Updates in Surgery

Massimo Carlini *Editor*

Abdominal Neuroendocrine Tumors

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Forewords by
Marco Montorsi
Eugenio Santoro

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Editor
Massimo Carlini
Department of Surgery
Division of General Surgery
S. Eugenio Hospital
Rome, Italy

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To Silvia, Elena, Tiziana, Elettra, Piero

Foreword

It is with great pleasure that I present this monograph on neuroendocrine tumors that is the result of the exhaustive and meticulous work of Massimo Carlini and his group.

Although neuroendocrine tumors are relatively rare, they represent a very heterogeneous class of neoplasms. Since the discovery in 1870 by the German physiologist Rudolf P.H. Heidenhain of the existence of a group of gastrointestinal cells that were separate from oxyntic, chief and enteric cells, these peculiar cells – named "enterochromaffin" in 1907 by Carmelo Ciaccio – have not finished surprising us. Part of the fascination of this pathology is surely due to the syndromes frequently associated with it.

Clinical manifestations observed for centuries have been formally associated with these tumors only in recent history. From the very first observations of Robert M. Zollinger and Edwin H. Ellison published in 1955 and the identification by Allen O. Whipple of the diagnostic hallmark of insulinoma (Whipple's triad), many decades have passed. Yet it is not infrequent to encounter patients with a late diagnosis of gastrinoma after years of ineffective medical therapy or, worse, patients with insulinomas treated for years by psychiatrists or neurologists for vague behavioral disorders, or even VIPomas mistreated by superficial physicians.

A century ago, Siegfried Oberndorfer presented his observations on carcinoid tumours using the diminutive name "Karzinoide Tumoren" to distinguish these seemingly benign tumours from malignant adenocarcinomatous lesions and starting to delineate the extremely peculiar behavior of these neoplasms, so similar and yet so different from other solid lesions. This difference has been widely studied with increasing interest over the last few decades.

Differences in the biological behavior of these neoplasms will affect the prognosis even more than the stage at the time of diagnosis or surgical resectability. This prompted the European Neuroendocrine Tumor Society (ENETS) to propose in 2010 a combination between tumor grading, based on mitotic rate and Ki-67 index, and the TNM classification.

The varied behavior of this type of neoplasm has led surgeons to approach

different clinical situations both with standard oncological resections and with parenchyma-sparing techniques. A multidisciplinary approach and tailored surgery are key elements in treating this family of neoplasms which, despite sharing some features with other solid tumors, are unique in many biological aspects.

No wonder the zebra had been chosen as one the symbols of this pathology.

The authors have to be commended for their tremendous efforts to guide the reader through a very large but somewhat conflicting body of knowledge. We hope this monograph can further spread the understanding of the complexity of neuroendocrine tumors and help us to manage appropriately the increasing number of cases we have to face in our daily clinical practice.

Milan, September 2017

Marco Montorsi
President, Italian Society of Surgery

Foreword

The Biennial Report that the Italian Society of Surgery has entrusted to the editorship of Massimo Carlini addresses a topic that is highly challenging and fascinating at the same time: abdominal neuroendocrine tumors. Traditionally, the purpose of the Society's Biennial Reports has been to provide a snapshot of knowledge acquired in the fields of pathology, clinical presentation and surgical technique. This time, instead, this knowledge is in continuous motion as there is so much still to be learnt about the neuroendocrine system, with areas of uncertainty pervading all aspects from genetics to embryology, anatomy, physiology, pathology, presentation and treatment. And this report can only acknowledge this state of affairs.

That the topic is complex is reflected in the many acronyms that have been used over the decades to indicate the various anatomical, nosographic and pathological entities of the neuroendocrine system: APUD, DNES, CNES, BANT, BINT, NEN, NEC, NEN-GEP, SSTR2, MEN 1, MEN2… Even the WHO has met with considerable difficulty describing the diseases and has, over the past 10 years, repeatedly reviewed its pathological classification of GEP-NETs, the focus of this report.

To Carlini goes the credit of having accepted a somewhat imprudent and intriguing topic, but he was able to do so on the strength of his extensive experience in oncology - gained over the course of 16 years of work and research alongside me at the Regina Elena National Cancer Institute of Rome - and his large surgical case series, gathered over more than 10 years as head of the Department of Surgery at Sant'Eugenio Hospital in Rome. Based on his excellent oncologic competency he could have developed and written the entire volume by himself; instead, he humbly chose to invite other outstanding specialists with expertise in different areas to contribute their own chapters. The result is an extraordinary treatise of current knowledge about not only neuroendocrine tumors but also multiple endocrine neoplasms, with special reference to those involving the gastrointestinal tract, which account for 60% of cases of this uncommon disease.

With an annual incidence between 2 and 5 per 100,000 individuals in the western world, this disease is objectively rare, although it does not belong to

the family of officially recognized rare diseases and hence does not attract the same amount of required political and scientific attention. As a consequence, knowledge about these tumors is not widespread and is rarely taught. Reading the text, there is much to be learned, and numerous biological and clinical questions will find answers in these excellent pages authored by Carlini himself and by the other major experts he called upon to share their in-depth and qualified knowledge by contributing specific chapters on given topics.

In addition to reviewing current knowledge on the topic, the volume is also meant as a much-needed source of information and analysis, given that only half of these benign and malignant tumors are diagnosed at an early, curable stage, while the other half are detected when they have become metastatic or have invaded other organs and tissues, and are thus beyond cure. It should also be remembered that the diagnosis and treatment of these tumors requires the collaboration of several specialists including gastroenterologists, endocrinologists, surgeons, radiologists, nuclear medicine physicians, pathologists, laboratory physicians, as well as primary care physicians.

Among the many invaluable Biennial Reports published by the Italian Society of Surgery over the past 25 years, which were predominantly focused on surgical pathology and thus intended for "internal use" by the surgical community, this Report on abdominal neuroendocrine tumors stands out for being written for multiple medical specialties in the hope that this rare and little-known disease will rightfully become part of the cultural background of the whole medical community.

Written in a clear and informative style, this book will no doubt also be an additional resource for the activity and development of the Italian Association for Neuroendocrine Tumors (A.I.NET), a deserving voluntary association constantly working not only to provide support to patients with neuroendocrine tumors and their families, but also to foster research, disseminate knowledge and educate healthcare personnel. The book will also provide an opportunity to exchange views with international specialists and reiterate the high scientific and cultural standards of Italian medicine.

Rome, September 2017

Eugenio Santoro
President Emeritus, Italian Society of Surgery

Preface

Neuroendocrine tumors (NETs) are rare neoplasms if compared to the corresponding exocrine tumors, but their incidence has been increasing in recent decades and surgeons caring for cancer patients may have already faced or will no doubt face, at some point in their careers, the challenge of managing a case of NET. A heterogeneous group of tumors located in the neck, head, lung and abdomen, the latter representing the most important localization for a general surgeon, NETs have emerged as paradigm tumors for which multidisciplinary care is required.

The last few years have seen an extraordinary development of novel therapeutic options for NETs that have deeply changed previous practices. These changes have generated some confusion, and physicians are now seeking guidance from experts and comprehensive reviews summarizing current information on treatments for patients with NET.

The aim of this book is to provide readers with a better understanding of abdominal NETs and their most appropriate management by offering current, up-to-date knowledge about the various aspects of the disease, from both the theoretical and practical point of view.

In order to shed light on all of the features of the disease, this volume presents all the most recent advances in epidemiology, genetics, molecular biology, biomarkers, pathology, clinical presentation, diagnostics, medical therapy and – above all – surgical treatment. The volume brings together the most recent findings on NETs covering all areas of research and development, and stands out as paradigm for investigators and clinicians to improve research designs and treatments. I have aimed to keep the spirit of a multidisciplinary approach in the thirteen chapters of this book, where readers will find data that may help them to choose the right options and optimize the care of patients with NET.

The monograph is divided into three parts: the first concerns the nosography of the disease, the second addresses the diagnosis, and the third looks at clinical management. In Part I a complete nosologic framework and up-to-date classification are provided, along with hereditary syndromes and sporadic abdominal NETs. In Part II modern diagnostics – such as biomarkers, endoscopy,

non-functional imaging, functional imaging and pathological analysis – are described in detail. In Part III the multidisciplinary management of gastric, pancreatic, duodenal, ileal, appendiceal, colorectal and hepatic metastatic NETs is described.

I am very pleased to have succeeded in completing this volume and I want to express my heartfelt thanks to the President and to the Board of the Italian Society of Surgery for giving me the honor and the task of creating it as the two-year report of our Society. I sincerely thank all the co-authors, true experts in the field, for their work and for having realized that their efforts will contribute to a better management of NETs. Finally, I would like to express my affectionate thanks to Professor Eugenio Santoro who taught me all I know and all I do in surgery.

