptomatic

response to assess patients' quality of life. Posttherapeutic ^{177}Lu-DOTATATE imaging can successfully be used to assess functional response between therapy cycles and at the end of the therapy. The time period for response evaluation depends on different situations according to clinical needs, but generally the first follow-up is recommended after 3 months and to be continued after 3–6 months.

PRRT for neuroendocrine tumours with ^{177} Lu- and ^{90}Y-labelled somatostatin analogues has been in clinical use for more than a decade. Most of the studies in the literature conclude that PRRT can give high absorbed doses to neuroendocrine tumours with overexpression of sstr2 which results with partial and complete objective responses in up to 30 % of patients. Gastroenteropancreatic neuroendocrine tumours have showed the best objective response rates, with partial responses ranging from 9 to 29 % and complete remission rates from 2 to 9 %. Almost similar response rates have been reported for neuroendocrine tumours of the lungs and neuroectodermal tumours such as paragangliomas. Dedifferentiated thyroid carcinomas, medullary thyroid cancers and neuroendocrine tumours of the thymus have displayed less satisfactory response rates. Besides these, promising results have been reported for other sstr-positive tumour types such as astrocytomas, medulloblastomas and meningiomas [8–12].

27.11.2 Survival

According to some studies in which survival analyses have been reported, patients who have high somatostatin receptor expression at the beginning of PRRT show significantly higher objective responses in correlation with significantly longer survival when compared with similar patients with lower receptor expression [10, 13, 14].

Some studies have reported that biochemical response is also good predictor for the overall survival of patients with dedifferentiated thyroid cancer and medullary thyroid cancer following ^{90}Y-DOTATOC therapy [9, 15]. Bushnell et al. concluded that significant symptomatic response after ^{90}Y-DOTATOC therapy has had an impact on progression-free survival of patients [16].

27.11.3 Side Effects and Toxicity

The kidney is the dose-limiting organ for PRRT. Pretherapy renal protection protocols are mandatory, and they reduce renal absorbed doses significantly and avoid serious renal dysfunction due to PRRT [5, 17]. Renal toxicity is more frequently seen with 90Y-DOTATOC; especially the patients with the diagnosis of longstanding and poorly controlled hypertension have high risk for renal toxicity [16]. Despite kidney protection, renal dysfunction can occur following PRRT with approximate creatinine clearance loss of 3.8 % per year for ^{177} Lu-DOTATATE and 7.3 % per year for ^{90}Y-DOTATOC [18].

Acute bone marrow toxicity may be seen in about 10–13 % of patients following ^{90}Y-DOTATOC and in 2–3 % following ^{177} Lu-DOTATATE PRRT [19]. Most of the bone marrow toxicities are reversible.

PRRT may cause a transient impairment in male fertility, and recovery is commonly complete within 2 years following the last cycle of the therapy [20].

PRRT may cause hepatic failure not in patients with extensive metastatic involvement of the liver. In these patients, ^{177} Lu-DOTATATE should be preferred to ^{90}Y-DOTATOC and administered activity should be reduced [18, 20].

Although there are somatostatin receptors in healthy pituitary, thyroid and adrenal glands, PRRT has never been reported to alter endocrine function [21].

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Management of Hormonal Symptoms

28

Beata Kos-Kudla

28.1 Introduction

Gastroenteropancreatic (GEP), neuroendocrine neoplasms (NEN), arising from the diffuse endocrine system (DES) cells disseminated in the gastrointestinal tract and in the pancreas, may produce excessive amounts of hormones and/or biogenic amines. In such circumstances, they create clinical symptoms of syndromes specific for oversecretion of particular hormones (Table 28.1).

The most common functional NENs include carcinoid (See Chaps. 12–16), insulinoma, and gastrinoma [1–3].

28.2 Clinical Characteristics

28.2.1 Insulinoma

Insulin-secreting pancreatic tumor is the most common functional pancreatic NEN. In approximately 1 % of patients, extra-pancreatic location is possible (duodenum, stomach, bile ducts, lungs) [4]. Clinical symptoms result from hypoglycemia and neuroglycopenia: skin pallor, increased perspiration, trembling of hands, nausea, palpitations, hunger, headache and vertigo, blurred vision, double vision, changed behavior, confusion, concentration disorders, retrograde amnesia, drowsiness, hallucinations, delusions, convulsions, and loss of consciousness (sometimes accompanied by grandmal convulsions).

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Table 28.1 Clinical syndromes in particular functional GEP NENs

Tumor type	Oversecretion of main substances	Clinical syndromes
All NENs	CgA	
Carcinoid	Serotonin	Carcinoid syndrome
Insulinoma	Insulin, C peptide or proinsulin	Hypoglycemia and neuroglycopenia
Gastrinoma	Gastrin	Zollinger-Ellison syndrome (ZES)
Glucagonoma	Glucagon, enteroglucagon	Necrolytic migratory erythema, weight loss, diabetes mellitus
VIPoma	VIP	Verner-Morrison syndrome; pancreatic cholera; WDHA syndrome (watery diarrhea, hypokalemia, achlorhydria)
Somatostatinoma	SST	Cholelithiasis, weight loss, steatorrhea,

Key: *CgA* chromogranin A, *SST* somatostatin, *VIP* vasoactive intestinal peptide

Insulinoma is characterized by Whipple's triad:

1. Clinical symptoms suggestive of hypoglycemia
2. Decreased blood glucose (<40 mg/dl; 2.2 mmol/l) measured at the time of the symptoms
3. Relief of symptoms after intake of carbohydrates [2, 5, 6]

28.2.2 Gastrinoma

They are tumors oversecreting gastrin and thus causing Zollinger-Ellison syndrome (ZES). The most common symptoms of gastrinoma include persistent pain in the upper abdomen, nausea, vomiting, and diarrhea, which disappears after the use of proton pump inhibitors (PPIs) – a very characteristic feature, body weight loss, and gastrointestinal bleeding. There are no differences between the clinical symptoms of pancreatic and duodenal gastrinoma [7].

Helicobacter pylori infection is less frequent in ZES patients compared to idiopathic peptic ulcer disease. Therefore, negative results of Helicobacter pylori tests in pat)ZESients with recurrent peptic ulcer disease who do not receive NSAIDs or acetylsalicylic acid should be suggestive of gastrinoma [2, 8].

28.2.3 VIPoma

VIPoma (Verner-Morrison syndrome, pancreatic cholera, WDHA syndrome): symptoms: diarrhea, hypokalemia, dehydration, acidosis, rarely skin reddening, hypercalcemia, glucose intolerance, and functional gallbladder disorders.

28.2.4 Glucagonoma

Symptoms of glucagonoma are migrating necrolytic erythema, glucose intolerance, body weight loss, stomatitis, diarrhea, and hypoaminoacidemia.

28.2.5 Somatostatinoma

Symptoms of somatostatinoma include cholelithiasis, diabetes, diarrhea, and body weight loss [2, 9–11].

28.3 Diagnostics

28.3.1 Biochemical Diagnostics

Biochemical diagnostics of hormones and markers secreted by neuroendocrine neoplasms may be helpful in:

- Initial diagnosis of a disease
- Assessment of treatment effectiveness
- Prognosis

Biochemical diagnosis of functional NENs (F-NENs) requires the evidence of increased serum concentrations of specific hormonal markers, e.g., gastrin in the ZES or insulin in insulinoma (in combination with 72-h fasting assay), etc. If clinical symptoms indicating the hypersecretion of a given hormone occur, other tests may be performed, for instance, demonstration of excessive secretion of gastric juice in ZES, or secretin test can be done in justified cases [12, 13].

Biochemical diagnostics of rare F-PNEN include confirmation of increased serum concentrations of specific biochemical markers, e.g., glucagon in suspected glucagonoma (positive result > 1,000 pg/ml), vasoactive intestinal peptide (VIP) (positive result >170 pg/ml), and somatostatin (positive result in pancreatic tumor location is over 50 times higher than the reference values).

Chromogranin A, which is a nonspecific marker, may only be used to confirm the presence of a neuroendocrine tumor and to monitor the course of the disease, but it cannot constitute the basis for the diagnosis of functioning NENs [3, 9].

Detailed guidelines for laboratory diagnostics are discussed in Chapter 7.

28.3.2 Pathomorphological Diagnostics

Pathomorphological diagnostics necessary to diagnose GEP NENs are discussed in other chapters.

It is worth emphasizing that demonstrating expression of specific hormones in an immunohistochemical assay does not justify the diagnosis of functional NEN. Only evidence of high blood concentrations of particular hormones confirms such diagnosis.

28.3.3 Imaging

In the diagnostics of GEP NENs, both anatomic imaging techniques and nuclear medicine tools are used. The choice of imaging examination depends on the primary focus location and the stage of the disease: USG/EUS, CT, MRI, and endoscopy. One of the functional examinations is the somatostatin receptor expression test with ^{68}Ga or ^{99}Tc-DOTA TOC, which enables the location of the primary lesion, but also assessment of the stage of the disease and qualification for treatment with "hot" somatostatin analogs. PET/CT scan with ^{18}FDOPA may be an alternative diagnostic method, if the results of the above functional tests are negative. In the diagnostics of insulinoma, the role of a new isotope-labeled GLP-1 analog in detecting small lesions of less than 1 cm is considered [9, 14].

28.4 Treatment

28.4.1 Surgical Treatment

Surgical treatment is the treatment of choice in GEP NENs [1, 2]. Primary tumor resection should be considered even in the presence of metastases, including hepatic metastases, if they are potentially resectable and the patient meets the criteria for the surgery. In advanced functional NENs, cytoreduction is intended to reduce the symptoms and the tumor mass.

Surgical treatment is the therapy of choice in the case of pancreatic neuroendocrine neoplasms (PNENs), as it is associated with a significantly prolonged patient survival. The development of diagnostic methods has improved the detection of small, asymptomatic tumors. Most nonfunctional neoplasms of ≤ 2 cm in diameter are benign and demonstrate a moderate risk of becoming malignant. Only 6 % of the nonfunctional, accidentally diagnosed PNENs present histopathological characteristics of malignancy. In certain cases, accidentally diagnosed tumors of ≤ 2 cm in diameter may be observed for the first year, with tests performed at 3-month intervals, then every 6 months for the next 3 years. Due to the lack of clear recommendations, the decision on the course of treatment should be taken by a multidisciplinary team of doctors experienced in the management of PNENs, and the surgical procedure should be performed in a center specializing in pancreatic surgery [2, 3].

In multiple endocrine neoplasia type 1 (MEN1), if multiple lesions occur, it is recommended to remove them preventively before they become malignant; however, this approach in the case of small, nonfunctional tumors is still controversial [15].

Liver transplantation is conducted in selected groups of patients with exacerbated symptoms associated with hormonal secretion. Patients who may benefit from transplantation are those under the age of 50 years, without metastases outside the liver, and with a low expression of Ki-67 [16–19].

In MEN1 syndrome, surgical resection of the parathyroids in primary hyperparathyroidism reduces excessive secretion of hydrochloric acid [2].

28.4.2 Locoregional Hepatic Therapies

In each case when resection of hepatic metastases is impossible, the recommended palliative treatment methods include hepatic artery embolization (HAE), transarterial chemoembolization (TACE), or HAE with the use of isotope. Radiofrequency thermoablation (RFA), cryoablation, and microwave ablation (MWA) can be used for tumors ≤5 cm. With these different modalities, symptoms improve in 40–80 % of patients [2, 17, 20, 21].

28.4.3 Endoscopic Treatment

It is of significant importance, and it may be used for symptomatic treatment of:

- Mechanical jaundice (prosthesis of the biliary duct)
- Obstruction of the gastrointestinal tract (prosthesis of the gastrointestinal tract lumen)
- Control of gastrointestinal bleeding (the use of endoscopic hemostatic methods)

Recently, single cases of using EUS for ablation of functional pancreatic tumors secreting insulin have been reported [22–24]. It is possible that in the future, endoscopic EUS-controlled ablation of pancreatic NENs, involving administration of cytotoxic agents, alcohol, or using thermoablation, will become an alternative, minimally invasive therapeutic method for patients who cannot be treated surgically [9, 17, 19].

28.4.4 Pharmacological Treatment

It is the main course of action in the management of hormonal syndromes. The main purpose of pharmacological treatment is maintaining the patient's good quality of life for the longest possible time, reduction of clinical symptoms, and, if achievable, prolongation of the patient's survival [8].

Prior to planning the treatment, the tumor size, presence of metastases, histological grading, and the profile of secreted hormones and other markers should be determined.

The choice of the treatment method depends on the symptoms, staging of the disease, the level of radiotracer uptake in receptor scintigraphy, and histological characteristics of the tumor [8, 25].

28.4.4.1 Symptomatic Treatment

Symptomatic treatment should be started when the clinical and biochemical symptoms indicate hormonal activity of the NEN, even before the precise location of the primary site or confirmation of metastases. The symptoms associated with excessive secretion of hormones by NENs may impair the patient's quality of life, and in certain cases, they may be life-threatening (e.g., severe diarrhea and hypokalemia in VIPoma or carcinoid crisis) [9].

Insulinoma

Pharmacological treatment of insulinoma is intended to prepare patients for a surgical procedure or to achieve the biochemical control in patients with symptoms of hypoglycemia in case of inoperable metastatic insulinoma.

Patients often require intravenous glucose infusion and multidirectional treatment (see Table 28.2). In most patients, diazoxide proved to be effective in the management of the symptoms of hypoglycemia [2, 6].

Diazoxide has hyperglycemic effect, as it inhibits insulin secretion by a direct action on pancreatic β-cells and activation of glycogenolysis. Recommended daily dose is 50–300 mg, by oral route, up to 600 mg/day. This is usually an effective treatment in controlling the symptoms of hypoglycemia. Adverse events include edema, increased body weight, hirsutism, and renal function disorders.

Diazoxide therapy is often supported with hydrochlorothiazide at a dose of 25 mg/day, which prevents edema and hyperkalemia, and increases the hyperglycemic effect of diazoxide.

Verapamil and diphenylhydantoin (phenytoin) can be used to control glycemia, as an alternative to diazoxide, in some patients with insulinoma.

Table 28.2 Pharmacological treatment of the symptoms of hypoglycemia in insulinoma [6, 9]

Pharmacological treatment of the symptoms of hypoglycemia in insulinoma	
Diazoxide	50–300 mg/day, max. dose 600 mg/day
Somatostatin analogs	Can be effective in patients with expression of sstr2 (may intensify hypoglycemia)
PRRT	
Everolimus	
Ca blockers (e.g., Verapamil)	
Diphenylhydantoin	
Glucocorticoids	In cases resistant to treatment (in refractory cases)
Interferon α	In selected, few cases
Systemic chemotherapy	"old": Streptozocin combined with doxorubicin and/5-FU, "new": capecitabine plus temozolomide

Key: *PRRT* Peptide receptor radionuclide therapy

Corticosteroids, including prednisolone, are usually used in patients with refractory hypoglycemia.

Somatostatin analogs (*SSA*) (octreotide and lanreotide) are often ineffective in controlling hypoglycemia (50–60 % of insulinomas), and their effect on the blood glucose concentration varies. In some cases they may even intensify hypoglycemia by inhibiting glucagon secretion.