Rome, September 2017 Massimo Carlini

Contents

All web addresses have been checked and were correct at time of printing.

Contributors

Daniela Apa Department of Surgery, Division of General Surgery S. Eugenio Hospital, Rome, Italy

Marialuisa Appetecchia Digestive Endoscopy Unit, Fondazione Policlinico Gemelli, Rome, Italy

Fabia Attili Digestive Endoscopy Unit, Fondazione Policlinico Gemelli, Rome, Italy

Claudio Bassi General and Pancreatic Surgery Unit, Pancreas Institute, University of Verona Hospital Trust, Verona, Italy

Davide Bellini Department of Radiological Sciences, Oncological and Pathological Sciences, Sapienza University of Rome – ICOT, Latina, Italy

Ivo Boškoski Digestive Endoscopy Unit, Fondazione Policlinico Gemelli, Rome, Italy

Maria Luisa Brandi Department of Surgery and Translational Medicine, University of Florence, Florence, Italy

Massimo Carlini Department of Surgery, Division of General Surgery S. Eugenio Hospital, Rome, Italy

Roberto Cianni Diagnostic Imaging, Interventional Radiology and Nuclear Medicine, Santa Maria Goretti Hospital, Latina, Italy

Sara Cingarlini Oncology Unit, Department of Medicine, University Hospital of Verona, Verona, Italy

Guido Costamagna Digestive Endoscopy Unit, Fondazione Policlinico Gemelli, Rome, Italy

Sophie Deguelte Department of Digestive Oncology, Beaujon Hospital, Clichy, France

Giuseppe M. Ettorre General Surgery and Organ Transplantation, Azienda Ospedaliera San Camillo-Forlanini, Rome, Italy

Luca Filippi Diagnostic Imaging, Interventional Radiology and Nuclear Medicine, Santa Maria Goretti Hospital, Latina, Italy

Francesca Giusti Department of Surgery and Translational Medicine, University of Florence, Florence, Italy

Elisabetta Grego Oncology Unit, Department of Medicine, University Hospital of Verona, Verona, Italy

Michele Grieco Department of Surgery, Division of General Surgery, S. Eugenio Hospital, Rome, Italy

Pascal Hammel Department of Digestive Oncology, Beaujon Hospital, Clichy, France

Annarita Ianniello Nuclear Medicine Unit, Cancer Center Institute, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), Meldola, Italy

Andrea Laghi Department of Radiological Sciences, Oncological and Pathological Sciences, Sapienza University of Rome – ICOT, Latina, Italy

Luca Landoni General and Pancreatic Surgery Unit, Pancreas Institute, University of Verona Hospital Trust, Verona, Italy

Rosa Lauretta Endocrinology Unit, Regina Elena National Cancer Institute, Rome, Italy

Erminia Manfrin Department of Diagnostics and Public Health, Section of Anatomical Pathology, University and Hospital Trust Policlinico G.B. Rossi, Verona, Italy

Francesca Marini Department of Surgery and Translational Medicine, University of Florence, Florence, Italy

Roberto L. Meniconi General Surgery and Organ Transplantation, Azienda Ospedaliera San Camillo-Forlanini, Rome, Italy

Stefano Minardi Medical Oncology Unit, Azienda Ospedaliera Cosenza, Cosenza, Italy

Marco Miotto General and Pancreatic Surgery Unit, Pancreas Institute, University of Verona Hospital Trust, Verona, Italy

Chiara Nessi General and Pancreatic Surgery Unit, Pancreas Institute, University of Verona Hospital Trust, Verona, Italy

Giovanni Paganelli Nuclear Medicine Unit, Cancer Center Institute Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), Meldola, Italy

Salvatore Paiella General and Pancreatic Surgery Unit, Pancreas Institute, University of Verona Hospital Trust, Verona, Italy

Salvatore Palazzo Medical Oncology Unit, Azienda Ospedaliera Cosenza, Cosenza, Italy

Giuseppe Pelosi Department of Oncology and Hemato-Oncology, University of Milan, and Interhospital Pathology Division, Science & Technology Park, IRCCS MultiMedica, Milan, Italy

Francesca Rota Endocrinology Unit, Regina Elena National Cancer Institute, Rome, Italy

Maddalena Sansovini Nuclear Medicine Unit, Cancer Center Institute Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), Meldola, Italy

Aldo Scarpa ARC-Net Research Center and Department of Diagnostics and Public Health, Section of Anatomical Pathology, University and Hospital Trust Policlinico G.B. Rossi, Verona, Italy

Stefano Severi Nuclear Medicine Unit, Cancer Center Institute Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), Meldola, Italy

Angelica Sonzogni Department of Pathology and Laboratory Medicine, Fondazione IRCCS National Cancer Institute, Milan, Italy

Domenico Spoletini Department of Surgery, Division of General Surgery S. Eugenio Hospital, Rome, Italy

Francesco Tonelli Department of Surgery and Translational Medicine, University of Florence, Florence, Italy

Salvatore Turano Medical Oncology Unit, Azienda Ospedaliera Cosenza, Cosenza, Italy

Marta Zerunian Department of Radiological Sciences, Oncological and Pathological Sciences, Sapienza University of Rome – ICOT, Latina, Italy

Part I

Nosography

Neuroendocrine Tumors: a Nosologic Framework

1

Massimo Carlini and Marialuisa Appetecchia

1.1 Neuroendocrine Cells of the Digestive System

The neuroendocrine system is a network of cells distributed throughout the human body, having structure similar to nerve cells (neurons) and releasing hormones into the bloodstream like endocrine cells. These hormones work like neurotransmitters and are released to transmit signals or impulses to other specialized or nerve cells.

The gastrointestinal (GI) tract and pancreatobiliary system contain a variety of endocrine cells that constitute a diffuse endocrine system. This is the largest population of hormone-producing cells in the body [1]. Specialized endoderm-derived epithelial cells, called enteroendocrine cells (EECs), are widely distributed throughout the GI tract [2, 3]. Intestinal EECs are restricted to the mucosa and represent only a small minority (<1%) of the overall epithelial cell population, often lying isolated from one another and interspersed by non-endocrine epithelial cells. These cells produce and release hormones [4] and play a key role in the control of GI secretion and motility, regulation of food intake, postprandial glucose levels and metabolism. When EECs interact with luminal content, they release signal molecules which can enter the circulation and act as classic hormones on distant targets. They also act locally on neighboring cells and on distinct neuronal pathways including enteric and extrinsic neurons [3]. The normal distribution of the neuroendocrine cells in the GI and pancreatobiliary tracts is reported in Table 1.1.

The role of the enteroendocrine system [3–5] is to detect the components of the intestinal lumen, to monitor the energy status of the body, and to elicit appropriate responses to control metabolic homeostasis in response to ingested

M. Carlini (✉)
Department of Surgery, Division of General Surgery, S. Eugenio Hospital
Rome, Italy
e-mail: maxcarlini@tiscali.it

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Table 1.1 Neuroendocrine cells in the gastrointestinal tract and pancreas

Cell Type	Hormones Produced	Distribution
alpha	Glucagon	Pancreas
beta	Insulin	Pancreas
delta	Somatostatin	Stomach, small intestine, pancreas, appendix, colon, rectum
EC	Serotonin	Stomach, small intestine, pancreas, appendix, colon, rectum
ECL	Histamine	Stomach
G	Gastrin	Stomach, duodenum
I	Cholecystokinin	Small intestine
K	Gastric inhibitory peptide	Small intestine
L	Glucagon-like peptide 1, peptide YY	Rectum, small intestine
M	Motilin	Small intestine
N	Neurotensin	Small intestine
P/D1	Ghrelin	Stomach, small intestine, appendix, colon
PP	Pancreatic polypeptide	Pancreas
S	Secretin	Small intestine, pancreas
D1	Vasoactive intestinal peptide	Pancreas, stomach, small intestine, appendix, colon, rectum

EC, enterochromaffin; *ECL*, enterochromaffin-like.

food. The GI tract's endocrine system produces more than 20 different hormones which mediate effects via neuro-, auto-, and paracrine mechanisms from at least 10 distinct EEC populations.

At least 15 gut neuroendocrine cells exist, all of which produce various bioactive peptides or amines, including serotonin, somatostatin, histamine and gastrin. These secretory products are stored in vesicles. Enteroendocrine cells are characterized by the presence of secretory vesicles, either large or smaller, synaptic-like or similar to those found in neurons [6, 7]. Other general markers for EECs include neuron-specific enolase and protein gene product 9.5, both located in the cellular cytoplasm [8]. As the most specific feature of EEC subtypes, the peptide/amine(s) contained within secretory vesicles has formed the basis for the classification of EECs [9].