In some patients using *interferon-α* may be beneficial in treating hypoglycemia.

An mTOR inhibitor – *everolimus* – is one of the medications controlling insulin secretion and hypoglycemia in patients with malignant insulinoma [2, 6, 9, 26].

Gastrinoma

Excessive secretion of gastric acid in gastrinoma must be inhibited pharmacologically in all patients with gastrinoma, in order to prevent complications.

The treatment of choice involves proton pump inhibitors (PPIs) (omeprazole, lansoprazole, pantoprazole, rabeprazole, esomeprazole), which reveal similar effectiveness. Administration of PPI once or twice a day is effective in most patients. Recommended initial dose for omeprazole in sporadic forms of ZES is 60 mg once a day. In patients with ZES complications (MEN1 with hypercalcemia, severe gastroesophageal reflux disease (GERD) symptoms, preceding Billroth II resection), higher doses of medications are used (e.g., omeprazole 40–60 mg twice a day). During a long-term therapy with PPI, the serum levels of vitamin B12 should be monitored once a year, and more frequent bone fractures in this population should be taken into account.

Histamine H2 receptor antagonists may also be used in patients with ZES. Patients with gastrinoma require higher and more frequent doses than patients with idiopathic peptic ulcer disease.

If oral administration of medications is not possible, PPIs are administered intravenously, as well as histamine H2 receptor antagonists, in large doses, in continuous IV infusion.

Long-acting somatostatin analogs are not first-line medications, and they should be used only in the case of PPI treatment-resistant, malignant gastrinoma.

Loperamide, cholestil, pancreatic enzymes, cholestyramine, bisphosphonates, and corticosteroids may be used for symptomatic treatment, according to clinical indications [8, 9, 13].

VIPoma

Hydration and supplementation of electrolytes are recommended, as they may considerably improve the patient's clinical condition. In patients with VIPoma accompanied by a rare life-threatening syndrome, administration of SSA significantly relieves the symptoms (in 80–90 % of patients) and lowers the concentration of vasoactive intestinal peptide (60–80 %). Biochemical improvement does not always correlate with clinical improvement, so a dose titration based on patient's clinical condition is necessary. Corticosteroids are used in patients with a life-threatening diarrhea which does not respond to the maximum doses of SSA [9, 27].

Glucagonoma

In 80–90 % of patients with glucagonoma, SSA treatment results in a visible clinical improvement of skin lesions due to necrolytic erythema, although the treatment is less effective in the management of diabetes and body weight loss. SSA reduces blood glucagon concentration in approximately 60 % of patients.

Zinc salts may be used in patients with glucagonoma to prevent further skin damage. Antithrombotic prophylaxis should be considered in all patients with NEN associated with an increased risk of thromboembolic complications [9, 28].

Somatostatinoma

Long-acting SSAs are effective in fighting the symptoms of hypersecretion in some cases of somatostatinoma [2].

28.4.4.2 Chemotherapy

Chemotherapy is considered in selected cases when clinical symptoms of functional GEP NENs have not been managed.

The effectiveness of the chemotherapy of well-differentiated or moderately differentiated GEP-NENs is different in case of neoplasms of pancreatic origin, compared to other locations [29].

In well-differentiated pancreatic NENs (PNENs), the highest activity in monotherapy (response rate: 20–40 %) is demonstrated by streptozotocin, doxorubicin, fluorouracil, dacarbazine, and temozolomide. Multidrug regimens are more effective than monotherapy regarding the response and survival rates (median survival: 15–30 months) [29, 30]. Combination of capecitabine and temozolomide demonstrated 70 % objective tumor responses, a median progression-free survival (PFS) of 18 months, and a 2-year survival of 92 % [3].

In palliative treatment of pancreatic neuroendocrine neoplasms, it is recommended to combine streptozotocin with doxorubicin and 5-fluorouracil (5-FU) and to use a two-drug chemotherapy in patients with a greater risk of complications or not qualifying for the treatment with anthracyclines [9].

28.4.4.3 Targeted Therapies

Another therapeutic option in the management of clinical hormonal syndromes involves targeted therapies using molecularly targeted drugs (sunitinib and everolimus). Sunitinib may be considered in the case of well-differentiated neoplasms (G1), and indications for everolimus comprise well- and moderately differentiated neoplasms (G1 and G2). Recommended dose: everolimus 10 mg/day or sunitinib 37.5 mg/day in the case of gastrinoma and other GEP NENs: everolimus 10 mg/day [9, 31, 32].

In patients with malignant insulinomas resistant to conventional treatment, using everolimus significantly improved glycemic control [33, 34].

However, using sunitinib and everolimus is associated with a number of unresolved problems (e.g., the order of use for various treatment methods, selection of patients for specific medications, the ability to anticipate the effectiveness and adverse events, as well as possibility of combining the treatment with other methods) [8, 31, 32, 35].

28.4.5 Radioisotope Treatment

Radioisotope therapy may be used for the treatment of hormonal syndromes.

Peptide receptor radionuclide therapy (PRRT) is now a recognized form of palliative treatment which enables stabilization or partial regression of the disease and less often a complete remission [36, 37].

In the case of malignant lesions, it is possible to conduct isotope treatment as a form of symptomatic treatment with temporary therapeutic response. In patients with diffuse gastrinoma and insulinoma, and with positive receptor scintigraphy results, eligibility for PRRT may be considered. However, this therapy requires studies involving a larger number of patients with functional tumors in order to establish its actual therapeutic value. It has been noted that gastrinomas respond to therapy faster, but early progression is relatively frequent [6, 7, 9].

PRRT is used mainly as palliative treatment, without any expectations regarding the impact on partial remission of the disease or patients' survival. If clinical symptoms recur, PRRT may be considered as the second-line therapy in case of disease progression, following stabilization or remission achieved with this method. Re-implementation of isotope treatment at the maintained expression of somatostatin receptors may prolong the patient's survival without a significant exacerbation of adverse reactions associated with this therapy. Radioisotope treatment is frequently combined with "cold" somatostatin analogs [9].

Among different forms of isotope treatment used in the management of the clinical symptoms of NENs, including PNENs, radioembolization with yttrium-labeled microspheres is used, in which the response rate is estimated at 52–66 % and the mean survival is 70 months [21, 38]. According to Ramage et al. [8], a complete remission of the disease is achieved in 2.7 % of patients and a partial remission in 60 %. Since there are no reports on the use of radioembolization in a larger group of patients, further studies in this field are necessary.

28.5 Summary

In functional NENs, the basic form of treatment is surgery. In the case of nonsurgical functional tumors, or tumors progressing after the surgical treatment, the therapy involves using "cold" somatostatin analogs. Isotope treatment may be considered as the second-line treatment. Chemotherapy may be introduced as the second-line treatment if the disease progresses, especially when somatostatin receptor expression is lost.

Targeted therapies using molecularly targeted drugs (mostly everolimus) may be used for symptomatic treatment of GEP-NENs.

If symptomatic refractory disease develops, this can be an indication to consider liver-directed therapies (embolization, chemoembolization, RFA, radiolabeled microspheres) which are reported to improve symptomatic control in many patients.

Management of hormonal symptoms is difficult, but fortunately with quite a number of new promising diagnostic and treatment options.

In order to determine best sequence and multimodality combinations of procedures to be applied, clinical trials need to be performed. Until then, choice must be based on decisions made by a multidisciplinary team of experts.

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Carcinoid Syndrome

29

Saadettin Kilickap and Suayib Yalcin

29.1 Background

Carcinoid tumor was firstly described as a group of small, benign-appearing tumor in the intestinal tract by Oberndorfer [1]. Carcinoid tumors are rare, slow-growing, well-differentiated endocrine tumors and mostly originated from three different sites including the bronchus, small intestine, or colon/rectum. They are malignancies originating from the enterochromaffin cells in the neuroendocrine system. The most common location of carcinoid tumors is the gastrointestinal tract. They account for 1.2–1.5 % of all gastrointestinal tract neoplasms [2, 3].

Carcinoid syndrome, which was described in 1953 by Rosenbaum [4], occurs in less than 10 % of patients with carcinoid tumors. It affects men and women equally. A number of symptoms including flushing, wheezing, diarrhea, asthma-like symptoms, abdominal pain, diaphoresis, skin lesions, hypotension, and valvular heart disease, especially pulmonary or tricuspid valve involvement, are principal features of the carcinoid syndrome. The signs and symptoms occur due to hormone and peptide and their metabolites secreting from the tumor cells into the bloodstream. Most cases only occur after metastatic spread to the liver [5].

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29.2 Symptoms

The most common signs and symptoms of carcinoid tumors are shown in Table 1.1. A number of chemical substrates secreting from neuroendocrine tumor cells such as serotonin and its metabolites account for the majority of symptoms of carcinoid syndrome. Symptoms are also triggered by physical exertion, stress, or foods and drinks such as chocolate, blue cheese, or red wine. Sometimes carcinoid syndromes are diagnosed as acute appendicitis due to tumor-related obstruction.

The two most common symptoms of carcinoid tumors are flushing and diarrhea. Both of them associate with releasing serotonin and its metabolites into the bloodstream. Flushing occurs in about 85 % of patients with carcinoid syndrome and usually is of sudden onset. It is observed on the skin of the face, neck, upper chest, and the nipple line, and the skin color varies from pink to red. Although the flush may be provoked by stress, exercise, some foods such as chocolate and cheese, drinking alcohol, certain drugs such as serotonin reuptake inhibitors, and catecholamines, sometimes it may happen spontaneously [6–8].

Foods that triggered the flush usually contain tyramines. Flushing episodes are usually brief and last a few minutes. The flushes observed with gastric and bronchopulmonary tumors tend to be more intense, and they may continue several hours or days but are not permanent. In these cases, the involvement of the upper trunk is uncommon.

Diarrhea occurs in 75–80 % of cases with carcinoid syndrome, and in 85 %, it is accompanied by flushing. The diarrhea observed in patients with carcinoid syndrome is a secretory diarrhea, and the episodes are up to 20 per day. The diarrhea is described as watery. Thus, it may cause fluid and electrolyte depletion. Abdominal cramps may be accompanied by a watery diarrhea. The diarrhea is unrelated to nutrition and may continue during fasting.

Other manifestations of carcinoid syndrome include cardiac and pulmonary symptoms and skin lesions. Cardiac disease in patients with carcinoid syndrome

Table 1.1 Carcinoid symptoms and their frequencies

Symptom	Frequency (%)
Flushing	85
Telangiectasia	25
Cyanosis	18
Pellagra	2–25
Diarrhea	75–80
Abdominal cramp	75
Bronchospasm	20
Valvular lesions	50
Right heart	40
Left heart	13
Asthenia	<5
Neuropsychiatric symptoms	<5
Musculoskeletal	<5

occurs in about 50 % of the cases and is usually characterized by valvular lesions [9, 10]. The lesions develop due to the formation of fibrotic plaques on the cardiac valves, especially on the right side. Retraction of the valves results in regurgitation and right-sided congestive heart failure [11]. The right-sided involvement in patients with carcinoid syndrome is predominant. Of these patients, 97 % have tricuspid insufficiency, 59 % tricuspid stenosis, 50 % pulmonary insufficiency, 25 % pulmonary stenosis, and 11 % (0–25 %) left-side lesions [12]. These patients may be present with tachycardia, palpitation, low blood pressure, and heart failure symptoms such as dyspnea, edema, and effusion.

The most common pulmonary symptoms in patients with carcinoid syndrome are wheezing, shortness of breath, and asthma-like symptoms developing due to bronchospasm [13]. They occur about 20 % of these patients. These symptoms occur due to hormone and peptide release such as serotonin and its metabolites or pulmonary fibrosis.

Carcinoid syndrome-related skin lesions include facial telangiectasis, pellagra-like skin lesions, and purplish areas. They occur about 2–25 % of the patients and appear on the face and upper lip. Other uncommon symptoms in patients with carcinoid syndrome include asthenia, irritability, agitation, aggression, arthritis, arthralgia, myopathy, fatigue, abdominal pain, and tenderness [14].

29.3 Causes

The etiology of carcinoid tumor is unclear. It occurs usually as sporadically but rarely to be hereditary. In small bowel carcinoid tumors, loss of arms 9p and 16q on chromosome 18 has been thought to be associated with these tumors [15]. Also, these tumors may be present as a part of multiple endocrine neoplasia.

Although most of carcinoid tumors are asymptomatic and silent tumor, they become symptomatic disease when carcinoid tumors metastasized to the liver. In this stage, serotonin and other vasoactive substances secreted by the tumor cells escape hepatic metabolization and reach to the systemic circulation. Thus, they are called as carcinoid syndrome. The principal chemical substrates which are responsible for these symptoms and signs are presented in Table 1.2. Some carcinoid tumors secrete certain and specific vasoactive amines and peptides. For example, gastric carcinoids produce usually histamine, but serotonin and adrenocorticotropic hormone (ACTH) are released by carcinoid tumors arising from the lung. Primary tumor location, peptides secreted from the tumor cells, and frequency of carcinoid syndrome were illustrated in Table 1.3.

29.4 Diagnosis

The first step for the diagnosis is medical history and physical examination. Patients should be questioned for symptoms and signs of carcinoid syndrome, when they have symptoms including unexplained recurrent flushing and severe diarrhea episodes. Secondly, to determine the levels of substances released by tumor cells,

Table 1.2 Substrates causing carcinoid tumors

Amines
Serotonin
5-Hydroxytryptophan
Norepinephrine
Dopamine
Histamine
Polypeptides
Gastrin
Corticotropin (ACTH)
Bradykinin
Pancreatic polypeptide (PP)
Somatostatin
Vasoactive intestinal peptide (VIP)
Substance P
Peptide YY
Neurokinin A and B
Motilin
Kallikrein
Growth hormone
Neuropeptide K
Glucagon
Beta-endorphin
Neurotensin
Chromogranin A

Table 1.3 Primary tumor location, peptides secreted from the tumor cells, and frequency of carcinoid syndrome

Location	Secretory products	Carcinoid syndrome
Foregut	5-Hydroxytryptophan histamine, multiple polypeptides	Rare
Midgut	Serotonin prostaglandins, polypeptides	Often
Hindgut	Variable	Rare

laboratory tests including blood and urine analyses are done. These laboratory tests include the following.

29.4.1 5-HIAA

5-Hydroxyindoleacetic acid (HIAA), a metabolite of serotonin, is extensively used for the diagnosis of the carcinoid syndrome. To measure 24-h urinary excretion of 5-HIAA is the most sensitive test and should be used as an initial diagnostic test. Its

sensitivity and specificity is over 90 % for the carcinoid syndrome [16]. To avoid false-positive elevation of urinary excretion of 5-HIAA, patients should prohibit intake of tryptophan- and serotonin-rich foods such as bananas, walnuts, pecans, avocados, and pineapples for 3 days prior to urine collection and discontinue certain drugs containing serotonin reuptake inhibitors, acetaminophen, salicylates, and L-dopa [17]. Reasons of false-positive and false-negative 5-HIAA urinary excretion are shown in Table 1.4. Urinary excretion of HIAA is more sensitive in patients with primary midgut than those with hindgut and foregut which rarely secreted serotonin.

29.4.2 Serotonin

Measurement of serum levels and urinary excretion of serotonin may be beneficial for the diagnosis. The benefit is higher in patients with midgut carcinoid tumor secreting serotonin, but its benefit is limited for the other carcinoids. Blood serotonin concentration may be measured by using different methods, but there is no described standard method to measure serum serotonin level.

Table 1.4 Reasons of false-positive and false-negative 5-HIAA urinary excretion

False positive	False negative
Drugs	*Drugs*
Phenobarbital	Corticotrophin
Phentolamine	Levodopa
Nicotine	MAO inhibitors
Acetaminophen	Phenothiazines
Fluorouracil	Aspirin
Melphalan	Isoniazid
Guaifenesin	Ethanol
Ephedrine	Streptozotocin
Phenacetin	Methyldopa
Methamphetamine	Heparin
Foods and drinks	
Pineapples	
Bananas	
Walnuts	
Avocados	
Tomatoes	
Pecans	
Hickory nuts	
Kiwi fruit	
Caffeine	

5-HIAA 5-hydroxyindoleacetic acid; MAO monoamine oxidase

29.4.3 Chromogranin A

Chromogranin A, a group of acidic glycoprotein, is stored and released from chromaffin granules of neuroendocrine cells. The measurement of serum chromogranin A is often used to diagnose well-differentiated neuroendocrine tumors. The serum level of chromogranin A is associated with tumor burden [18, 19]. As a tumor marker, chromogranin A is moderately sensitive and nonspecific [17]. Foods and drugs such as proton pump inhibitors may affect serum chromogranin concentration [20]. Due to a nonspecific marker, plasma chromogranin A is useful with urinary excretion of 5-HIAA. For patients who have elevated plasma chromogranin A at the baseline, it is an appropriate marker for detection of recurrence and monitoring of response to different treatments.

29.5 Imaging

Imaging studies including computed tomography, magnetic resonance imaging, and scintigraphy are available for carcinoid tumors. For diagnosis of carcinoid tumors and related syndromes, the first step for imaging tests is computed tomography of the abdomen. Computed tomography is usually recommended to localize the site of primary tumor and to assess the extension of tumor metastases. Carcinoid tumor and its metastasis may appear as a hypervascular lesion. Carcinoid tumor is isodense with liver on a noncontrasted imaging, whereas it appears as a hyperdense lesion at the early arterial phase after the injection of intravenous contrast. Carcinoid tumors originating in the small bowel may not be seen on computed tomography because of their small size.

Magnetic resonance imaging (MRI) is a limited advantage for diagnosing and staging carcinoids and related syndromes [21]. MRI should be used for detection of liver metastases of carcinoid tumors because its sensitivity for liver metastasis is higher than computed tomography [22, 23].

Somatostatin receptors have been expressed in patients with carcinoid tumor. Octreotide is a somatostatin analogue, and a radiolabeled octreotide scintigraphy with indium-111 DTPA, called as OctreoScan or somatostatin receptor scintigraphy, is usually used to localize the site of primary tumor and to assess response to therapy and tumor progression or recurrence for carcinoid tumors. The sensitivity and specificity of OctreoScan is higher for the tumors which are highly expressed somatostatin receptors, especially well-differentiated neuroendocrine tumors. However, its sensitivity is lower for lesions smaller than 1.5 cm in diameter [23]. Metaiodobenzylguanidine (MIBG) scintigraphy which is an alternative to Octreoscan for the diagnosis of carcinoid tumor may also be useful to localize the disease. MIBG scans have been found to be positive in about two thirds of all patients with carcinoid tumors [24].

Functional PET imaging techniques including 18-F-dihydroxy-phenyl-alanine (18F-DOPA), 11-C-5-hydroxytryptophan (11-C-5-HTP), and 68-Ga-DOTA- D-Phe^{1}-Tyr^{3}-Octreotide (68-Ga-DOTATOC) have recently been used for the diagnosis and follow-up of carcinoid tumors. These techniques have higher sensitivity for detection of small tumors. However, 68-Ga-DOTATOC PET may be more sensitive than OctreoScan for detection of the bone and lung carcinoid tumors [25]. It is not clear whether functional PET imaging techniques should be preferred over somatostatin receptor scintigraphy or they should be used for patients whom OctreoScan is negative [26, 27].

29.6 Treatment

The first treatment of carcinoid tumor is surgical resection of the tumor if it is completely removed. For patients whose primary tumor cannot be entirely removed, surgical debulking may be useful. This may reduce the signs and symptoms of carcinoid syndrome. Hepatic resection may be useful for symptomatic palliation and should be considered in patients who have potentially resectable liver metastasis. The benefit of hepatic resection is unclear for patients who have widespread extrahepatic metastases. However, patients with the carcinoid syndrome should be treated with systemic therapies and/or tumor-targeted local therapies including chemo- or radioembolization techniques because the tumor has spread to the liver.

Somatostatin receptors are present in 70–95 % of neuroendocrine tumors [28]. They are extensively expressed by carcinoid tumors, whereas this expression is lower in poorly differentiated tumors. Somatostatin inhibits the release of a majority of bioactive amines by binding to its receptors. Synthetic analogues of somatostatin such as octreotide and lanreotide are widely used agents to improve the symptoms of patients with carcinoid syndrome. Using somatostatin analogues, the symptoms are reduced in over 80 % of these patients [29]. Patients should be treated with monthly intramuscular long-acting octreotide, is a depot form of the drugs, at a dose of 20–30 mg [30]. However, subcutaneous short-acting octreotide should be given as supplementation at the initial therapy or should be used in the treatment of symptomatic patients. Lanreotide, another long-acting somatostatin analogue called as Somatuline Autogel, is available at a dose of 60–120 mg as subcutaneous deep injection for every 4 weeks. Although the efficacy and tolerability of lanreotide are similar with octreotide for treatment of carcinoid syndrome, there are no randomized studies comparing the efficacy of octreotide versus lanreotide in carcinoid syndrome patients [31–36]. The antiproliferative effect of somatostatin analogues has also been shown. The objective response rate of these drugs is less than 5 %. But, they are very effective drugs for symptomatic control of the carcinoid syndrome, and they also improve the biological marker response. Although there are several clinical studies that indicate that higher doses of long-acting somatostatin analogues may be useful, the guidelines have not yet been recommended [37–39].

Interferon stimulates T cell function. Thus, it inhibits angiogenesis and tumor growth. Although an interferon has lower antitumor effect, it has been used to control symptoms of carcinoid syndrome [40]. Symptom control is achieved by 50 % due to be reduced hormonal hypersecretion.

The liver is the most common metastatic site of carcinoid tumors. And the liver metastasis accounts for the symptoms. The use of liver-targeted therapies such as radiofrequency ablation and transhepatic chemoembolization has been considerably increased. Radiofrequency ablation is a minimal invasive treatment delivering heat through a thin needle into the tumor in the liver. Thus tumor cells are destroyed. Cutting off via arterial catheterization the blood supply to tumors that have metastasized to the liver provides symptom control. Infusion of chemotherapy via hepatic arteries also improved symptoms as well as its antitumoral effect.

These therapy options play a vital role for symptomatic improvement. They are repeated in the treatment of the recurrent disease. Similarly, radioembolization using 90Y-labeled resin may be beneficial to reduce symptoms [41]. These procedures should be performed only in experienced medical centers.

Although cytotoxic chemotherapy is very effective in treatment of poorly differentiated neuroendocrine tumors, its benefit is limited in treatment of well-differentiated tumors. Because these tumors grow slowly, response rate with conventional cytotoxic chemotherapy is less than 10 % in clinical studies. Targeted therapies are novel therapeutic agents used for cancer therapy. A number of targeted therapy agents including sunitinib, bevacizumab, sorafenib, and everolimus have recently been used in the treatment of carcinoid tumors. Antitumoral effect of targeted therapies is lower in carcinoid tumor, but symptomatic improvement and reduction in biochemical response are achieved.

Radiolabeled somatostatin analogues such as ^{90}Y-DOTA tyr3-octreotide, ^{90}Y-edotreotide, or ^{177}Lu-DOTA^{0},Tyr^{3}-octreotate could be beneficial in the treatment of carcinoid tumors expressing somatostatin receptor. Most of the patients treated with radiolabeled somatostatin analogues have been observed with higher response rate and have achieved symptomatic improvement [42].

29.7 Management of Carcinoid Crisis

Carcinoid crisis is characterized by a severe episode of flushing, low blood pressure, arrhythmias, confusion, and shortness of breath and can be a life-threatening form of carcinoid tumors. The crisis may be provoked by tumor manipulation, anesthesia used during surgery, and local therapies and may be life-threatening [43, 44]. Octreotide infusion should be used to prevent and to treat the crisis [45]. Octreotide is administered as a bolus dose of 500–1,000 mcg or a continuous intravenous dose of 50–200 mcg/h [37].

Other symptomatic therapies of carcinoid syndrome include loperamide, ketanserin, and diphenoxylate for treatment of diarrhea, bronchodilators for bronchospasm, serotonin receptor antagonists or somatostatin analogues, and histamine H_1 and H_2 receptor antagonists such as diphenhydramine and cimetidine.

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Somatostatin Analogs and Interferon in the Treatment of Neuroendocrine Tumors

30

Jonathan Strosberg

30.1 Introduction

Biotherapy for neuroendocrine tumors (NET) emerged in the 1980s when somatostatin analogs (SSAs) and interferons (IFNs) were investigated in small series of patients with malignant carcinoid syndrome. While early clinical trials focused on palliation of hormonal symptoms, subsequent studies have been designed to assess the inhibitory effects of SSAs and IFNs on tumor progression. The recognition that biological therapies may be synergistic has led to several randomized studies investigating IFNα in combination with SSAs. This chapter will review the mechanism of action and key clinical trials investigating SSAs and IFNs in advanced, well-differentiated NETs.

30.2 Somatostatin Analogs

Native human somatostatin is a cyclic peptide hormone that exists in two natural forms consisting of 14 and 28 amino acids [1, 2]. It is widely distributed in multiple tissues including the central nervous system, pancreas, and gastrointestinal tract. It has been characterized as a universal endocrine "off switch" due to its inhibitory paracrine, exocrine, and endocrine effects. In the digestive tract, somatostatin inhibits multiple intestinal functions such as bowel motility and absorption [3]. Somatostatin also inhibits secretion of other pancreatic and intestinal hormones including gastrin, glucagon, and serotonin. The physiological actions of somatostatin are mediated through five subtypes of somatostatin receptors (sst_1-sst_5) belonging

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to a family of G-protein-coupled receptors with 7 transmembrane domains [4]. Approximately 80 % of well-differentiated NETs express high levels of somatostatin receptors, particularly receptor subtypes 2 and 5 [5].

Native somatostatin is rapidly cleared in the circulation due to enzymatic cleavage sites, limiting its clinical utility [5, 6]. In order to improve somatostatin's pharmacokinetics, synthetic SSAs have been developed by truncating the polypeptide chain while retaining somatostatin receptor binding sites [7, 8]. Two commercially available analogs, octreotide and lanreotide, are cyclic peptide SSAs which bind with high affinity to sst_2 and with moderate affinity to sst_3 and sst_5. SSAs have had a profound impact on neuroendocrine tumor treatment. They were initially developed as antisecretory agents designed to control symptoms such as flushing and diarrhea associated with the carcinoid syndrome. In recent years, high-level data has emerged demonstrating that SSAs can also control neuroendocrine tumor growth [9–11].

The initial formulation of octreotide was as a subcutaneously administered drug with an elimination half-life of approximately 2 h. A landmark phase II trial evaluated octreotide 150 mcg t.i.d. in 25 patients with malignant carcinoid syndrome [12]. Rapid palliation of flushing and diarrhea was reported in 22 patients (88 %), associated with major reductions of urine 5-HIAA in 18 patients (72 %). Multiple subsequent studies have confirmed that both octreotide and lanreotide are effective for palliation of the carcinoid syndrome [13–15]. A crossover study of 33 patients comparing octreotide to lanreotide demonstrated equivalency in control of flushing and diarrhea as well as improvement in quality of life [13]. Both drugs are also effective at controlling hormonal symptoms associated with pancreatic neuroendocrine tumors such as VIPomas, glucagonomas, and gastrinomas [16–20]. Patients with advanced insulinomas may also respond to SSAs [21]; however, there is potential for exacerbation of hypoglycemia due to inhibition of the counter-regulatory hormone glucagon [22].

During the past decade, a long-acting repeatable (LAR) depot formulation of octreotide (Sandostatin LAR®) has been available, which releases drug slowly by cleavage of a polymer ester linkage and enables monthly intramuscular dosing. A randomized phase III study compared subcutaneous octreotide every 8 h to octreotide LAR 10, 20, or 30 mg every 4 weeks [23]. Control of diarrhea was comparable in each of the four study arms; however, control of flushing was least effective in the 10 mg LAR cohort. Based on this trial, the recommended starting dose of octreotide LAR was set at 20 mg every 4 weeks with subsequent dose titration suggested for symptom control [24]. A second somatostatin analog, lanreotide, was licensed in Europe in 1998 for the treatment of hormonal symptoms-advanced NETs. A long-acting depot formulation of lanreotide (Somatuline Autogel®) has also been developed as a deep subcutaneous injection. It is supplied as a prefilled syringe consisting of a viscous aqueous solution available at doses of 60, 90, and 120 mg every 4 weeks.

Side effects of SSAs are generally mild and include nausea, bloating, and steatorrhea. Most side effects resolve over time. Malabsorptive symptoms caused by SSAs can often be controlled with pancreatic enzyme supplementation. Inhibitory effects of SSAs on gallbladder function increase the long-term risk of gallstones.

Therefore, prophylactic cholecystectomy should be considered in patients undergoing abdominal surgery [25]. Other reported side effects include bradycardia, pancreatitis, and hyperglycemia. However, it is important to note that the only randomized trial of octreotide LAR versus placebo did not identify any increase in serious adverse events [26].

30.2.1 Treatment and Prophylaxis of the Carcinoid Crisis

Carcinoid crisis is defined as an episode of severe hypotension caused by an acute release of serotonin and other vasoactive substances into the circulation. It occurs most commonly in the operative setting since triggers include general anesthesia, epinephrine, and physical manipulation of tumors. Patients with carcinoid syndrome or elevated urine 5-HIAA who undergo an invasive procedure should receive a supplementary dose of octreotide 250–500 mcg subcutaneously or intravenously 1 h prior to the procedure [27]. Hypotension occurring in the setting of carcinoid crisis can be managed with bolus intravenous doses of octreotide 500–1,000 mcg until control of symptoms is achieved. Alternatively, continuous intravenous infusion of 50–10 mcg/h may be given after a bolus dose [28].

30.2.2 Antiproliferative Effects of SSAs

In recent years, strong evidence has emerged that SSAs can inhibit the growth of neuroendocrine tumors. Antitumoral activity, also known as the "antiproliferative effect" of SSAs, can occur via direct and indirect mechanisms [6, 9, 29]. Direct mechanisms involve the binding of SSAs to somatostatin receptors on tumor cells leading to activation of intracellular signaling transduction pathways. Multiple in vitro studies using cell lines transfected with somatostatin receptors indicate that all receptor subtypes (sst1-5) can participate in inhibition of cellular proliferation and that specific receptor subtypes (sst2,3) mediate apoptosis [30, 31]. Indirect antiproliferative mechanisms include inhibition of circulating growth factors such as vascular-endothelial growth factor (VEGF) and insulin-like growth factor (IGF) [32], as well as inhibition of tumor angiogenesis through interaction with somatostatin receptors on endothelial cells [33].