Gut hormones are responsible for glycemic control, appetite stimulation and suppression, regulation of gastric emptying, and trophic effects on the intestinal epithelium. Additionally, EECs have unique direct connections to the enteric nervous system enabling precise transmission of sensory data and communication with the central nervous system [10]. These hormones (Table 1.2), identified in

Table 1.2 Main locations of enteroendocrine cells (EECs) and physiological functions of gut hormones

EEC	Hormones	Locations	Targets	Physiological functions
A	Ghrelin	Stomach		Appetite control, food intake, growth hormone release
D	Somatostatin	Stomach, small intestine		Gastrin release (stomach)
G	Gastrin	Stomach (pyloric, antral)	NE cells of gastric gland (ECL cells, parietal cells)	Gastric acid secretion, mucus growth, gastric contraction
I	CCK	Proximal small intestine	Gallbladder, pancreas, gastric smooth muscle	Gallbladder contraction, inhibits stomach emptying, pancreatic enzyme secretion
K	GIP	Proximal small intestine	Pancreatic beta cells	Insulin release, gastric acid secretion, LPL activity in adipose tissue
L	GLP-1, GLP-2, PYY, oxyntomodulin	Distal small intestine, colon	Endocrine pancreas	Nutrient uptake, intestinal motility, appetite regulation, insulin release, inhibits glucagon release, slows gastric emptying
M	Motilin	Small intestine	Smooth muscle of stomach, duodenum	Regulation of migrating myoelectric complex in pig, dog, human gut motility
N	Neurotensin	Small (distal) and large intestine		Gastric acid secretion, biliary secretion, intestinal mucosal growth, intestinal peristalsis
P	Leptin	Stomach		Appetite regulation, food intake
S	Secretin	Proximal small intestine	Pancreas, stomach	Bicarbonate release, gastric acid secretion, colonic contraction, motility, pancreatic growth

NE, neuroendocrine; *LPL*, lipoprotein lipase; *ECL*, enterochromaffin-like.

the late 70s, include cholecystokinin (CCK) that is responsible for stimulating the digestion of dietary fat and protein, the antidiabetic hormones glucagon-like peptide 1 (GLP-1) and glucoinsulinotropic polypeptide (GIP), the pro-satiety hormone peptide YY (PYY), the hunger hormone ghrelin and the inhibitory hormone somatostatin as well as the neurotransmitter 5-hydroxytryptamine (5-HT, serotonin).

The EEC population of the large bowel is generally less diverse than in the small intestine [11]. For instance, cholecystokinin-secreting cells are found in the small bowel but are absent in the colon [12]. From duodenum to rectum, the frequency of EECs is highest proximally and falls steadily reaching a trough in

the colon, before rising again within the rectum. After proximal small bowel, the rectum is the location with the next greatest frequency of EECs and the only location in the GI tract where EECs are occasionally seen adjacent to each other or in clusters [5].

More generally, GIP-secreting EECs are located in the proximal duodenal region, CCK-secreting EECs in the duodenal and jejunal regions, GLP-1-secreting EECs in the jejunal, ileal, and colonic regions, and PYY-secreting EECs appear more restricted to the ileal and colonic regions [13]. Enterochromaffin (EC) cells reside in the epithelium of the GI tract, secrete serotonin and regulate secretory and peristaltic reflexes. They also activate vagal afferents through 5-HT3 receptors to signal to the central nervous system [10]. EC cells are the most abundant EECs of the GI tract and are distributed widely, populating the gastric antrum, duodenum, jejunum, ileum and appendix as well as the colon and rectum. EC cells have been shown to make up over 70% of the EEC population in the proximal large bowel. They fall to around 40% in the rectum [5].

Other EECs such as the appetite-stimulatory ghrelin-secreting A cells in the stomach appear buried within the epithelial mucosa and make contact with the serosal blood supply only [10]. These cells are presumed to detect mechanical, neuronal, and paracrine stimulations since they do not make contact with the luminal cavity. In addition to gastric ghrelin-secreting A cells, D cells secrete somatostatin, G cells secrete the acid-releasing hormone gastrin, and P cells secrete the satiety hormone leptin.

A second population of EC cells also resides in the gastric mucosa, but does not contain 5-HT. These cells respond to gastrin secreted by G cells by releasing histamine and stimulating the secretion of gastric acid from parietal cells.

Hormone output from the EECs is also regulated by other EEC subtypes, including somatostatin released by delta cells, which inhibits GLP-1 secretion from L cells in the intestine, presumably through SSTR5.

The histamine-producing enterochromaffin-like (ECL) cells have been recognized as the leading cell type involved in the most significant alterations of gastric neuroendocrine cells. The trophic stimulus exerted by circulating gastrin has been demonstrated to have a crucial role in proliferative changes of ECL cells, through a sequence of hyperplasia-dysplasia-neoplasia.

In the pancreas, the endocrine cells constitute from 1% to 2% of the volume of the organ and most of them form well-circumscribed nests called islets of Langerhans, while a few scattered endocrine cells are also present in the main pancreatic and larger interlobular ducts, but are not observed in the smaller ducts. There are at least five types of cells with a specific hormone secretion: alpha, beta, delta, PP, Y, and epsilon cells. These cells produce several peptide hormones, including insulin, glucagon, somatostatin, pancreatic polypeptide (PP), vasoactive intestinal peptide (VIP) and ghrelin. The most common cells are insulin-producing beta cells, which account for 60% to 80% of all islet cells and are centrally located in the islets, while glucagon-producing alpha cells are located at the periphery of islets and constitute from 15% to 20% of the islet

volume. Somatostatin-producing delta cells and PP-producing cells constitute the remaining portions. Extrahepatic biliary epithelia also contain scattered endocrine cells in the intrapancreatic portion of the common bile duct.

1.2 Tumors Arising from Neuroendocrine Cells

Neuroendocrine tumors (NETs) are a group of malignancies with different clinical presentation and heterogeneous pathogenesis, which have a common origin from diffuse neuroendocrine cells. NETs can arise in any organ although the most frequent are those of the gastroenteropancreatic tract (GEP-NETs) and of lungs.

Tumors arising from neuroendocrine cells tend to have the typical histologic appearance of the site of origin. Diagnostic difficulties arise in the rare cases with unusual morphology [14].

NETs arising at different anatomical sites of the digestive system represent tumor entities that differ in their biology and clinical presentation [15].

NETs can be divided into functioning NETs and non-functioning NETs. Functioning NETs secrete hormones which give rise to specific clinical syndromes. Non-functioning NETs can secrete hormones in low amounts (not sufficient to determine symptoms) or secrete clinically inactive substances, such as chromogranin, neuron-specific enolase, pancreatic polypeptide, neurotensin, and ghrelin. In most cases NETs are asymptomatic. When symptomatic, the clinical picture is linked to the mass effect. Non-functioning pancreatic NETs (P-NETs) represent 60–90% of P-NETs. The most frequent functioning P-NETs are gastrinoma and insulinoma. Glucagonoma, VIPoma, somatostatinoma, GRFoma are less frequent [16].

1.2.1 Tumors by Site

Approximately 65% of all NETs are GEP-NETs which thus arise in the digestive system. Classically they are divided by site into: foregut, midgut and hindgut tumors. Organs that originate from the fetal foregut are the esophagus, stomach, duodenum, and pancreas (as well as the lungs). The midgut includes the jejunum, ileus, appendix and right colon. The hindgut is the transverse colon, left colon and rectum. All the GEP-NETs arising in the digestive system are listed in Table 1.3.

1.2.1.1 Esophagus

Endocrine cells in the esophagus are relatively rare. Clusters in cardiac-type glands have been reported within the 2 cm of the esophagus proximally to the gastroesophageal junction. Neuroendocrine tumors of the esophagus are particularly uncommon, and reports are limited to single cases and small series.