30.2.2.1 Direct Antiproliferative Mechanisms

In vitro studies in various cell lines, including breast, pancreatic, and prostate carcinomas, demonstrate that SSA binding to somatostatin receptors activates a subset of phosphotyrosine phosphatases (PTPs), including SHP-1 and SHP-2, leading to cell cycle arrest [34, 35]. In a pituitary adenoma cell line, activation of sst_2 arrested cell proliferation via stimulation of SHP-1 [36]. SHP-1 has also been implicated in sst_3-dependent apoptosis in transfected Chinese hamster ovary (CHO) cells [37]. The precise effects of SSAs on mitogen-activated protein kinase (MAP-kinase) signaling are uncertain since both inhibition and stimulation of this pathway have been

linked to the antiproliferative effects of SSAs. In a glioma cell line, the receptor-like PTP, PTPeta, mediated the antiproliferative effects of somatostatin through inhibition of ERK1/2 [38]. In another study, somatostatin activated MAP-kinase in sst_1-expressing CHO cells, thereby stimulating the cyclin-dependent kinase inhibitor p21 and inhibiting cell proliferation [39].

30.2.2.2 Indirect Antiproliferative Mechanisms

Suppression of tumor growth may occur through inhibition of various circulating growth factors, including growth hormone (GH) [40]. Inhibition of GH is thought to be mediated through sst_2 and sst_5, which are expressed in the anterior pituitary [41]. Octreotide has been shown to suppress circulating levels of IGF-1, both by suppressing pituitary GH and by inhibiting IGF-1 production in the liver [32, 42]. SSAs also appear to have an inhibitory effect on tumor angiogenesis [33, 43]. Octreotide has been shown to inhibit proliferating endothelial cells that express sst_2 and sst_5 [44]. The primary antiangiogenic mechanism may be suppression of endothelial nitric oxide release [45]. Inhibition of circulating vascular-endothelial growth factor (VEGF) appears to also play a role in suppression of peritumoral vessel growth [46].

30.2.3 Clinical Evidence of the Antiproliferative Effect

Multiple phase II and retrospective studies have investigated the effects of SSAs on tumor progression in patients with GEP-NETs. In general, these studies have documented low rates of objective radiographic response (<5 %) but high rates of disease stabilization [11, 14, 47–50]. One of the first prospective studies to report on the antiproliferative effects of SSAs in GEP-NETs was conducted by the German Sandostatin Study Group [51]. In this study, 103 patients with GEP-NETs were treated with octreotide 200 μg t.i.d. until evidence of radiographic progression. Among patients who had disease progression documented at treatment outset, 37 % experienced disease stability lasting at least 3 months, whereas among patients with stable disease at baseline, disease stability lasting at least 12 months was documented in 54 % of patients. Another phase II clinical trial testing octreotide in 34 patients with progressive metastatic NETs reported disease stabilization rate in 50 % of patients lasting a median of 5 months [49].

The antiproliferative activity of lanreotide was tested in several phase II clinical trials. In a study of 55 patients with GEP-NETs, 7 % of 31 assessable patients achieved a partial response to lanreotide, and 81 % experienced disease stability [17]. In another study of 46 patients with GEP-NETs, 2 patients (4 %) achieved an objective radiographic response, while 19 patients (41 %) had stable disease for a mean duration of 9.5 months [11]. In one study of patients with progressive tumors, participants received either octreotide LAR 30 mg or lanreotide SR 60 mg. Among 31 assessable patients, 14 (45 %) achieved disease stability [52]. In multivariate analysis, tumor control appeared to be significantly higher in small bowel NETs compared to pancreatic NETs. Extrahepatic metastases were associated with a poorer prognosis.

Definitive evidence of the antiproliferative effect of SSAs emerged with the PROMID trial, the first randomized placebo-controlled study performed in the field of neuroendocrine tumors [26]. Eighty-five patients with metastatic well-differentiated NETs originating in midgut were randomized to receive either octreotide LAR 30 mg or placebo. Patients with severe uncontrolled carcinoid syndrome were excluded. The primary endpoint was time-to-tumor progression. Median time-to-tumor progression (TTP) was 14.3 months in the octreotide LAR 30 mg group versus 6.0 months in the placebo group ($P = 0.000072$). Serious adverse events were nearly evenly balanced (11 patients in the octreotide LAR 30 mg arm and 10 patients in the placebo arm). On multivariate analysis, patients with resected primary tumor and low hepatic tumor burden (<10 %) appeared to benefit most significantly from octreotide treatment versus placebo. The small number of deaths in each treatment arm and the high rate of post-trial crossover precluded any analysis of differences in overall survival. Based on the PROMID data, octreotide LAR therapy is now considered an appropriate first-line treatment for patients with metastatic midgut NETs that are not surgically resectable, regardless of presence or absence of the carcinoid syndrome.

Non-midgut NETs tend to progress more aggressively than midgut NETs and are less likely to express somatostatin receptors. It is still uncertain whether SSAs are appropriate for use as antiproliferative agents in non-midgut NETs. The CLARINET study (clinical trial on nonfunctioning enteropancreatic endocrine tumors) is intended to address this question by randomizing patients with hormonally non-functioning GEP-NETs to treatment with depot-lanreotide versus placebo. The target enrollment of 200 patients has been achieved, and results are pending [53].

30.2.4 Above-Label Doses of SSAs

While the highest dose of octreotide LAR tested prospectively in patients with metastatic NET has been 30 mg every 4 weeks, higher doses and/or frequencies are often prescribed for patients with suboptimal control of hormonal syndromes. Data supporting this practice derive primarily from retrospective studies. In a longitudinal database conducted by the National Comprehensive Cancer Network (NCCN) encompassing 7 cancer centers, 40 % of carcinoid tumor patients and 23 % of pancreatic NET patients who received SSAs underwent dose escalations beyond 30 mg every 4 weeks [54]. The most common dose/frequency combinations were 40 mg every 4 weeks and 40 mg every 3 weeks. In one retrospective institutional study, 100 patients out of a total of 338 with metastatic midgut NETs (30 %) received octreotide LAR at doses and/or frequency above the standard label dose of 30 mg every 4 weeks [55]. Indications for dose increase were worsening carcinoid syndrome (60 patients), radiographic progression of tumors (33 patients), and rising urine 5-HIAA (6 patients). Among patients whose doses were increased for refractory carcinoid syndrome, 34 patients (62 %) experienced improvement in diarrhea, and 28 patients (56 %) experienced improvements in flushing. A single prospective trial investigated octreotide LAR 30 mg every 3 weeks among 28 patients with disease

progression on 30 mg every 4 weeks [56]. Complete and partial control of clinical symptoms were observed in 40 and 60 % of treated patients, respectively.

30.2.5 Novel Somatostatin Analogs

Pasireotide is a novel somatostatin analog which binds avidly to four of the five somatostatin receptors ($sst_{1,2,3}$ and sst_5) [57]. Compared with octreotide, pasireotide has a 40-, 30-, and 5-fold higher binding affinity for sst_5, sst_1, and sst_3, but a 2.5-fold lower affinity for sst_2. An open-label trial evaluated the activity of short-acting subcutaneous pasireotide in 45 patients with carcinoid syndrome whose symptoms (flushing and diarrhea) were suboptimally controlled with octreotide LAR [58]. In this trial, 27 % of patients experienced some degree of improvement in their symptoms. However, a randomized phase III trial of long-acting intramuscular pasireotide vs. octreotide LAR in patients with refractory carcinoid syndrome was halted at interim analysis for futility [59]. The main toxicity observed with pasireotide is hyperglycemia, caused by suppression of insulin secretion which is mediated through the binding of pasireotide to sst_5. In the phase III trial of pasireotide vs. octreotide, Grade 3 or 4 hyperglycemic events occurred in 11 % of patients in the pasireotide arm vs. 0 % in the octreotide arm. Clinical trials are currently testing the antiproliferative effects of pasireotide in advanced GEP-NETs.

30.3 Interferon

Interferon-α (IFNα) was first introduced to treatment of midgut NETs in 1983 when a small study reported symptom palliation in 6 patients with carcinoid syndrome treated at doses of three to six million units daily [60]. Subsequent single-arm studies have reported objective response radiographic rates in the range of 5–10 %, with higher rates of disease stabilization and palliation of hormonal symptoms [10, 61, 62]. More recently, randomized studies have offered evidence for the antiproliferative activity of interferon combined with somatostatin analogs.

30.3.1 Mechanisms of Action

Interferons are a complex array of cytokines with antiviral and antitumoral properties. They are divided into several classes. Type I interferons consist of IFNα (leukocyte interferon) and IFNβ (fibroblast interferon) which bind to a unique interferon receptor [63]. IFNs activate STAT (signal transducer and activator of transcription) complexes including the Janus-kinase-STAT (JAK-STAT) signaling pathway leading to signaling cascades involving the tumor cell and the host immune system [64]. Immune-related mechanisms of action include induction of major histocompatibility complex (MHC) type I expression in tumor cells which leads to enhanced immune surveillance. Other effects of IFNs include promotion of dendritic cell

proliferation and differentiation and alterations of T-cell CD4:CD8 ratios [65]. Angiogenesis inhibition appears to be another indirect mechanism of action. In a study of neuroendocrine tumor xenografts in nude mice, IFNα treatment reduced microvessel density and suppressed vascular-endothelial growth factor (VEGF) gene expression [66].

IFNs may also exert a direct antiproliferative effect on tumor growth through induction of cell cycle arrest; however, the precise mechanism remains elusive. In one in vitro study of lymphoid cell lines, G0/G1 cell cycle arrest was mediated through induction of the cyclin-dependent kinase inhibitors (CKIs) p21 and p15 and the expression of p27 [67]. However, another in vitro study of neuroendocrine tumor cells demonstrated that IFNα delayed progression from S-phase to G2 due to reduction of cyclin B levels and inhibition of Cdc2 kinase activity [68]. A third study demonstrated that IFNα induced apoptosis through activation of caspases-1, -2, -3, -8, and -9 in a variety of malignant cell lines [69].

30.3.2 Studies of IFNα Monotherapy

An early study of IFNα three to six million units/day reported objective tumor responses in 4 of 36 patients (11 %) with advanced carcinoid tumors and major reductions in urine 5-HIAA in 16 patients (53 %) [70]. Another study of 22 patients with pancreatic NETs reported high rates of symptomatic and biochemical responses [71]. A more recent phase II study of 49 patients with advanced NETs evaluated IFNα-2a at a daily dose of six million units daily for 8 weeks followed by the same dose 3 times weekly thereafter [72]. Among 34 patients with carcinoid tumors, partial radiographic responses were observed in 4 patients (12 %), and major improvements in carcinoid syndrome were reported in 64 % of cases. The most frequently observed side effects were fever, flu-like symptoms, and leukopenia. In the United States, Moertel et al. treated 27 patients with metastatic carcinoid tumors with IFNα at a high dose of 24 million units/m^2 [73]. Despite an objective radiographic response rate of 20 % and major urine 5-HIAA reductions in 39 % of patients, the authors concluded that any therapeutic gains seemed to be outweighed by severity of side effects which included fevers/chills, anorexia, weight loss, leukopenia, and liver function abnormalities.

30.3.3 Studies of IFNα Combined with SSAs

Studies evaluating the combination of IFNα and SSAs emerged in the 1990s, based on in vitro data demonstrating that interferons can upregulate the expression of somatostatin receptors. One trial of patients with carcinoid syndrome who had become refractory to octreotide reported symptomatic improvement in 49 % of patients after addition of IFNα [74]. Another study reported disease stabilization in 14 of 21 patients in whom IFNα was added to preexisting octreotide therapy [75]. A more recent trial investigated pegylated IFN (PEGIFN), a long-acting

formulation which enables weekly dosing. Of 17 patients who progressed on SSA therapy, inhibition of tumor growth was observed in 13 patients. The side effect profile of PEGIFN appeared to be superior to that of IFNα [76].

Several randomized clinical trials have investigated SSAs alone versus in combination with IFNα. One study of 68 patients with metastatic midgut carcinoid tumors who had undergone prior hepatic embolization evaluated octreotide alone versus octreotide combined with IFNα three million units administered five times per week [77]. The 5-year survival in the combination arm was 57 % versus 37 % in the octreotide monotherapy arm. The overall survival results were not statistically significant ($p=0.13$), possibly due to the small number of patients. However, the hazard ratio of 0.28 for tumor progression was highly significant ($p=0.008$). Another randomized study of 109 gastroenteropancreatic NET patients compared octreotide alone versus octreotide combined with alpha-interferon 4.5 million units administered three times per week [78]. The median survival was prolonged in the combination arm (51 vs. 35 months), but results did not achieve statistical significance ($p=0.38$). Objective response rates in both arms were less than 6 %. A three-arm trial of 80 therapy-naïve patients with advanced carcinoid and pancreatic NETs compared subcutaneous lanreotide alone to IFNα five million units three times weekly, or the combination of the two drugs [79]. In this trial, time-to-tumor progression was similar in all three arms. Among 11 patients who were treated with lanreotide monotherapy and crossed over to combination therapy, 1 patient showed measurable inhibition of tumor growth.

Given the underpowered nature of these randomized trials, it is difficult to draw any definitive conclusions regarding the effects of alpha-interferon on overall survival or PFS. The most compelling data favoring IFNα is in midgut NETs where data supports its use in combination with SSAs for palliation of the carcinoid syndrome and inhibition of tumor growth. Enthusiasm for IFNα is somewhat tempered by potential toxicities which include chronic fatigue, flu-like symptoms, and depression. Other abnormalities in laboratory values can include anemia, thrombocytopenia, leukopenia, and increases in liver enzymes. However, it is important to note most neuroendocrine trials have studied low doses of IFNα which are fairly tolerable. Some experts recommend titration of IFNα dose aiming at reduction of leukocyte count to 3×10^9/L [80].

Conclusion

Biotherapy plays a vital role in neuroendocrine tumor treatment, both for control of hormonal syndromes and inhibition of tumor growth. SSAs are exceptionally well tolerated and are appropriate as first-line systemic therapy for most patients with advanced well-differentiated midgut NETs. In this setting, they have clearly demonstrated efficacy at palliating the carcinoid syndrome as well as prolonging time to progression. SSAs also have significant activity in other rare hormonal syndromes such as VIPoma and glucagonoma syndromes. Their role in non-midgut NETs remains to be defined, but emerging evidence suggests that they can inhibit tumor growth in low-proliferative tumors.

IFNα can also inhibit tumor growth and control hormonal symptoms, but its use is somewhat more limited by toxicity. It appears to be most highly active in well-differentiated midgut NETs where trials have demonstrated a significant improvement in PFS in combination with SSA. Most guidelines suggest that IFNα be considered as a second-line agent in patients with refractory carcinoid syndrome or tumor progression on SSA; however, consensus has yet to be reached on optimal dose and formulation. Even as progress is made in the targeted therapy of advanced NETs, it is important to regard IFNα as a relevant treatment option for long-term control of tumor growth and symptom control.