Table 1.3 Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) arising from the digestive system

Site	% of all NETs	Subtypes	Subtype %
Esophagus	<1%		
Stomach	5%	Type 1 - Associated with atrophic gastritis	70–80%
		Type 2 - Associated with Zollinger-Ellison syndrome	5%
		Type 3 - Sporadic tumors	15–20%
		Type 4 - Poorly differentiated carcinomas	Rare
Pancreas	9%	Non-functioning tumors	70–80%
		Gastrinoma	5–10%
		Insulinoma	5–10%
		Glucagonoma	Rare
		Somatostatinoma	Rare
		VIPoma	Rare
		Others	Extremely rare
Duodenum	5%	Gastrinoma	90%
		Somatostatinoma	5%
		Non-functioning	5%
		Gangliocytic paragangliomas	Extremely rare
Jejunum	2%		
Ileum	14%		
Appendix	7%	Classical NET	>95%
		Goblet cell carcinoids	Rare
Colon	8%		
Rectum	15%	Classical NET	>95%
		Adenoneuroendocrine carcinomas	Rare
Other site	3%		

1.2.1.2 Stomach

Gastric mucosa contains several varieties of neuroendocrine cells. The gastrin-producing G cells are the most common. The gastric mucosa also contains D cells, A cells, and X/A-like cells, as well as smaller numbers of less well-characterized endocrine cells.

Stomach NETs differ from the rest of the GI tract and are therefore divided into several subtypes, depending on the background in which they arose. Assessing the etiology has important implications for the prognosis and treatment of these tumors.

Type 1 gastric NETs arise in the fundus/body, and are related to atrophic gastritis with important elevation of gastrin levels due to absent negative feedback of acid. These are the most common gastric NETs, encompassing approximately 75% of the total cases [17].

Type 2 gastric NETs are less common (5% of cases). They occur in patients with Zollinger-Ellison syndrome (ZES) and multiple endocrine neoplasia 1 (MEN1). Similar to type 1 NETs, these tumors occur in the fundus/body.

Type 3 gastric NETs are sporadic. They arise in normal or inflamed mucosa, but do not show any evidence of atrophy or hyperplasia. They occur with greater frequency in the antrum.

Type 4 gastric NETs are equivalent to poorly differentiated carcinomas.

1.2.1.3 Duodenum

The duodenum contains G, D, and EC cells, though with a greater proportion of EC cells. Even though NETs are common in the small bowel overall, those arising in the proximal portions of the duodenum and ampulla are less frequent [18].

These tumors are classified by the protein expression patterns in: duodenal gastrinomas (the most common), somatostatinomas and non-functioning tumors. Duodenal/ampullary gangliocytic paragangliomas are also included as NETs but these are very rare, with only 192 cases described in the literature [19]. Ampullary NETs appear to have a more aggressive phenotype, generally with poor outcome.

1.2.1.4 Pancreas

Pancreatic NETs originate in islet cells. Although they may be similar or identical in histologic appearance to NETs of the GI tract, differences in their underlying biology and likely differences in response to therapeutic agents suggest that they should be treated and investigated as a distinct entity. Most pancreatic NETs are sporadic, but some occur as part of the multiple endocrine neoplasia type-1 (MEN1). When part of the MEN1 syndrome, there may be multiple pancreatic tumors.

Islet tumors may either be functioning or non-functioning. More than 70% of sporadic tumors are non-functioning and the diagnosis is based on the mass effect of the tumor itself, pain, nausea or bleeding. The pancreatic neuroendocrine tumors, which usually present with symptoms of hormone hypersecretion, are listed in Table 1.4.

The first two are the most frequent, representing 15–20% of all pancreatic NETs, while the others are extremely rare. All these tumors produce a clinical hormone-related syndrome [20].

1.2.1.5 Small Bowel

Neuroendocrine tumors are the most common small bowel malignancies. They are frequently associated with the classic carcinoid syndrome of diarrhea, flushing, and heart damage. Tumors arising in the mid to distal duodenum through the ileum, appendix, and proximal colon are derived from the serotonin-expressing

Table 1.4 Pancreatic neuroendocrine tumors (P-NETs) (~9% of GEP-NETs)

Non-functioning P-NETs (70% of P-NETs)

Functioning P-NETs (30% of P-NETs)

- Gastrinoma, excessive gastrin production, Zollinger-Ellison syndrome
- Insulinoma, excessive insulin production, hypoglycemia syndrome
- Glucagonoma, excessive glucagon production, glucagonoma syndrome
- VIPoma, excessive production of vasoactive intestinal peptide (VIP), watery diarrhea, hypokalemia-achlorhydria syndrome
- Somatostatinoma, excessive somatostatin production

Extremely rare functioning P-NETs (rare reported cases)

- CRHoma, excessive production of corticotropin-releasing hormones
- Calcitoninoma, excessive calcitonin production
- GHRHoma, excessive production of growth hormone-releasing hormone
- ACTHoma, excessive production of adrenocorticotropic hormone
- GRFoma, excessive production of growth hormone-releasing factor
- Parathyroid hormone-related peptide tumor

EC cells of the midgut. Most of them appear in the distal ileum (70% of total), and the tumors in the jejunum and ileum have often multiple lesions.

Midgut NETs, even if small, have a stronger tendency to metastasize to local lymph nodes and liver compared to other gastrointestinal NETs. Even for metastatic disease, survival is often good, and these tumors are rather indolent. Unfortunately, they respond poorly to most chemotherapies. Most of the midgut NETs are found incidentally on endoscopy, owing to their small size and lack of specific symptoms [21]. Tumors of the small bowel classically produce intense fibrosis of the bowel and vascular structures, and the classical clinical presentation of bulky lesions is bowel occlusion.

1.2.1.6 Appendix

Small NETs of the appendix are sometimes present in appendices removed for acute appendicitis, approximately 1 every 200/300 appendectomies. Bulky lesions are frequently metastatic, but low-grade appendiceal NETs have a better outcome than most GEP NETs.

Throughout the midgut, but most frequently in the appendix, it is possible to find goblet cell carcinoids. Rather than an origin in the ECL cells of the mucosa, these are thought to derive from a pluripotent stem cell [22]. Goblet cell carcinoids express neuroendocrine markers and have a worse prognosis than typical NETs.

1.2.1.7 Colon and Rectum

Approximately 70% of colonic NETs are located in the rectum, and most of them are discovered during screening colonoscopy (1 new diagnosis every 2,500 colonoscopies) and represent 1% of all rectal tumors. Usually they are small lesions, 6/10 cm above the sphincters, with a good prognosis, and a low metastatic rate. In rectal NETs, size and grading of the primary tumor are the most important prognostic data [23].

In the rectum some adenoneuroendocrine carcinomas are also reported. Adenocarcinomas can express neuroendocrine markers, but these are adenocarcinomas with a high grade of neuroendocrine cellular component [23].

1.3 NET Epidemiology

NETs are rare, but their incidence is increasing [25–31]. It is unclear whether the increased incidence is real or dependent on the improvement of diagnosis. Sources of data on GEP-NET epidemiology are the National Cancer Registries from the US and Europe (Norway, Sweden, Ireland, Netherlands, Denmark, Scotland, United Kingdom) [32–38], while epidemiological studies outside the US and Europe are few [39–44]. Furthermore, important information comes from national registries dedicated to NET patients, but these are not population-based registries and are therefore unable to report on incidence rates [27, 45–47].

Much information on the epidemiology of NETs derives from the Surveillance, Epidemiology and End Results (SEER) Program of the National Cancer Institute of the US. The SEER registries covered about 9% (SEER 9), 14% (SEER 13) and 26% (SEER 17) of the total US population. From 1973 to 2004, 35,618 patients with NETs entered the SEER database. A progressive increase in NET incidence has been detected from 1973 (1.09/100,000) to 2004 (5.25/100,000) (Fig. 1.1). Statistically significant increases in incidence were reported at all primary sites (Fig. 1.2). A recent study analyzed the data collected in the SEER registries in the period 1973-2007. Among 49,012 NETs, GEP-NETs were 60.5%. In the SEER 17 registry, covering from 2000 to 2007, GEP-NETs were 61% of all NETs. In this registry, the rectum is the most common site (17.7%), followed by the small bowel (17.3%), colon (10.1%), pancreas (7%), stomach (6%), and appendix (3.1%) [26].