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Chemotherapy

31

Barbro Eriksson

31.1 Introduction

31.1.1 Epidemiology and Classification

Neuroendocrine neoplasms (NENs) are relatively rare and constitute a heterogenous group of tumors with a wide range of morphologic, functional, and biological behavior. According to the North American Surveillance, Epidemiology, and End Results (SEER) database, NENs occur most frequently in the gastrointestinal (GI) tract (60.9 %) with the second most common location in the bronchopulmonary tract (27.4 %), followed by less frequent locations such as the ovaries, testes, hepatobiliary system, and pancreas [1]. The incidence of GEP-NENs has been increasing over the last decades, and because of the long survival of patients with NENs compared to other GI cancers, they are now the second most prevalent GI cancer after colon cancer [2].

The major negative factors which affect patient survival are the presence and extent of metastases (tumor–node–metastasis (TNM) stage) [3, 4], degree of differentiation (well or poorly differentiated), Ki-67 index, tumor size, angioinvasion, slope of tumor growth, and site of the primary tumor. The pancreas, right hemicolon,, and small intestine are the most frequent primary tumors associated with distant metastases at initial diagnosis. In European and US referral centers, up to 77 % of pancreatic NENs and 91 % of small intestinal NENs present distant metastases, i.e., have TNM stage IV at diagnosis [5, 6]. The new World Health Organization (WHO) classification from 2010 categorizes NENs of the gastrointestinal tract according to Ki-67 index into well-differentiated neuroendocrine tumors (NETs)

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G1 (Ki-67 ≤ 2 %) and G2 (Ki-67 3–20 %) and neuroendocrine carcinomas (NECs) G3 (Ki-67 > 20 %) [7]. Ki-67 is a proliferation antigen, present in G1, S, G2, and M phases of the cell cycle, and is probably the best available prognostic marker. For pancreatic NETs a Ki-67 cutoff level of 5 % has been suggested as a better prognostic marker than 2 % [8]. Bronchial NENs are classified according to WHO [9] into typical and atypical "carcinoids": typical should have <2 mitoses per 2 mm^2 (10 high-power fields (HPF)) and no necroses, whereas atypical have 2–10 mitoses per 2 mm^2 (10 HPF) and necroses present. A comparison between mitotic count and Ki-67 index in bronchial NETs has not been performed yet.

The discussion of this chapter will focus on chemotherapy in well-differentiated NETs G1 and G2. Poorly differentiated tumors or NEC will be discussed in another chapter (see Chap. 26).

31.1.2 Therapeutic Considerations

Therapeutic options for the management of metastatic disease include surgical, locoregional procedures, nuclear medicine modalities, and medical treatment including chemotherapy, somatostatin analogs (SSA), interferon, and new molecular targeted therapies, mainly everolimus and sunitinib. Therapeutic decisions should be made by a multidisciplinary team consisting of specialists in oncology, endocrinology, gastroenterology, endocrine surgery, pathology, radiology, and nuclear medicine. The initiation of chemotherapy can be controversial because of the slow growth of the tumors and the fact that only palliative treatment is available. When palliative treatment is offered, it is extremely important to weigh the expected side effects and quality of life against the expected response to treatment. Because of the heterogeneity and varying degree of aggressiveness, there is no standard approach to medical or chemotherapeutic management. In addition to the above mentioned negative factors affecting survival, also functionality, performance status, and age of the patient should be considered in the therapeutic decision. Most of the published studies describing the use of chemotherapy in NEN patients have been single-center, retrospective series, using inconsistent response criteria, including tumors with different primary sites and histopathologies.

31.2 Response Criteria

In earlier studies of chemotherapeutic agents, the so-called WHO criteria were used to evaluate the efficacy [10]: *complete remission (CR)* was defined as a complete disappearance of tumor lesions on conventional imaging; *objective or partial remission (OR or PR)* was defined as a decrease of more than 50 % in tumor size. Tumor size was defined as the product of the maximum diameter and its perpendicular. *Stable disease* was defined as a decrease of less than 50 % in tumor size and *progressive disease (PD)* an increase of more than 25 % in tumor size or the appearance of new metastases. In earlier studies, in addition, a reduction by >50 % of biochemical markers was reported as part of the overall response (OR).

In most clinical trials currently performed, the so-called Response Evaluation Criteria in Solid Tumors (RECIST) are being employed [11]: *partial remission (PR)* is defined as a reduction of at least 30 % in the tumor load estimated as the sum of the longest diameters of all measurable lesions, taking as the reference the baseline sum of the longest diameter. *Progressive disease (PD)* is defined as at least a 20 % increase in the tumor load, taking as the reference the smallest sum of longest diameter recorded since the start of treatment. *Stable disease (SD)* is defined as a less than 30 % decrease or less than 20 % increase in the sum of the longest diameters.

If one compares the WHO and RECIST criteria, PR with both methods will require approximately the same tumor volume reduction, whereas PD will require a much greater increase in tumor volume with RECIST than with WHO criteria. This fact should be kept in mind when results of clinical trials are compared.

With the introduction of new biological and molecular targeted therapies, which exert cytostatic rather than cytotoxic effects on tumors, the concept of "disease control" or progression-free survival (PFS) is becoming accepted, whereby stable disease or a delay in progression is considered as a positive response to treatment. Using disease control or progression-free survival (PFS) as a criterion/primary end point should require demonstration of disease progression within 3–12 months of entry for inclusion into phase II and III trials [12]. Treatment may also produce symptomatic relief and reduction in hormone levels, and there is an ongoing debate whether biochemical response should also be an end point. In addition, health-related quality of life should be assessed during treatment, possibly as part of a so-called composite score [12].

31.3 Systemic Chemotherapy in Well-Differentiated NEN

A number of chemotherapeutic agents have been tested in metastatic well-differentiated G1 and G2 NETs, mainly pancreatic and other foregut tumors. Results with systemic chemotherapy in well-differentiated small intestinal NETs have until now been poor with response rates of about 15 % [13], and therefore, these patients have not generally been recommended for chemotherapy. There is also limited data on systemic chemotherapy in "hindgut" NETs.

Strategies on when to start treatment are sometimes difficult to define, but patients with bulky, symptomatic tumor burden and progressive tumors are candidates for treatment. In addition, patients with locally advanced tumors, which may be down-staged for resection, should be considered. Not all patients will respond, and patient selection is essential to increase the chance of response and therapeutic benefit and minimize unnecessary side effects. Primary tumor location and tumor proliferation, and differentiation have been used to guide therapeutic decisions, and therefore, histological characterization has been assessed using WHO criteria and Ki-67 index. Ki-67 has been shown to predict biological behavior and correlate with survival [14]. It has, however, not yet been established at which Ki-67 index different chemotherapy protocols should be chosen, and the predictive value

for sensitivity of treatment is unknown. In the future, hopefully other predictive markers for sensitivity to chemotherapeutic agents will be developed. For neuroendocrine G1/G2 tumors (Ki-67 ≤ 20 %), streptozotocin-based combinations have been used as first-line treatment for more than 3 decades.

31.3.1 Single Agents

31.3.1.1 Streptozotocin

The alkylating agent streptozotocin, later renamed as streptozocin (STZ), has been used since 1967, when Murray-Lyon et al. reported improvement in hypoglycemic symptoms and tumor load in a malignant pancreatic NET in 1968 [15]. The US Food and Drug Administration approved the use of STZ for "islet cell" carcinoma in 1982. STZ binds to DNA and inhibits DNA repair, which leads to a blockade of DNA and RNA synthesis and fragmentation of DNA [14]. It blocks cells in all stages of the cell cycle, but it particularly blocks progression from G2 to M phase [16]. STZ does not seem to produce any significant clinical diabetogenic effect in humans, which is in contrast with findings in animal species [16].

31.3.1.2 Other Single Agents

Other old-generation cytotoxic DNA-damaging agents, 5-fluorouracil (5-FU) and doxorubicin (Dox) [17], have been administered with low efficacy. A phase II trial conducted by the Eastern Cooperative Oncology Group (ECOG) assessed the efficacy of dacarbazine (DTIC) [18] in 50 patients with pancreatic NETs. The partial response (PR) rate was 34 %, but a total of 16 patients (30 %) developed grade 3–4 toxicities and 2 (4 %) lethal toxicity.

31.3.1.3 Temozolomide

Temozolomide (TMZ) is a relatively new oral chemotherapeutic agent with clinical activity in metastatic melanoma and glioma [19, 20]. It is an alkylating agent, sharing its active metabolite with DTIC, with a rapid penetration through the blood–brain barrier. The very first patient who received this drug in our department in 1999 had recurrent brain metastases of a thymic NET after conventional radiotherapy of the brain. The brain metastases and metastases in other locations decreased significantly in size, and the response lasted for more than a year. Subsequently, 36 patients with different types of the so-called foregut NETs (12 pancreatic, 13 bronchial (10 typical), 7 thymic, 1 gastric, 1 "foregut," 1 paraganglioma, 1 cecal) received TMZ as monotherapy as second- to fourth-line treatment [21]. The first cycle of TMZ was administered orally at 150 mg/m^2 for 5 consecutive days. In subsequent cycles repeated every 28 days, the dose was increased to 200 mg/m^2, if treatment was well tolerated. Partial response according to RECIST was seen in 5/36 patients (14 %): 1 pancreatic NETs (8 %) and 4 bronchial NETs (31 %). Stable disease was noted in 19/36 (53 %): 8 pancreatic (67 %), 4 bronchial (31 %), and 5 thymic NET (71 %). Median time to progression was 7 months. Chromogranin A (CgA) was reduced by >50 % in 5 patients (19 %) and stabilized in 10 (37 %). The treatment was

well tolerated; grade 3/4 anemia occurred in 1 patient (3 %), thrombocytopenia in 5 (14 %), leukopenia in 1 (3 %), neutropenia in 1 (3 %), and fatigue in 3 (9 %). Gastrointestinal bleeding occurred in one patient (with tumor growth into the duodenum) and hormonal crisis in one patient with a metastatic VIP-producing tumor after the first dose of TMZ. There was a tendency that tumors with a low expression of the DNA repair enzyme, O^6-methylguanine DNA methyltransferase (MGMT) assessed by immunohistochemistry (IHC), had a higher response rate than those with a medium or high expression. The results were promising, considering that patients had received a mean of 2.4 palliative treatments and were given TMZ as salvage treatment. Bronchial and thymic NET, for which there is no established systemic treatment, showed a clinical benefit (response or stabilization) in 62 and 71 % of cases, respectively. Median overall survival from the start of treatment was 16 months, but 23 patients were still alive at the end of clinical follow-up.

The safety and efficacy of single-agent TMZ was explored in another study of 21 patients with progressive (within the past 3 months) digestive NETs [22]. All patients except one had received prior treatment (chemotherapy, hepatic resection). TMZ was administered orally at 200 mg/m^2/day for 5 days every 28 days. According to RECIST criteria, PR was obtained in 1 patient (5 %) and SD in 17 patients (81 %), and the median time to progression was 9 months. Grade 3 hematological toxicity occurred in 5 patients.

A recent retrospective update of the efficacy and safety of TMZ in 31 patients with progressive metastatic bronchial NETs (14 typical, 15 atypical, and 2 unclassified) was performed at our center [23]. More than half of the patients had received several lines of treatment. Partial response was seen in 3 patients (14 %) and SD in 11(52 %) with a median progression-free survival (PFS) of 5.3 months. Overall survival from the start of treatment in the whole patient cohort ($n = 31$) was 23.2 months. Notably, all patients with PR had atypical carcinoids with a high proliferative activity (10, 18, and 25 %, respectively). There was no grade 4 toxicity, but grade 3 adverse events were noted in 4 patients (14 %): thrombocytopenia ($n = 3$) and leukopenia ($n = 1$).

31.3.1.4 Other New Chemotherapeutic Agents

Other "newer" chemotherapeutic agents, such as paclitaxel, capecitabine, gemcitabine, and topotecan, have all been inactive as single agents [24–27].

31.4 Combination Chemotherapy: Streptozocin Based

31.4.1 Pancreatic NETs

The advantage of the combination of STZ plus 5-FU over STZ alone was first reported in a small randomized trial in 1980 [28]. STZ was given intravenously (i.v.) at 500 mg/m^2 and 5-FU at 400 mg/m^2 i.v. for 5 days every 6 weeks. The overall response rates (RR) were 63 and 36 %, respectively. STZ was subsequently approved by the FDA as a treatment for pancreatic NET. The same investigators

then compared two regimens, STZ plus Dox (given i.v. at 50 mg/m^2 D1 and D22) and STZ plus 5-FU [28], and STZ/Dox was shown to be superior to STZ/5FU (69 % vs. 45 % overall RR) with a median time to progression (20 vs. 6.9 months) and overall survival (2.2 vs. 1.4 years), respectively [29]. The doxorubicin arm was, however, associated with more side effects, including alopecia, myelosuppression, and cardiotoxicity. No group has since been able to reproduce the same response rates, most likely because of the use of hormonal responses and clinical assessment as response criteria in the Moertel study. The most recent reports of results with STZ/Dox describe RR of 6–36 %, and these are exclusively radiologic responses according to WHO or RECIST [30–32]. The low response rates in some of the recent studies may reflect the fact that in the last 20 years, biotherapy (SSA and interferon) may have been used as first-line or second-line treatment before the initiation of chemotherapy.

In a more recent study, a triple combination of STZ/5-FU/Dox was used, demonstrating an overall RR in 84 pancreatic NETs of 39 % and SD in 50 % with a median duration of response of 9.3 months and a PFS of 18 months [33]. The median interval to response was approximately 4 months, and the median overall survival from the start of treatment was 37 months. Grade 3–4 toxicity was noted in 23 % of patients, mainly leuko-/neutropenia. Analysis showed that metastatic replacement of more than 75 % of the liver and prior chemotherapy were independently associated with inferior PFS.

Our experience of the two combinations in earlier studies is that STZ plus 5-FU (given to 31 patients) is more effective than STZ/Dox (given to 25 patients) with radiologic PR in 35 % vs. 25 % in pancreatic NETs according to WHO criteria [34] and that responses can last for up to 10 years (median 23 months). Median overall survival in patients with liver metastases was 50 months from the start of treatment, some of the patients receiving other therapies after chemotherapy. These results have been confirmed in a recent unpublished update comprising 140 patients. Several centers including ours use an alternative schedule that is less cumbersome for the patient, administrating an induction course (STZ 500 mg/m^2 i.v. for 5 days and 5-FU 400 mg/m^2 i.v. D1–3) and then giving a 1-day course every 3 weeks (STZ 1,000 mg/m^2 and 5-FU 400 mg/m^2), which results in the same yearly doses of the drugs as the Moertel regimen [34, 35]. In responding patients, the cycle length is extended to 4–6 weeks after 1 year to allow for long-term treatment.

One problem with the Dox combination is the cardiotoxicity of Dox, which necessitates the withdrawal of the drug at a cumulative dose of 550 mg/m^2 and the replacement with 5-FU. STZ can cause nausea and vomiting, but these side effects can nowadays be almost completely avoided by modern antiemetics, e.g., 5-HT3 receptor blockers. Bone marrow toxicity is not a problem with STZ/5-FU. Renal dysfunction, including proteinuria and decreased creatinine clearance, which occurs in 20 % of patients, is the dose-limiting toxicity, but no grade 3–4 toxicity has been described in recent series [32–35]. Creatinine clearance should be checked before each course, and dehydration during or after the course should be avoided. In some tumors, especially those with production of vasoactive intestinal polypeptide (VIP), chemotherapy may induce hormonal crises, causing severe dehydration, and "protective" SSA treatment should be given.

31.4.2 Other Foregut NETs

There is as yet no established systemic treatment for foregut NETs. STZ combinations have been given with varying results. One publication described PR in 6/17 patients (35 %) and SD in 5/17 patients (29 %) with STZ/5-FU in patients with atypical bronchial carcinoids [36].

31.4.3 Recent Trials of STZ Combinations

The possibility of improving the response rates by adding cisplatin to STZ/5-FU was tested by Turner et al. in a mixed group of 79 patients, mainly pancreatic NETs but also gastrointestinal and lung NETs and in patients with unknown primary NETs [37]. The regimen FCiSt (5-FU 500 mg/m^2, STZ 1 g/m^2, and cisplatin 70 mg/m^2) was given i.v. on D1 of a 21-day cycle. The RR was 38 % for pancreatic NETs and 25 % for non-pancreatic. Stable disease was seen in 51 % of patients and time to progression was 9.1 months. Their conclusion was that the three-drug regimen was not superior to the two-drug regimen with STZ/5-FU or STZ/Dox.

In the BETTER study [38], bevacizumab (Bv) (7.5 mg/kg i.v. on day 1 every 3 weeks) was combined with STZ/5FU every 6 weeks in 34 patients with progressive, metastatic well-differentiated pancreatic NETs (G1 25 %, G2 75 %). The disease control rate was 100 % *(n = 34)*, including PR seen in 19/34 patients (55.9 %) and SD in 15/34 patients (44.1 %) with a median PFS of 23.7 months. Grade 3–4 toxicity occurred in 67 % of patients, gastrointestinal in 14.7 %, bevacizumab-related hypertension in 20.6 %, and thromboembolism in 11.8 %.

In another study recently performed, the aim was to investigate whether capecitabine (CAP) plus STZ +/− cisplatin warranted further studies [39]. Altogether 86 patients with pancreatic NETs (48 %), foregut (20 %), and unknown primary (33 %) were included (low grade 11 %, intermediate 67 %, and high grade 21 %). Courses were given every 3 weeks with STZ 1 g/m^2 i.v. D1, CAP 625 mg/m^2 twice daily D1–21, and cisplatin 70 mg/m^2 i.v. D1. In the STZ/CAP group (without cisplatin), PR was 8 % and SD 74 %, whereas with the triple combination (with cisplatin), the PR was 14 % and SD 64 %, respectively. PFS for the groups was similar 10.2 vs. 9.7 months. Hence, the response rates were similar between the two groups, but as expected, grade 3–4 adverse events were more frequent with the triple combination.

31.4.4 Current European Neuroendocrine Tumor Society (ENETS) Guidelines

According to current ENETS guidelines [40], STZ combinations should be considered as first-line treatment in patients with advanced unresectable progressive G2 pancreatic NETs. There is currently no cutoff value for Ki-67 for the recommendations of chemotherapy, but STZ could also be considered in G1 pancreatic NETs in

case of tumor progression (has historically been used in this subgroup in many centers before the assessment of Ki-67 was introduced). Ki-67 is a prognostic factor for survival and has been used to guide therapeutic decisions, but its predictive value for responsiveness to treatment is unknown. Predictive markers for sensitivity to STZ treatment should be developed in the future. The low or absent expression of MGMT might be such a marker, suggested by older studies in malignant gliomas and melanomas [41]. The cytoplasmic expression of phosphatase and tensin homologue (PTEN) has also been reported to predict sensitivity to STZ treatment [42].

31.5 Combination Chemotherapy: Temozolomide Based

31.5.1 Temozolomide Plus Capecitabine

Based on in vitro data indicating synergistic effects for induction of apoptosis between TMZ and CAP in neuroendocrine tumor cell lines, the combination has been administered to NET patients. In two retrospective series, the response rates have been relatively high. In one such study, 17 patients with mixed NETs, 11 (65 %) having received prior chemotherapy, were treated with TMZ at 150–200 mg/m^2 D10–14 every 4 weeks and CAP 1,000 mg twice daily D1–14. Complete response (CR) was noted in 1 patient (9 %) and PR according to RECIST in 9 patients (53 %) with a median duration of 9 months [43]. Toxicity was mainly grade 3 thrombocytopenia ($n = 2$) and hand–foot syndrome ($n = 1$). In the other study, the combination was given as first-line treatment in 30 patients with pancreatic NETs (16 well differentiated, 9 moderately, 5 not graded). TMZ was given at 200 mg/m^2 D10–14 and CAP 750 mg/m^2 twice daily D1–14 [44]. Partial response was noted in 21/30 patients (70 %) and SD in 8/30 (27 %) with a median PFS of 18 months and median duration of response of 20 months (responders). Overall survival at 2 years was 92 %.

31.5.2 Temozolomide in Combination with Molecular Targeted Agents

31.5.2.1 TMZ Plus Thalidomide

In a phase II prospective study, TMZ was combined with thalidomide, an agent with postulated antiangiogenic effect in metastatic NETs [45]. A dose-intensive TMZ regimen of 150 mg/m^2/d for 7 days every other week was administered together with thalidomide at 50–400 mg daily, median 100 mg. Twenty-nine patients were included (11 pancreatic NETs, 14 carcinoids, 4 pheochromocytomas) of whom 16 (55 %) had received no prior treatment. Radiologic response was seen in 25 % of patients: 5/11 (45 %) of pancreatic NETs and 1 of 14 (7 %) with carcinoids. Stable disease was observed in 68 % of patients, and the duration of response was 13.5 months (median). More than half of the patients had to stop treatment due to side effects, mainly due to thalidomide toxicity (neuropathy). Grade 3–4 lymphopenia occurred in 69 % of patients and 10 % developed opportunistic infections.

31.5.2.2 Temozolomide Plus Bevacizumab

In another phase II study, the efficacy of TMZ in combination with bevacizumab was evaluated in 34 patients with locally advanced or metastatic NETs (carcinoids 19, pancreatic 15) [46]. TMZ was given at 150 mg/m^2 D1–7 and D15–21 and Bv 5 mg/kg i.v. every other week in a 28-day cycle. Twelve patients had received prior chemotherapy, 7 chemoembolizations, and 17 octreotide (continued during study). At inclusion, 20 % of patients had stable disease. All patients received prophylaxis against *Pneumocystis carinii* and *Varicella zoster*. Overall radiologic response was 15 % (5 of 34), and response rates differed between patients with pancreatic NETs (5/15 (33 %)) and those with carcinoid tumors (0 of 19). The median PFS was 11.0 months (14.3 months for pancreatic NETs vs. 7.3 months for carcinoid tumors). The median overall survival was 33.3 months (41.7 months for pancreatic NETs and 18.8 months for carcinoids). Grade 3–4 adverse events included lymphopenia in 53 % of patients, thrombocytopenia in 18 %, leuko-/neutropenia in 6 %, and vomiting in 9 %.

31.5.2.3 Temozolomide in Combination with Everolimus

A recently published article described the safety and efficacy of temozolomide in combination with everolimus [47], a mammalian target of rapamycin (mTOR) inhibitor with single-agent activity in pancreatic NETs associated with improvement in PFS by 6.4 months compared with placebo as shown in the RADIANT-3 trial [48]. Patients with pancreatic NETs were treated with TMZ at a dose of 150 mg/m^2 per day D1–7 and D15–21 in combination with everolimus daily in each 28-day cycle. In cohort 1, TMZ was combined with everolimus at 5 mg daily. Following demonstration of safety in this cohort, subsequent patients in cohort 2 were treated with TMZ plus everolimus 10 mg daily. Patients were followed for toxicity, radiologic and biochemical response, and survival. The majority of patients (77 %) had received no prior systemic treatment with the exception of octreotide (40 % continued during study). Thirty patients (70 %) had evidence of radiologic progression of disease at inclusion. A total of 43 patients were enrolled, 7 in cohort 1 and 36 in cohort 2. Among 40 evaluable patients, 16 (40 %) experienced PR according to RECIST and 21 (53 %) had SD. The median PFS was 15.4 months and median overall survival was not reached. Biochemical responses with >50 % reduction of chromogranin A were seen in 45 % of evaluable patients. Twenty-one patients in cohort 2 discontinued treatment due to disease progression. An additional 9 patients discontinued therapy due to adverse events, including pneumonitis ($n = 3$), edema ($n = 1$), rash ($n = 2$), elevated creatinine ($n = 2$), and elevated liver enzymes ($n = 1$). Nearly half of the patients experienced grade 3 or 4 lymphopenia and 16 % grade 3 or 4 thrombocytopenia. One patient had a reactivated hepatitis B infection. Other toxicities included mild mucositis, hyperglycemia, hypercholesterolemia, or hypertriglyceridemia. The authors' conclusion was that patients with advanced pancreatic NETs can be safely and effectively treated with the combination. The optimal dosing schedule of TMZ and everolimus is, however, an open question because of the high toxicity rates.

31.5.2.4 Metronomic TMZ

Interestingly, another recent report described the activity of metronomic TMZ [49]. Administration of protracted low-dose TMZ may deplete MGMT, an important factor in cases of TMZ resistance, and/or inhibit endothelial cell proliferation and formation of tumor vasculature via a so-called metronomic effect [50]. Bevacizumab, an anti-VEGF humanized monoclonal antibody, has been shown to produce objective tumor responses in carcinoid tumors [51]. Preclinical experiments suggest that also somatostatin analogs can exert antiangiogenic effects [52]. Given the high vascularity in NENs, antiangiogenic treatment attacking multiple pathways was the rational for combining metronomic TMZ, bevacizumab, and octreotide in a prospective study of 15 patients with advanced progressive G2 NETs (14 stage IV) (7 pancreatic, 3 small intestine, 1 lung, 1 stomach, 1 rectum, and 1 unknown), of whom 80 % had received prior chemotherapy. TMZ was given at a continuous daily dose of 100 mg before bedtime, Bevacizumab 7.5 mg/m^2 i.v. every 3 weeks, and octreotide long-acting release (LAR) 30 mg intramuscularly every 4 weeks. Chromogranin A levels were also followed. Radiologic responses included one CR (7 %), eight PR (57 %), three SD (21 %), and 2 PD (14 %). Fifty-seven percent (8/14) of patients achieved PR after a median of 12.5 cycles. The median time to progression (TTP) in evaluable patients was 36 weeks. Among these patients, only five had Ki-67 > 6 % (6–19 %). The overall response rate in this study (CR and PR rate) of 64 % should be compared to RR in previous studies, in which higher doses of TMZ were given for fewer days. The high response rate may suggest that protracted administration of TMZ may lead to depletion of MGMT activity. Toxicity was mild compared with earlier trials with higher doses of TMZ. The results warrant further studies with this combination.

31.6 Predictive Value of MGMT Expression

MGMT is a DNA repair enzyme believed to induce cancer cell resistance to *O6-alkylating* agents, e.g., TMZ. The cytotoxic effect of TMZ has been attributed to its ability to induce methylation at the 0^6 position of guanine. Methylation of guanine results in DNA mismatch, which in turn leads to apoptosis and cell death [53]. The sensitivity of tumor cells to alkylating agents, including TMZ, has been associated with decreased levels of MGMT, which through its ability to restore DNA, can prevent chemotherapy-induced cell death [54]. Among patients with advanced melanoma or glioblastoma treated with TMZ, low MGMT expression was associated with longer survival [55, 56]. In a recent study, the prevalence of MGMT deficiency in NENs was evaluated by IHC and correlated to treatment response to TMZ-based regimens [57]. In archival specimen, MGMT deficiency was found in 19/37 of pancreatic NETs (51 %) and none of 60 carcinoid tumors. Among patients having received TMZ-based treatment, 18 of 53 patients (34 %) with pancreatic NETs but only 1 (bronchial carcinoid) of 44 with carcinoid tumors (2 %) experienced CR or PR ($p < 0.001$). Among 21 patients with evaluable tumor tissue who had received TMZ treatment, 4 of 5 with MGMT-deficient tumor (all pancreatic)

and none of 16 patients with tumors showing intact MGMT expression responded to treatment ($p = 0.001$). We had similar results in our single-arm trial of TMZ in 36 patients, i.e., low expression of MGMT correlated to response [19]. Both studies measured the expression of MGMT using IHC which is more relevant for clinical use. There is, however, controversy regarding the optimal method of MGMT analysis, and a discrepancy between results with IHC and methylation-specific PCR has been described in other tumors.

A standardization of techniques to assess MGMT status in tumor tissue and prospective trials to confirm a correlation between MGMT status and response to treatment with TMZ is warranted. This analysis may also serve as a predictive test for sensitivity to other alkylating agents, e.g., streptozocin and dacarbazine, since they have a common cytotoxic mechanism, i.e., induction of methylation at the O^6 position of guanine, and may have a similar resistance mechanism [57]. All three drugs seem to be more effective in pancreatic NET than in small intestinal tumors.

31.7 Other Chemotherapeutic Combinations

Bajetta et al. reported the result of a trial with the combination of oxaliplatin plus capecitabine in a group of 27 well-differentiated neuroendocrine G2 tumors [58]: oxaliplatin given i.v. 130 mg/m^2 D1 and CAP 2,000 mg/m^2 D2–15 every 3 weeks. Partial response was noted in 8/27 patients (30 %) and SD in 13/27 (48 %). Symptomatic improvement was observed in 50 % of patients and biochemical response in 20 %. This combination seems promising but needs further evaluation.

Other triplet chemotherapy regimens such as DTIC, 5-FU plus epirubicin or carboplatin, and gemcitabine plus irinotecan have been tested without achieving better response rates and unacceptable toxicity [59, 60].

31.8 New Molecular Targeted Agents in NETs

Recently, the mTOR inhibitor everolimus and the tyrosine kinase inhibitor sunitinib have been approved for the treatment of well-differentiated pancreatic NETs based on two multicenter placebo-controlled randomized trials [48, 61]. In both trials, the primary end point was PFS, and progression within the past 12 months was required for inclusion. The results were very similar with an improvement in PFS from 4.6 to 11.0 (hazard ratio for disease progression, 0.35; 95 % CI, 0.27–0.45; $p < 0.001$) for everolimus and from 5.5 to 11.4 months (hazard ratio for progression, 0.42; 95 % CI, 0.26–0.66; $p < 0.001$) for sunitinib. Overall survival as a secondary end point could not be assessed since about 70 % of patients crossed over from the placebo to the active arm in both studies. Tumor stabilization was achieved in both trials, but the objective response rates according to RECIST were low, 5 % for everolimus and 9.3 % for sunitinib. The proliferative rate or Ki-67 was not assessed except for a small number of patients in the sunitinib trial. Sunitinib was associated with diarrhea, nausea, asthenia, vomiting, and fatigue in more than 30 % of patients, and

grade 3 or 4 neutropenia and hypertension occurred in 12 and 10 %, respectively. With everolimus treatment, stomatitis, rash, diarrhea, and fatigue occurred in at least 30 %, and the most common grade 3 or 4 adverse events were anemia (6 %), hyperglycemia (5 %), stomatitis (7 %), and pneumonitis (2 %). The hyperglycemic effect has turned to be beneficial in patients with hypoglycemia due to malignant insulinoma.