NETs are a rare disease even in Europe and in Italy, where the incidence rate is 4.15/100,000 (data from the European RARECARE NET database) and 3.5/100,000, respectively [28]. Italian data about NET epidemiology are available in the AIRTUM database (January 2015), which includes all cancer cases diagnosed from 1976 to 2010. GEP-NETs were about 46% of all NETs. The most frequent primary GEP sites were the small intestine (25%), pancreas (22%), colon (19%), stomach (17%) and rectum (10%). The appendix was the primary site only in 5% of GEP NETs. Between 2000 and 2010, 9,197 NET cases were registered in

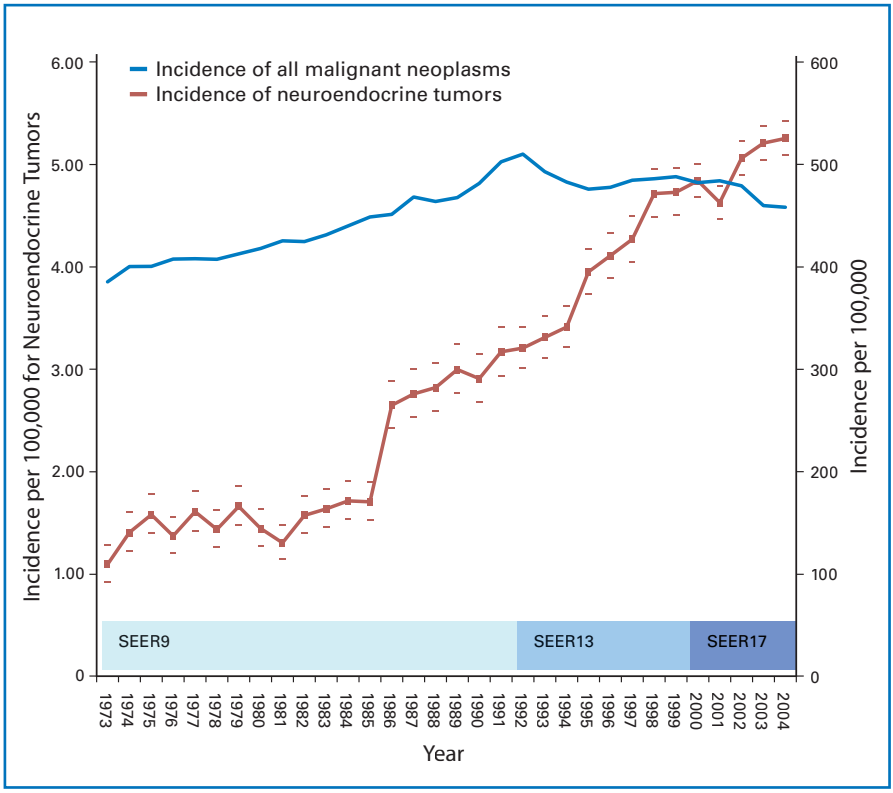

Fig. 1.1 Incidence of NETs compared to the incidence of all malignant neoplasms by year (data from the SEER database, 1973-2004) (reproduced with permission from [25])

the AIRTUM database, and in 2015 in Italy there were 2,697 estimated new cases of NET [28]. Italian data are available also in the NET Management Study. Also in the Italian study the incidence of NETs progressively increased from 1990 to 2007, although this was not a population-based study [27].

Berge et al. in a large Swedish autopsy series collected from 1958 to 1969 reported an average annual incidence of carcinoids of 8.4/100,000, which was sevenfold higher than that reported in Swedish clinical series. This supports the hypothesis that many NETs have no clinical relevance, consistent with the increase of incidental NETs detected in parallel to the wider use of imaging techniques [48].

Small bowel NETs (SB-NETs), including duodenal, jejunal and ileal NETs, are among the most frequent GEP-NETs in some European and US countries. In the SEER database, in the period 1973-2007 SB-NETs were the most common GEP-NETs (18.6% of all NETs) with a threefold increased incidence from 1973 to 2007. SB-NETs are much less common in eastern Asia (Japan, Korea, Taiwan) [38–40, 42, 43]. In the SEER 2000-2007 database rectal NET was slightly more

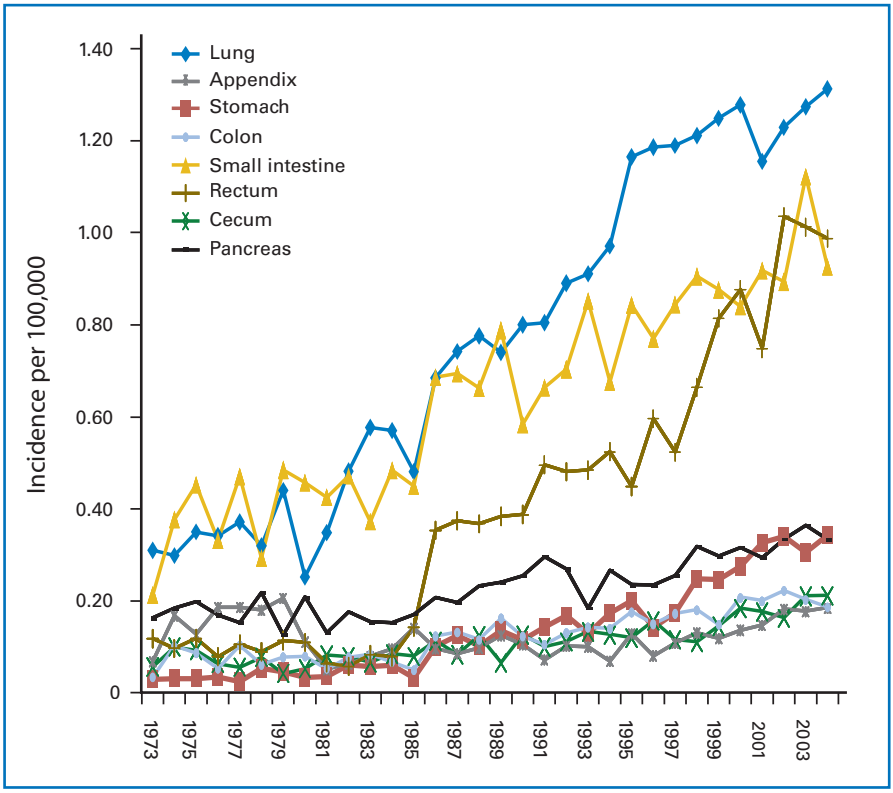

Fig. 1.2 Incidence of NETs by site (data from the SEER database, 1973-2004) (reproduced with permission from [25])

frequent than SB-NET, comprising about 17% of all NETs [26]. In the US, from 1973 to 2007, the incidence of rectal NET increased tenfold [26, 49]. Most likely this increase is not only due to the increased spread of endoscopy. In Europe rectal NETs have a low incidence, while they are the most common GEP-NETs in some countries of eastern Asia (Taiwan, Korea) [39–41]. Pancreatic NETs (P-NETs) are much more common in European NET registries dedicated to NET patients than in population-based studies, and in some of these registries they are the most frequent among GEP-NETs [27, 45, 47]. In the Italian NET Management Study the pancreas was the most common primary site, representing 31% of all NETs and 50% of GEP-NETs [27]. The incidence of P-NETs was also high in Japan (1.27/100,000) [44]. Conversely, they had a low incidence in the US SEER population. The incidence of P-NETs detected by the SEER 17 registry (2000-2004) was 0.32/100,000, with a slight predominance of males (0.38/100,000) over females (0.27/100,000) [25].

Despite the rarity of NETs, the total number of new cases per year is not negligible. Furthermore, as NETs have a low mortality rate, although the

incidence is low, their prevalence is high. In Italy the complete prevalence was 40.73/100,000 and estimated prevalent cases were about 24,000 in 2010. Well-differentiated, non-functioning NETs were the most prevalent [4]. In the US the estimated prevalence of NETs in 2004 was 103,312 cases or 35/100,000 [25], more prevalent than esophageal (28,664), gastric (65,836) and pancreatic (32,353) cancer [50].

There are gender and racial differences, which change depending on the primary site. In the US SEER population, 52% of patients were women and 48% were men. The mean age at diagnosis was 62 (median 63, standard deviation 15) and it ranged significantly according to primary tumor site (range 48-66). The lowest average age was detected in appendiceal NET and the highest in cecal NET. No differences in age at diagnosis by sex were observed. Therefore, the primary NET sites varied significantly by sex. The stomach, appendix or cecum were more frequently involved in women; the duodenum, pancreas, jejunum/ileum, and rectum in men. Patients from the SEER database belonged to different races: Caucasian (81%), African American (12%), Asian/Pacific Islander (5%) and American Indian/Alaskan Native (1%). Statistically significant differences by race were observed. Among GEP-NETs, jejunum/ileal NET occurred more frequently in Caucasian and African/American patients, rectal NET had a greater incidence in Asian/Pacific Islander and American Indian/Alaskan native patients [25]. The locations of primary NET varied also in different countries in the world, suggesting ethnic differences. In the AIRTUM database the incidence increased with age and was 1.49/100,000 in the age range 0–54 years, 7.37/100,000 in the 55–64 age range, and 11.27/100,000 in patients aged over 65 years [28]. Italian patients from the NET Management database had a mean age at diagnosis around 60 years [27].