Everolimus was also studied in non-pancreatic NETs with the carcinoid syndrome in combination with octreotide long-acting repeatable (LAR) in the RADIANT-2 trial [62]. Compared with octreotide LAR alone, the PFS improved from 11.3 to 16.4 months (hazard ratio, 0.77; 95 % CI, 0.50–1.00; one-sided log-rank test, $p = 0.026$) with the combination, but this did not reach the prespecified p-value of $p \leq 0.0246$. The lack of significance may be due to imbalances between treatment arms.

Conclusions

More than half of NENs are metastatic at discovery. Even though the indications for surgery have been broadened to include aggressive surgery of locally advanced disease and cytoreductive procedures to reduce the tumor burden in the liver, the majority of patients will require additional medical/systemic treatment.

As opposed to NETs of other origin, G1/G2 pancreatic NETs have been shown to respond to STZ-based combinations, and these combinations are recommended in current guidelines for patients with symptomatic, bulky, or progressive pancreatic NET. In these tumors, the response rates with STZ combinations are superior to other interventions/options. It should be established at which Ki-67 levels STZ (and other chemotherapies) should be administered. Adverse events, mainly renal toxicity, should be monitored carefully.

Another per oral alkylating agent, TMZ, has demonstrated promising results as monotherapy and in combination with CAP and/or Bevacizumab with higher response rates in pancreatic and bronchial NETs compared to small intestinal NETs. Mode of administration and low toxicity of TMZ are advantageous for the patients.

In the future, randomized phase II trials comparing the efficacy and toxicity of STZ and TMZ should be performed in well-defined patient groups. Additionally, the assessment of antitumor activity and toxicity of TMZ alone or in combination with other agents is warranted.

The combination of new molecular targeted therapies with chemotherapy is another interesting option. Even though the new drugs have been demonstrated to prolong PFS, patients with a high tumor burden may benefit from chemotherapy due to much higher response rates with chemotherapy. In such combinations, assessment of side effects and quality of life is essential. Such combinations could also prevent the development of escape or resistance mechanisms [63].

Progression-free survival should be preferred as an endpoint in future studies, in turn requiring demonstration of progressive disease prior to inclusion. Prolonged survival is the ultimate goal of treatment, but with more options being available, most patients will receive several lines of treatment, and hence, it will

be difficult to attribute prolonged survival to any one specific treatment. The treatment algorithm or sequence of therapy should be individualized according to several factors, including primary tumor location, grade and stage of the disease, functionality, and performance status. Tumor grade according to Ki-67 index has been used to guide therapeutic decision, but the appropriate cutoff level of Ki-67 has not been established. With improved knowledge in molecular biology, predictive markers of sensitivity or responsiveness to different therapies may become available to stratify patients optimally. Because all available therapies are palliative and accompanied by more or less severe side effects, early and accurate monitoring is important. In the future, new biomarkers and functional imaging with positron emission tomography (PET) or diffusion-weighted MRI may improve and be integral parts of the response evaluation.

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Targeted Therapies

32

Marianne Ellen Pavel

32.1 Introduction

32.1.1 Special Features of Neuroendocrine Tumours and Rationale to Use Targeted Drugs

Neuroendocrine neoplasms (NEN) represent a widely heterogenous group of tumours with distinct functionality, proliferative activity and growth behaviour. The vast majority of neuroendocrine tumours (NETs) (~90 %) are well to moderately differentiated tumours (NET G1/G2) and display slow to moderate tumour growth. Except for NEN with pancreatic primary tumour site and poorly differentiated NEN, (neuroendocrine carcinoma G3), NET are rather resistant to systemic chemotherapy. Their special features, such as a high degree of vascularity and high density of somatostatin receptors as well as the importance of different signalling pathways involved in neuroendocrine tumour cell proliferation and angiogenesis, make NET patients appear attractive candidates for the use of targeted drugs. Although the genetics and epigenetics of neuroendocrine tumorigenesis have yet to be fully elucidated, some studies have provided important insights.

In contrast to other non-endocrine malignancies, recent molecular studies in NET indicate that somatic mutations are rare, especially in small intestinal NET [1, 2]. Although mutations in MEN-1 gene and DAXX/ATRX gene are frequently found in pancreatic NET (Table 32.1), the significance of these alterations remains unclear, and there is no druggable target related to these alterations. Mutations in the mTOR pathway have been identified in 15 % of patients with pancreatic NET and ~2 % of intestinal NET. Genotype-phenotype correlations are, however, still lacking.

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Table 32.1 Somatic mutations in sporadic pancreatic and intestinal NET

Gene	Pancreatic NET (%) $n=48$	Intestinal NET (%) $n=68$
MEN-1	44	2
DAXX	25	0
ATRX	18	0
TSC2	9	0
PTEN	7	0
PIK3CA	1	2
VHL	0	2
P53	4	0
KRAS	0	0

From Jiao et al. [2] and Banck et al. [1]

In the absence of known major genetic drivers of tumour growth, advances in the field of targeted therapies are mostly related to inhibition of known signalling transduction pathways involved in proliferation of tumour cells, angiogenesis and cell survival.

32.1.2 Signalling Transduction Pathways and Targets in NETs

A better understanding of the mechanisms driving secretion and tumour growth has led to the development of several targeted antitumour agents in these rare tumours. NETs express a variety of peptide receptors including somatostatin receptors (sstr) and express interferon (IFN) receptors. Inhibition of sstr and IFN signalling by somatostatin analogues and interferon-alpha is an established approach to control secretion and growth of NET. Introduced in the mid 1980s, both drug classes can be considered as the oldest targeted drugs in the management of NET (see Chaps. 28, 29, 30).

Several growth-promoting targets are expressed in NET cells [3, 4]. These comprise not only growth factor receptors but also their ligands or other molecules, including vascular endothelial growth factor (VEGF), platelet-derived growth factor-α (PDGFR-α), platelet-derived growth factor-ß (PDGFR-ß), insulin-like growth factor 1 (IGF-1), transforming growth factor-α (TGF-α) and TGF-ß, and their cognate receptors [5]. VEGF-driven angiogenesis may play an important role in neuroendocrine tumorigenesis and tumour progression [6]. High vascularity is however rather a feature of well-differentiated than poorly differentiated NET. The epidermal growth factor (EGF) receptor is frequently expressed in NET, and binding of EGF or TGF-α induces RAF/MAP-ERK signalling in tumour cells; however, mutations of the EGFR tyrosine kinase which are predictive of a response to EGFR tyrosine kinase inhibitors in other types of cancers are rather uncommon in NET [7]. Activation of the IGF-1R by IGF-1 and IGF-2 plays an important role in tumour cell proliferation, and in NET cell lines (pancreatic

BON cells), it has been demonstrated that IGF-1 stimulates tumour cell growth by an autocrine loop. IGF-1 also plays a role in the upstream activation of the mammalian target of rapamycin (mTOR) pathway [8, 9].

The protein kinase mTOR exerts a central control function integrating multiple signalling pathways in response to growth factors and intracellular signalling by nutrients. mTOR is involved in the regulation of growth-related cellular functions, and the best known function is the regulation of translation initiation [10]. Inhibiting mTOR pathway may reduce cell growth and proliferation and impair the metastatic potential of tumour cells. It also acts at the endothelial cells. In NET constitutively activated PI3K-Akt-mTOR signalling seems to be a crucial event with 75 % of NET expressing activated Akt and 45 % mTOR [11]. Loss or downregulation of the expression of PTEN or TSC2 has been reported recently in a high percentage of pancreatic NET and may lead by loss of inhibition of mTOR to overactivation of this pathway [12].

In NETs, mTOR inhibitors and angiogenesis inhibitors have been most extensively studied. The following sections focus on the results from clinical trials with both kinds of drugs.

32.2 Targeted Therapies in Pancreatic NET

32.2.1 Inhibitors of the Mammalian Target of Rapamycin (mTOR) Pathway

Temsirolimus and everolimus are inhibitors of mTOR structurally related to rapamycin. Temsirolimus was the first mTOR inhibitor used in NET patients in a phase II study in advanced progressive NET. The objective response rate was less than 6 % and median time to disease progression (TTP) 6 months [13]. However, a significant percentage of patients experienced disease stabilisation (~60 %) with temsirolimus, and the 1-year progression-free rate of 40 % suggests drug activity beyond the natural course of the disease.

In a phase II trial, everolimus in combination with the long-acting SSA octreotide LAR (30 mg q 28 days) led to a tumour response rate of 22 % (with a more favourable response rate in pancreatic NET compared to NET of other origins, 27 % vs. 17 %, respectively). In the majority of the patients (70 %), stable disease was the best response in patients treated with everolimus [14]. The higher remission rate in pancreatic NET might be explained by the pathophysiological findings related to mTOR pathway [12].

Antitumour activity has been confirmed with everolimus in patients with progressive metastatic pancreatic NET after failure of cytotoxic chemotherapy in the phase II RADIANT-1 trial. 160 patients were included in two strata –/+ octreotide. The partial remission rate was low with 9.6 and 4.4 %, respectively. The rate of disease stabilisations was however high with 67.8 and 80 %, respectively. The corresponding figures for progression free survival (PFS) were 9.7 and 16.7 months [15]. Although data of this study are in favour of combination therapy of everolimus with octreotide, it remains

an open issue if combination therapy of everolimus and somatostatin analogues is indeed superior to everolimus alone to inhibit tumour growth.

The benefit of everolimus in advanced pNET was confirmed in a randomised placebo-controlled phase III trial (RADIANT-3) recruiting 410 patients. PFS was 11 months with everolimus, and 5.4 months with placebo [16]. Tumour remissions occurred in 5 % of the patients. Based on these data, everolimus was approved for progressive pNET patients. A survival benefit could not be demonstrated with everolimus compared to placebo; however, there was a high crossover rate from placebo to open-label drug upon progression. Treatment is continuous oral intake of everolimus 10 mg per day. Treatment is stopped if progressive disease or intolerable toxicity occurs. Most frequently reported adverse events include stomatitis (62 %) and rash (37 %). Patients have to be followed carefully for infections (20 %) and pulmonary events (12 %), such as pneumonitis [16] (Table 32.2).

To improve objective response rates and overcome acquired resistance to the drug, multiple combination therapies of everolimus with somatostatin analogues or angiogenesis inhibitors and dual inhibitors targeting upstream and downstream signalling of the mTOR pathway are currently under investigation. Since other treatments are available (somatostatin analogues, chemotherapy with either temozolomide or streptozotocin + 5-fluorouracil, angiogenesis inhibitors), it has still to be defined which patients will benefit most of targeted therapy with everolimus and in which sequence everolimus should be used.

32.2.2 Inhibitors of VEGF Receptor Signalling

The antiangiogenic strategies currently used in clinical practice in NET are specific monoclonal antibodies against VEGF (anti-VEGF A), such as bevacizumab and tyrosine kinase inhibitors (TKIs) targeting receptors for VEGF or PDGF expressed by tumour vessels. Further antiangiogenic drugs are evaluated in combination with somatostatin analogues (SSA), everolimus or systemic chemotherapy. SSA and everolimus also display antiangiogenic properties that include inhibition of

Table 32.2 Frequent side effects (>20 %) of novel targeted agents

Sunitinib		Everolimus	
Adverse event	Frequency (%)	Adverse event	Frequency (%)
Diarrhoea	59	Stomatitis	64
Nausea	45	Exanthema	49
Asthenia	34	Diarrhoea	34
Emesis	34	Fatigue	31
Fatigue	33	Infections	23
Grey hair	29	Nausea	20
Leucopenia	29	Oedema	20
Hypertension	27	Pneumonitis, lung infiltrates	17

Data from Yao et al. [16] and Raymond et al. [21]

endothelial cell proliferation and inhibition of secretion of angiogenic factors such as VEGF synthesised by tumour cells or microenvironment among other mechanisms [6, 17].

32.2.2.1 Tyrosine Kinase Receptor Inhibitors

Several small molecule tyrosine kinase inhibitors (TKIs) have been evaluated in NET, including sunitinib, pazopanib, axitinib and sorafenib, all of which have activity against diverse VEGF receptors and PDGF receptors. Some have additional activity against c-kit, RET or FGFR (Table 32.3), thus blocking multiple targets involved in growth, proliferation and metastatic spread of tumour cells [18, 19, 20].

Sunitinib is the only TKI that has been evaluated in a placebo-controlled phase III study based on promising results in a phase II trial including 66 patients with pNET. The objective response rate was 17 % in the latter study, and 68 % had stable disease [18]. In the phase III study, progressive disease was required before enrolment, and the objective response rate was <10 %. The primary endpoint, median PFS, was 11.4 months with sunitinib compared to 5.5 months with placebo. Most frequently reported adverse events include diarrhoea (59 %), nausea (45 %) and asthenia (34 %). Patients need to be followed carefully for hypertension (27 %) and leucopenia (29 %) [21]. Based on these results, sunitinib was approved for the treatment of pancreatic NET. In an updated survival analysis, no survival benefit could be demonstrated; however, most patients crossed over from placebo to open-label drug upon progression, a finding that may have biased these results.

32.2.2.2 Antibody-Based Therapy

Bevacizumab was evaluated in combination with somatostatin analogues, systemic chemotherapy, everolimus and other drugs. Results of larger and comparative studies (e.g. bevacizumab + everolimus vs. everolimus) are still lacking [22]. In small studies promising results were achieved with the combination of bevacizumab with either temozolomide or metronomic capecitabine plus octreotide (Table 32.3) [23, 24]. However, some combination therapies such as sorafenib and bevacizumab seem too toxic [25], and it remains unanswered yet to which extent bevacizumab might increase objective response rates or PFS in pNET.