GEP-NETs occur either sporadically or in the context of genetic syndromes (5–10% of all GEP-NETs) such as multiple endocrine neoplasia type 1 (MEN1), von Hippel-Lindau disease (VHL), neurofibromatosis 1 or von Recklinghausen's disease (NF1) and tuberous sclerosis (TS) [51–54]. The most frequent NETs in genetic syndromes are the pancreatic NETs. The P-NETs are observed in 75% of patients with MEN1, in 10–20% of patients with VHL, whereas they are less frequent in NF1 (less than 10%) and in TS, in which they are only found occasionally [55].

1.4 NET Natural History

Although Oberndorfer first described NETs in 1907, they still remain poorly understood. The only known risk factors are genetic syndromes in which NETs may arise. In particular, GEP-NET are expected in MEN1, VHL, NF1 and TS [53, 54].

Concerning the risk factors, in 740 US NET patients, among smoking, alcohol consumption, diabetes mellitus and a family history of cancer, only the latter

was associated with the disease [56]. A systematic review and meta-analysis confirmed that a family history of cancer is the most relevant risk factor for NETs at all investigated sites (lung, stomach, pancreas, small bowel, appendix and colon), followed by body mass index and diabetes [57]. Metabolic factors seem to intervene in the onset of rectal NETs: hypertriglyceridemia, low high-density lipoprotein cholesterol levels and high cholesterol levels, high fasting plasma glucose levels and metabolic syndrome [58–60].

Other risk factors have been proposed for P-NETs: origin from rural areas, chronic pancreatitis [61]. In a recent meta-analysis diabetes mellitus and a family history of cancer were associated with P-NETs [62]. The incidence of NETs in Canada was higher in rural areas than in urban areas (3.01/100,000 vs 2.82/100,000). The urban-rural difference in incidence could be related to environmental factors [63].

NETs are often indolent and the diagnosis is delayed and occasional, carried out in diagnostic procedures performed for other reasons. At diagnosis 40–60% of G1 and G2 NET patients are metastatic [64] and a higher percentage of NET patients are G3 [65]. In the functioning NETs, due to the presence of a more specific clinical picture, the diagnosis can be early. However, the carcinoid syndrome occurs late in NETs that secrete serotonin, typically in the presence of massive liver metastases. The wide involvement of the liver, compromising its function, prevents the degradation and elimination of amines and promotes the increase of their circulating levels, which causes the carcinoid syndrome.

The metastatic potential of NETs changes deeply depending on the primary site. Metastases are present mainly in small bowel and pancreatic NETs, whereas their prevalence in appendix and rectum NETs is low, and the most common site of metastases is the liver (46–93%) [64, 66, 67]. Bone metastases were found in less than 15% of cases, although it is likely that their prevalence is underestimated. Other rare metastases sites are lung, brain and peritoneum [64]. In SEER population (28,515 patients with known disease stage), 40% of patients had a localized NET (tumor confined to the organ of origin), 19% had a regional disease (tumor extended to surrounding organs or tissues and/or with regional lymph nodes metastases) and 21% had a distant NET (distant metastases). Male sex, Caucasian race and, first of all, site and grading were linked to stage disease. In the rectum, duodenum, stomach, appendix, NETs metastases were less likely to be found [25].

1.5 Mortality

GEP-NETs in many cases have a more indolent clinical course than adenocarcinomas of the same organs, as they are mainly low or intermediate grade malignant tumors. On the other hand, high grade neuroendocrine carcinomas (NEC) are very aggressive and have a poor prognosis [65]. In the US SEER population

(1973-2007) the 5-year overall survival (OS) rate of all GEP-NETs was 68.1% and varied depending on the primary site, resulting in 37.6%, 54.6%, 64%, 68.1%, 81.3% and 88.5%, respectively, for the pancreas, colon, stomach, small bowel, appendix, and rectum. Therefore, the lowest 5-year survival was observed in pancreatic NETs, while the highest was in rectal NETs [26]. The low survival rate for gastric NETs could be negatively affected by the collection of SEER registry data, which included up to 1986 only malignant gastric NETs. Type I and II gastric NETs, which are mostly benign, are therefore underestimated [26, 29, 30]. Other studies have reported an OS rate in gastric NETs ranging between 45% and 100% [33, 40]. As type I, II and III gastric NETs have a different malignant potential, it would be desirable that the data be collected by type [26].

Grading and staging have a great prognostic significance which was fully demonstrated in several GEP-NETs and mostly in upper digestive organs including the pancreas and the stomach [68–72]. Older age at diagnosis, male gender and African American race are also predictive of worse OS [25]. In Italy, the OS of NETs was 79% and 63% at 1 and 5 years, respectively, while in Europe it was slightly lower (71% and 54%, respectively). The small intestine was the NET site with the best prognosis [28]. The OS of GEP-NETs has increased by 23% from 1973 to 2002, perhaps as a result of greater awareness of the disease, early detection and more effective treatments [26]. However, the improvement of survival is lower than expected.

In centers of excellence, such as the Uppsala Centre for endocrine tumors, patients with NETs had a better survival [73]. Probably, a multidisciplinary approach could optimize patient care and improve their survival. For this reason, patients should be treated by a dedicated team with several experienced specialists in the field (surgeons, endocrinologists, pathologists, nuclear medicine physicians, oncologists, radiologists, interventional radiologists, gastroenterologists, and internists).

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Classification of Abdominal Neuroendocrine Tumors

2

Erminia Manfrin, Angelica Sonzogni, Aldo Scarpa, and Giuseppe Pelosi

2.1 Introduction

Abdominal neuroendocrine tumors make up a heterogeneous family of neoplasms ranging from low to high grade lesions [1]. These tumors, which are collectively called neuroendocrine neoplasms or NEN [2], are thought to arise from or differentiate towards elements of the neuroendocrine system in different anatomical sites [3], irrespective of the embryological derivation, which in turn includes either disseminated cells present in the wall of the gastrointestinal tube or parenchymal cells dispersed or clustered into classical organs such as the endocrine pancreas [4]. The neuroendocrine phenotype is largely determined by both positive and negative transcription factors, acting on precursor/ancestor or committed neuroendocrine cells during phylogenesis and ontogenesis [5, 6].

Neuroendocrine is a collective name widely used to indicate normal and neoplastic cells which share a common phenotype resulting from the simultaneous activation of a certain number of genes encoding for a wide variety of neuronal, epithelial and endocrine traits when evaluated by morphology, immunohistochemistry, molecular assays, functional properties and ultrastructural features [2, 7–9]. These traits include general markers of neuroendocrine differentiation common to all or almost all the elements of the neuroendocrine system, and cell-specific markers comprising regulatory peptides, biogenic amines, growth factors and cytokines restricted to individual cell lineages [2, 10]. Neuroendocrine tumors are supplied with specific cytoplasmic organelles related to the synthesis, packaging and secretion of single amino acid-derived hormones (biogenic amines), polypeptide hormones, growth factors and cytokines [3]. These

G. Pelosi (✉)
Department of Oncology and Hemato-Oncology, University of Milan,
and Interhospital Pathology Division, Science & Technology Park, IRCCS MultiMedica
Milan, Italy
e-mail: giuseppe.pelosi@unimi.it

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organelles involve the endoplasmic reticulum, small synaptic-like (40–80 nm) vesicles and large electron dense-core (100–200 nm) secretory granules [2, 7, 11]. Secreted substances are extruded directly into the bloodstream or released locally into the extracellular matrix via paracrine/autocrine regulatory mechanisms. This complex functional and structural organization of neuroendocrine cells accounts for their pleiotropic physiological effects and the protean presentation of the tumors hence derived [1].

Neuroendocrine cells, either normal or neoplastic, are easily identified on surgical specimens, biopsies and cytological samples by using immunohistochemistry for general/pan-neuroendocrine markers and cell-specific hormones [3, 8–10]. The former group includes a variety of diverse molecules, among which the most employed are those associated with small synaptic-like (synaptophysin) and/or large dense-core granules (chromogranins), while the latter group is made up of biogenic amines, polypeptides, cytokines and/or growth factors reflecting the specific functions and cell differentiation lineages [3]. On clinical grounds, abdominal neuroendocrine tumors are pragmatically classified into functioning and non-functioning neoplasms according to the occurrence or absence of clinical symptoms due to endocrine activity, respectively, either specific to the organ of origin or frankly ectopic [7]. Once believed to originate from neuro-ectodermal anlages of neural crests and collectively termed as carcinoids, abdominal neuroendocrine tumors are currently deemed to take rise from endoderm-derived precursors/stem cells along different molecular pathways [4], where the different anatomical sites and/or cell differentiation lineages are responsible for the different clinicopathologic presentation of such tumors [10].

These tumors are easily detectable on conventionally stained histologic sections when dealing with well-differentiated neoplasms by using a stepwise diagnostic process, in which the neuroendocrine nature of tumor cells is at first suspected by recognizing organoid nesting, trabeculae, rosettes and peripheral palisading and then eventually characterized by using chromogranin A and synaptophysin immunoreactivity [3]. Biogenic amines and/or polypeptide hormone markers are usually applied in selected cases when specific clinical purposes are incumbent (e.g., in the event of unknown primary site tumors). Conversely, poorly differentiated neuroendocrine tumors may be much more deceptive and diagnostically challenging because they may closely resemble unrelated exocrine tumors [3]. Sometimes, diverse neuroendocrine and non-neuroendocrine differentiation lineages may coexist within the same tumor mass giving rise to either combined or composite mixed lesions, where the neuroendocrine tumor component (conventionally accounting for at least 30% of the cell population, although this criterion is not universally accepted) is likely to be differently graded (NET or NEC) or even display concurrent neuroendocrine/non-neuroendocrine lineages featuring amphicrine traits within the same individual cells. This uncommon spectrum of tumors, which has been documented throughout the body in different anatomical sites, such as pituitary, thyroid, nasal cavity, larynx, lung, digestive system, urinary system, male and

female genital organs and skin, has recently been defined in the unifying concept of mixed neuroendocrine/non-neuroendocrine neoplasms (MiNENs) [12]. This umbrella definition includes mixed exocrine-endocrine carcinomas of the WHO 2000-2004 classification [13, 14] and mixed adeno-neuroendocrine carcinoma (MANEC) of the WHO 2010 classification [1].

The term neuroendocrine tumor has completely substituted, in the gastro-enteropancreatic (GEP) tract, the old-fashioned and time-honored terminology of carcinoid or APUDoma [7], which held field until the end of 80s on the basis of embryological, architectural or physio-anatomical classification schemes [15]. Currently, there is a large body of literature supporting the view that all abdominal/GEP tract neuroendocrine tumors have a malignant potential, which needs to be stratified into low- to high-grade for clinical purposes [1–3, 9, 10]. The malignant potential depends on several unrelated factors such as the anatomical location, tumor cell lineage, tumor grade and stage. Of note, tumor differentiation is only part of grading, as high-grade tumors may sometimes retain certain levels of neuroendocrine differentiation on morphology (rosettes and trabecular growth) and/or immunohistochemistry (unexpected strong decoration for secretory granule-related markers) [16]. Grading is an intensive property of tumors, also independent of their extension (and hence stage), exactly as the temperature of a body is proportional to the amplitude of molecular agitations but not to its free enthalpy [16]. Grading and staging are often but not always related to each other [10, 17], because small-size and localized (hence low-stage) tumors may display a high-grade and, vice versa, low-grade tumors may be extensively metastatic at the time of the initial diagnosis [17]. In other words, grading correlates with the levels of biological recruitment of tumors closely related to patient survival indicating, so to speak, a kind of "tumor temperature" [16]. Therefore, such information needs to be always clarified in the pathology report in keeping with current international guidelines, even though the defining criteria are different, not superimposable and depend on the organs of origin [1–3, 16, 18, 19].

Once the neuroendocrine nature of GEP tract tumors has been unveiled, the next step regards the diagnostic separation into well-differentiated and poorly differentiated neuroendocrine tumors as a part of the grading procedure [3, 8, 10, 20]. Diagnostic criteria for well-differentiated neoplasms include organoid features, absent or only focal/point necrosis, typical to slightly atypical cytology, less than 20 mitoses/2 mm^2 or 10 high power field (HPF) and less than 20% of proliferation activity as assessed by Ki-67 labeling index (Ki-67 LI) [1, 2]. Nuclear pleomorphism, vascular invasion or perineural permeation do not affect the ultimate diagnosis of well-differentiated tumors, while they may suggest a more aggressive clinical course. On immunohistochemistry grounds, these tumors are strongly decorated with chromogranin A, synaptophysin and hormones (the latter, not necessarily diffuse in all tumor cells) [8, 10]. Over-expression of p53 or CD117 is usually lacking. Diagnostic criteria for poorly differentiated neoplasms include in turn solid features, strongly atypical cytology, more extensive to geographic necrosis, small or large cell morphology, more than 20 mitoses/2 mm^2 or

10 HPF and more than 20% of Ki-67 LI [1–3, 8, 10]. On immunohistochemistry grounds, poorly differentiated tumors usually retain strong labeling for synaptophysin, while chromogranin A and hormones are usually vanishing, even though sometimes they may be unexpectedly preserved especially in the case of ectopic hormone secretion [1, 2, 7, 8, 10, 14]. Over-expression of p53 and CD117 is quite common, especially in small cell carcinoma [21, 22].

The terminology to adopt in abdominal neuroendocrine tumors on pathology reports, once this dichotomous separation into well and poorly differentiated neoplasms has been accomplished, is clinically determined by the need to precisely stratify for clinical purposes the malignant potential of these lesions. A detailed analysis of the different grading systems developed over time is beyond the scope of the present work, but suffice it to say that the WHO 2000/2004 classification has revolutionized the world of NEN for several reasons [13, 14]. It introduced for the first time the concepts of cell differentiation and anatomical sites as clinically relevant, emphasized the activity status as functioning or non-functioning, replaced the term of carcinoid with neuroendocrine tumor or neoplasm, and proposed a common classification scheme to adopt in all organs, with benign, uncertain behavior and malignant categories being recognized as a general taxonomy to all NEN. In this classification, malignancy was tautological in poorly differentiated neoplasms regardless of their extent or revealed by synchronous or metachronous distant metastases, intestinal wall invasion or extension to neighboring organs in well-differentiated tumors. The major criticism to this classification, however, was the mix of grading and staging concepts, the confusing term of uncertain behavior and the lack of further prognosis stratification in the well-differentiated category [19, 23, 24]. The increasing awareness and knowledge of GEP tract neuroendocrine neoplasms [25], especially as a function of their natural history revealed by long-term follow-up observations [1], and the substantial amelioration of early diagnosis procedures made it indispensable to develop a more simple, reproducible, behaviorally effective and widely agreed-upon system. This new scheme, which has recently been integrated into the WHO 2010 classification of digestive tract tumors [1] and is an integrant part of the European Neuroendocrine Tumor Society (ENETS) [26, 27] and North American Neuroendocrine Tumor Society (NANETS) [28] guidelines, will be here briefly discussed in its clinical strengths and weaknesses.

2.2 Grading

The pathological bases for establishing tumor grade according to the WHO 2010 classification, independently of the primary or secondary nature of tumors, include at first the separation between well and poorly differentiated neoplasms as outlined above [1]. The term “neuroendocrine” has replaced the previous

adjective "endocrine" and the term "neoplasm" (more adherent to the biological concept of new growth) was introduced instead of "tumor" (more akin to the definition of space-occupying mass) for indicating all these lesions [2]. All neuroendocrine neoplasms are thought to be potentially malignant if enough follow-up time is made available, and therefore need to be graded [1]. Even, a minimum 10-year follow-up is advocated to highlight very low malignant tumors.

Well-differentiated neuroendocrine neoplasms are split into two separate categories, both heralded by the term "tumor" (NET), regardless of being primary or metastatic (hence, irrespective of stage), while neuroendocrine carcinoma (NEC) indicates poorly differentiated neoplasms (once again, irrespective of tumor extension) [2]. NEC in turn comprises large and small cell morphological variants, whose diagnostic criteria are similar to those established for the homologous tumors of the lung. Representative histologic features of digestive neuroendocrine neoplasms according to tumor grade are depicted in Fig. 2.1. NETs are further separated into G1 NETs and G2 NETs, while NEC is tautologically G3 on the basis of the mitotic count on 2 mm^2 or 10 HFP and different cut-off thresholds of Ki-67 LI, as supported by several studies conducted on a variety of neuroendocrine neoplasms with confirming data [1, 2]. Ki-67 antigen is a 359-kD non-histone nuclear protein, which plays an essential role in the control and timing of cell proliferation, because it is expressed during the entire cell cycle with a maximum in the G2 and M phases. In neuroendocrine neoplasms, Ki-67 antigen was at first and then largely investigated as a prognostic factor in the pancreas [17, 29, 30], then exported to many other types of intestinal neuroendocrine tumors [13, 14, 26] until it was operatively incorporated into the grading system of digestive tract neuroendocrine neoplasms in the 2010 WHO classification [1]. Ki-67 LI should be counted on 500–2000 tumor cells in hot spot areas [31], with high correspondence between biopsies and surgical specimens when strict counting guidelines are applied [32] to obviate biological and methodological intratumor heterogeneity due to sampling, tumor fragment sizing or subtyping [33]. Eyeball estimation of Ki-67 LI should be usually discouraged because of higher inter- and intraobserver variability [34], but an eye count evaluation may be worthwhile in expert hands [35]. In general, an accurate manual counting of Ki-67 LI should always be recommended, because it is not more time-consuming than perceiving other grading parameters, such as mitoses or necrosis, and avoids cumbersome setting up of image analysis systems [32]. Individual values of Ki-67 LI should be expressed as entire percentages rounding up to lower value when 0.1–0.4 and to a higher value when 0.5–0.9 especially in the range between 2% and 3% to assign more robust prognostic assignment avoiding borderline categorization of G1 NET and G2 NET [2]. It is reasonable to report the actual Ki-67 LI value and mitotic count along with grading, thereby making cross-study comparison available [31]. Retrospective and prospective tumor series have largely demonstrated the substantial validity of this grading approach in the stomach, pancreas and small bowel, with some