32.3 Targeted Therapies in Non-pancreatic NET (Carcinoids)

32.3.1 Inhibitors of the Mammalian Target of Rapamycin (mTOR) Pathway

Following promising results from a phase II study, a combined therapy of everolimus plus the somatostatin analogue octreotide LAR was evaluated in a large placebo-controlled phase III trial (RADIANT-2) enrolling 429 patients with progressive NETs from various disease sites with a history of carcinoid syndrome [14, 26]. Due to the heterogeneity of tumour types (the most frequent tumour site was the intestine, followed by the lung) included in this trial but also differences in local

Table 32.3 Molecular targeted drugs in pancreatic NETs

Author	Drugs	Targets	Study phase	Patients (*n*)	Objective response (%)	PFS/TTP* (months)
Duran et al. [13]	Temsirolimus	mTOR	II	15	10.6	6.7*
Yao et al. [14]	Everolimus + octreotide	mTOR	II	30	27	12.5
Yao et al. [15]	Everolimus Everolimus + octreotide (RADIANT-1)	mTOR SSTR-2/SSTR-5	II	115 45	9.6 4.4	9.7 16.7
Yao et al. [16]	Everolimus vs. placebo (RADIANT-3)	mTOR	III	207 203	5.0 2.0	11.04 4.6
[a]	Everolimus vs. everolimus + bevacizumab	mTOR VEGF	II	138	Results pending	
[a]	Everolimus vs. everolimus + pasireotide (COOPERATE-2)	mTOR SSTR-1, SSTR-2, SSTR-3, SSTR-5	II	160	Results pending	
Kulke et al. [18]	Sunitinib	VEGFR-1, VEGFR-2; PDGFR, c-kit	II	66	17	7.7*
Raymond et al. [21]	Sunitinib vs. placebo	VEGFR-1, VEGFR-2; PDGFR, c-kit	III	86 85	9.3 0	11.4 5.5
Hobday et al. [19]	Sorafenib	C-RAF, B-RAF, VEGFR-2, VEGFR-3, PDGFR-β; c-kit	II	41	10	–
Ahn et al. [20]	Pazopanib	VEGFR-1, VEGFR-2, VEGFR-3, PDGF-α, PDGF-β, c-kit	II	12	18.9[b]	15.5
Chan et al. [23]	Bevacizumab + temozolomide	VEGF MGMT	II	15	33	14.3
Castellano et al. [25]	Bevacizumab + sorafenib	VEGF C-RAF, B-RAF, VEGFR-2, VEGFR-3, PDGFR-β; c-kit	II	13	7.7	N.R (>15 mo.)
Berruti et al. [24]	Bevacizumab + octreotide + metronomic capecitabine	VEGF C-RAF, B-RAF, VEGFR-2, VEGFR-3, PDGFR-β; c-kit; antimetabolite	II	19	26.3	14.3

[a] www.clinicaltrials.gov
[b] Includes other types of NET
*Indicates TTP
NR not reached

and central reading of radiographs, the results of the study are less clear. Median PFS by central reading (primary endpoint) was 16.4 months with everolimus + octreotide, and 11.3 months with placebo + octreotide, but did not reach prespecified statistical significance, probably due to imbalanced informative censoring that occurred following earlier assessment of tumour progression by local reading compared to central reading of radiographs and imbalances in baseline characteristics of the study population. However, despite discrepancies in local and central reading, the risk reduction (hazard ratio) was similar for both assessments [26]. The study provides evidence that subgroups of patients with functioning progressive non-pancreatic NET may have a benefit of everolimus. The activity of everolimus as monotherapy is currently further evaluated in intestinal and lung NET within a placebo-controlled phase III trial (RADIANT-4).

32.3.2 Inhibitors of VEGF Receptor Signalling

All studies are small in size and most deal with mixed patient populations including pancreatic NET and carcinoids. In a phase II study with the multiple tyrosine kinase inhibitor sunitinib, the objective response rate was low (2 %) in the subgroup of carcinoids. Its activity against carcinoid tumours could not be definitively determined in this nonrandomised study [18]. Sunitinib is currently evaluated in combination with lanreotide Autogel in a randomised phase II trial vs. lanreotide Autogel in progressive advanced or metastatic midgut carcinoid tumours (SUNLAND study; www.clinical trials.gov).

The combination therapy of bevacizumab and octreotide was assessed over a limited study period of 18 weeks in carcinoids. The objective response rate was 18 % with this combination and superior to a combination therapy of octreotide and pegylated interferon-alpha [27]. The results of a large US trial including 400 patients with carcinoids to further explore the combination of bevacizumab + octreotide vs. interferon-alpha + octreotide are still pending.

In the absence of results of comparative trials, it remains unclear what the add-on value of bevacizumab therapy might be in distinct types of non-pancreatic NET (Table 32.4).

32.3.3 Other Compounds

Other drugs targeting growth factor receptors or ligands that bind to growth factor receptors have been explored in NET. There are two published negative studies on the use of the insulin-like growth factor 1 (IGF-1) receptor inhibitor, a human monoclonal antibody that binds to the IGF-1R (MK-0646), in patients with metastatic well-differentiated NET [28] who had very advanced disease. While the drugs were inactive as single agents in well-differentiated NETs, the outcome of combined use of other IGF-1 receptor inhibitors and octreotide, or IGF-1 receptor inhibitors with octreotide and everolimus, is not reported yet (Fig. 32.1).

Table 32.4 Molecular targeted drugs in non-pancreatic NET ("carcinoids")

Author	Drugs	Targets	Study phase	Patients (*n*)	Tumour site	Objective response (%)	Median PFS/ TTP* (months)
Duran et al. [13]	Temsirolimus	mTOR	II	21	Carcinoids	4.8	6.0*
Yao et al. [14]	Everolimus + octreotide	mTOR SSTR-1, SSTR-2	II	30	GI, carcinoids	17	15.8
Pavel et al. [26]	Everolimus + octreotide vs. placebo + octreotide	mTOR SSTR-1, SSTR-2	II	429	GI Lung Others	2.3 1.9	16.4 (12.0)† 11.3 (8.6)
[a]	Everolimus vs. placebo (RADIANT-4)	mTOR	III	279	GI Lung CUP	Results pending	
[a]	Bevacizumab + octreotide vs. interferon-alpha + octreotide	VEGF SSTR-1, SSTR-2; IFN-R SSTR-1, SSTR-2	III	400	Advanced NET	Results pending	
Kulke et al. [18]	Sunitinib	VEGFR, PDGFR	II	41	Carcinoids	2.4	10.2*
Yao et al. [27]	Bevacizumab + octreotide	VEGF SSTR-2, SSTR-5	II	22	Carcinoids	18	–
Berruti et al. [24]	Bevacizumab + octreotide + metronomic capecitabine	VEGF SSTR-2, SSTR-5	II	26	Intestinal NET; CUP	11.5	14.3
Reidy et al. [28]	MK 0646	IGF-1R	II	15	Carcinoids	0	2.7

[a] www.clinicaltrials.gov; *Indicates TTP
† number in parentheses indicates values of local reading
CUP carcinoma of unknown primary

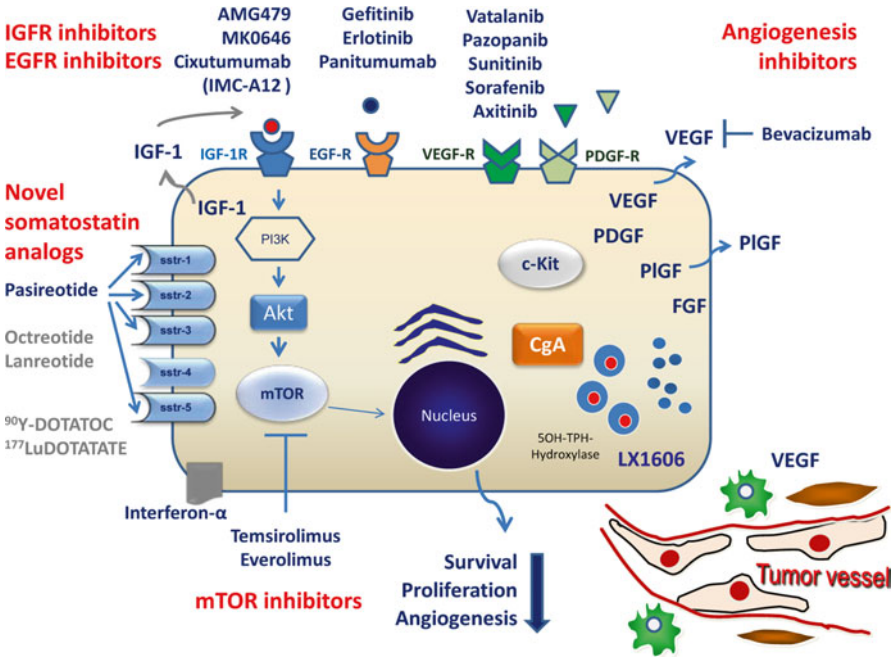

Fig. 32.1 Signalling pathways, drug targets and targeted therapies in NET (Modified from Pavel [30])

32.4 Summary and Conclusions

The approval of sunitinib and everolimus for the management of advanced pancreatic NET has extended therapeutic options in this rare type of tumour. In contrast, their role is less clear in NET of other sites, and both drugs are currently evaluated in ongoing clinical trials. Partial tumour remissions occur in less than 10 % of the patients with both molecular targeted drugs; however, stabilisation of the disease including minor tumour shrinkages is more frequently observed, and prolongation of PFS may be achieved and considered as a clinical important outcome parameter. Although prolongation of PFS is the major benefit of these targeted drugs, and an impact on overall survival could not be demonstrated in clinical trials in part due to a high crossover rate from placebo to open-label treatment, the use of these drugs in sequencing different kind of treatments may prolong individual patients' life. However, the clinical results achieved with sunitinib and everolimus in pancreatic NET are limited by de novo or acquired drug resistance. In consequence, treatment strategies utilising combinations of agents targeting multiple pathways have been initiated in an attempt to increase response rates and improve clinical outcomes. One of the major challenges is to improve the molecular understanding of diverse types of NET and to validate biomarkers for the selection and stratification of patients for distinct targeted therapies.

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Societies and Guidelines for Neuroendocrine Tumors

33

Kjell Öberg

33.1 Historical Background

Neuroendocrine tumors (NETs) are increasing considerably in both incidence and prevalence in the last decades [1]. In previous years, the beginning of the 1980s, there was very little interest in this field. There were discussions whether the tumor should be called endocrine tumors or NETs. Although one had the old publications from Obendorfer (1907) on carcinoid tumors and later in the 1950s publications on Zollinger-Ellison syndrome and Verner-Morrison syndrome, there was very little known and written about these so-called rare diseases. In the late 1970s and early 1980s, the most important center in the world for taking care of patients with NETs was the Mayo Clinic in the USA with Professor Charles Moertel and Dr. Larry Kvols with documented interest in managing these patients. The only treatment in metastatic disease at that time was streptozocin plus 5-fluorouracil or doxorubicin which was given to all the different subtypes of neuroendocrine tumors. At the end of the 1970s and early 1980s, the era of radioimmunoassays was established, and a lot of different antibodies to hormones and amines were developed which were used both for immunohistochemistry and for developing radioimmunoassays and Elisa. These developments became a new dawn of the NET field. At the same time, the development of new therapies came along with somatostatin analogues as well as alpha interferon for slow-growing NETs. Later on, in the 1990s, long-acting formulations of somatostatin analogues were developed. New cytotoxic treatments such as temozolomide were presented in the beginning of 2000, and during the most recent days, we have the development of the so-called targeted agents such as everolimus and sunitinib. In parallel, new imaging techniques were developed, somatostatin receptor scintigraphy and positron emission tomography.

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Another development during this last decade was prospective randomized controlled trials: the PROMID study with octreotide for carcinoids, the RADIANT 2 and 3 trials with everolimus, as well as the sunitinib trial for pancreatic NETs. Most recently, we also have the CLARINET study for nonfunctioning enteropancreatic tumors. The most important general biomarker chromogranin A was established worldwide. Molecular imaging paved the way for radioactive treatment with peptide receptor radionuclide therapy (PRRT). All these factors contributed to the accelerated increase in the incidence and prevalence of NETs together with increased awareness. Educational programs and the establishment of societies contributed to the increased interest in NETs. The first professional society that was developed in the field of NETs was the European Neuroendocrine Tumor Society (ENETS) which started as a small discussion group of 30 people in 1995. The aim of this group was to try to establish common diagnostic and therapeutic models. In 2001, this small group decided to establish a society (ENETs) which increased the number of members. The first guidelines were developed in 2005/2006 for different kinds of NETs [2]. This was the first comprehensive guideline that was developed in the field and was also appreciated by WHO which in 2010 made a new classification system [3]. The grading of NETs into NET G1, NET G2, and NEC G3 is based on the ENETS classification system. The work by the ENETS was appreciated by colleagues in North America which in 2006 formed the North American Neuroendocrine Tumor Society (NANETS), which has started to develop their own guidelines, partly based on the previously developed guidelines by the ENETS [4]. Later on, in 2010, the Latin American Neuroendocrine Tumor Society (LANETS) was established, including countries in South America and Mexico. In 2013, the Asia Pacific Neuroendocrine Tumour Society (AP-NETS) was founded in Kuala Lumpur covering the countries in Asia and the Pacific region, including Australia, ranging from India to Japan. All these latter societies are spin-offs from the ENETS, which have had yearly conferences in Europe since 2001. For these conferences, colleges from all over the world have been attending, and last year, almost 2,000 participants were registered at the meeting in Barcelona (2014). Today, the ENETS has around 1,100 members. The sister organizations are significantly smaller, even the North American Neuroendocrine Society which has around 200 members and around 250 participants in their yearly conference, the last one in Nashville, TN (2014). I myself, being a chairman of ENETS (2011–2014), have tried to establish a close collaboration between our sister organizations to develop programs for the management of NETs and to establish common clinical trials and educational programs.

Furthermore, ENETS has developed an accreditation program for hospitals interested in taking care of patients with NETs, so-called centers of excellence. At the moment, 27 centers have been accredited all over Europe. These centers are supposed to follow the ENETS guidelines in terms of diagnosis and treatment of NETs. They are also expected to work in a multidisciplinary way and to have regular tumor boards to discuss the patients. Furthermore, the patient's influence of their management is secured. It is obvious that centers working in multidisciplinary teams significantly improve the outcome for patients with NETs. Not only in Europe but also in the USA. The centers of excellence are reviewed every

3 years by a special organization based in Germany. The work by these specific societies such as the ENETS, NANETS, APNETS, and LANETS to develop guidelines has generated a new interest for NETs in larger oncology organizations such as the European Society for Medical Oncology (ESMO) and American Society for Clinical Oncology (ASCO) to further work on these guidelines and to spread them out to the general oncology practice. Therefore, the ESMO has generated their own guidelines based on the ENETS guidelines [5], and National Comprehensive Cancer Network (NCCN) in the USA has developed guidelines that are based on the NANETS guidelines as well as ENETS. Today, NETs are well recognized at different conferences and symposia in the fields of oncology, surgery, gastroenterology, endocrinology, and pathology which usually have large sessions related to neuroendocrine tumors. Besides the international societies, most countries have their own national societies for NETs.

Patient organizations have developed their own societies over the last two decades in parallel with the professional societies. Most countries in Europe have a well-developed patient organization. These European societies are working in networks with other societies in the USA, Latin America, and Australia to form an International Neuroendocrine Cancer Alliance (INCA).

Conclusion

The development of professional societies in the field of NETs with established guidelines for the management of NETs has significantly contributed to the development of the field and also improved the quality of life and the survival of many patients with malignant NETs. The patient organizations are important supporters. Patients with these diseases are regularly in contact with professionals from different organizations to get the latest developments in the field of diagnosis and treatment of NETs. One example of the developments in the management of NETs is that patients with carcinoid tumors and carcinoid syndrome had a median survival in the early 1980s of about 2 years and today, with modern treatment, follow-up, and the availability of different treatment modalities, have a median survival of more than 16 years at centers of excellence.

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