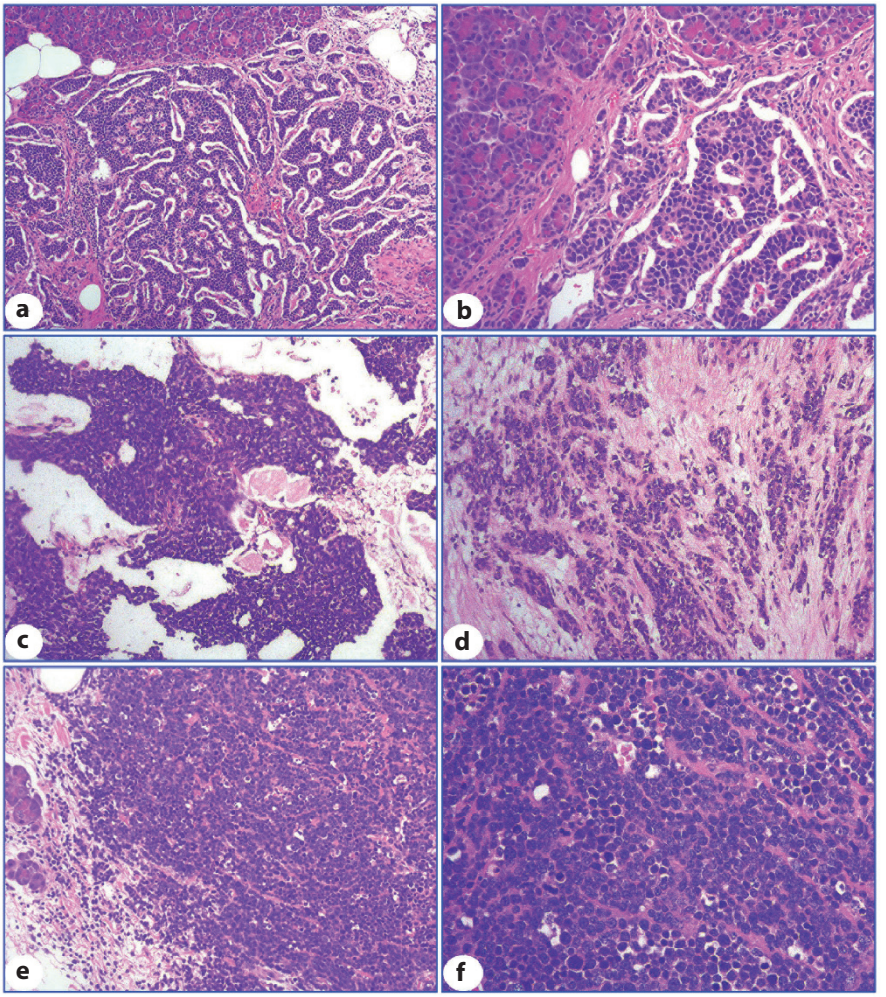

Fig. 2.1 Histologic features of digestive neuroendocrine neoplasms as exemplified in the pancreas according to morphologic criteria for tumor grading. Well-differentiated neuroendocrine tumors of grade 1 exhibit organoid appearance featuring trabecular, insular or gyriform growth, mild atypia and abundant vasculature (**a**,**b**). Well-differentiated neuroendocrine tumors of grade 2 show trabecular to solid appearance with moderate cell atypia (**c**) and modified desmoplastic stroma (**d**). Poorly differentiated neuroendocrine carcinomas are characterized by confluent and solid growth with necrosis, marked atypia, large or small cells and plentiful mitoses (**e**,**f**)

concern in neuroendocrine tumors of the appendix [1, 2, 36]. Differences in the choice of cut-offs between G1 NET and G2 NET or carcinoids in accordance with the different origin organs have been proposed for the pancreas [17, 30, 37] and the lung [16, 35], because it is a backbone observation that NEN arising in different anatomical sites behave differently [25].

Further complexity to the issue of grading abdominal neuroendocrine tumors is provided by the category of NEC. Traditionally considered a monolithic group of poorly differentiated and high-grade carcinomas with uniformly dismal prognosis and consistently hopeless therapy options [1], this category has recently been re-thought on the basis of the differential distribution of defining criteria, in particular Ki-67 LI and differentiation level. As a matter of fact, the wide range of cell differentiation (from well to poorly differentiated features) and of Ki-67 LI distribution (>20 up to virtually 100%) suggest that this tumor category is inherently heterogeneous in terms of molecular characteristics, clinical behavior and response to therapy [20, 38–40]. A 55% cut-off threshold has been proposed for Ki-67 LI to separate true NEC with small to large cell morphology, dismal prognosis and susceptibility to platinum agent chemotherapy from neoplasms bearing NET-like or large cell morphology, intermediate prognosis and lower response to platinum agent treatments according to the provisional term of G3 NET or well-differentiated NEC [20, 38–40]. This newly challenging tumor category would show response to alkylating agents and, to some extent, biological drugs [41].

Another puzzling observation regards the coexistence of higher and lower grade components in the same neoplasms of abdominal neuroendocrine neoplasms as highlighted by heterogeneous morphology, mitotic count, Ki-67 LI and molecular alterations [42], which challenges the concept of secondary NEC as an entity developing from preexisting NET as a result of tumor progression. This finding suggests a paradigm shift to the current knowledge on the pathogenesis of GEP NEN, but the same phenomenon is shared by NET arising in the thymus and the lung [43].

To ensure reproducibility of results, both parameters should be assayed on areas of highest immunostaining for the Ki-67 antigen or greatest concentration of mitoses (the so-called hot spots), possibly assessing the same tumor areas to minimize discrepancies due to imperfect collinearity of Ki-67 antigen and mitotic activity. To minimize interobserver variability, an actual area of 2 mm^2 has become a standard reference in NET of the lung [44]. In the event of grading discrepancy, the criterion of the highest value of Ki-67 LI or mitotic count was advised, mostly identifying Ki-67 LI as the closest predictor of tumor behavior (a higher Ki-67 LI compared to the mitotic count is more likely than the opposite taking into account technical artifacts and greater difficulties in recognizing mitoses in small biopsy samples) [2, 45, 46]. The grading assignment was proven to be independent of morphology in providing NET and NEC characterization, inasmuch as either tumor category may show disconnection between morphology (more deceptive and challenging in small-sized material) and subsequent clinical behavior [2, 45]. This phenomenon is well known not only in the NET category revealing G1 NET and G2 NET, but even in the NEC category (realizing the so-called G3 NET or well-differentiated NEC as above detailed), whose heterogeneous composition has recently shown important clinical implications in terms of response to different therapies [41]. Necrosis

does not play any role in the grading procedure of GEP tract NEN at variance with lung neuroendocrine tumors [35, 44, 47], although this parameter has been proposed to meliorate the prediction of prognosis [48]. This grading system has improved the definition and diagnosis of abdominal neuroendocrine tumors and identified more homogeneous populations of patients amenable to personalized treatments. Another advantage regards the better management of stage IV patients, who can be treated according to behavioral and biological characteristics of tumors rather than tumor extent, thereby avoiding overtreatment of lower tumor grade patients [16].

Potential sources of discrepancy could arise from differences in Ki-67 LI and mitotic count between primary and secondary lesions, either synchronous or metachronous, inside the same tumor mass or in different metastatic foci due to clonal selection or epigenetic changes [17]. Furthermore, different methods for assaying Ki-67 LI, either manual or automated, the use of biopsy or cytology samples vs. surgical specimens and interobserver variation of defining criteria may further account for discrepancy in tumor grade definition [33, 49]. Several tools have been proposed to minimize these drawbacks, such as multi-parametric definition combining necrosis, mitoses and Ki-67 LI, additional biopsies in case of large size tumors and/or multifocal metastasis, and the use of instrumental guidance reflecting metabolic or cell differentiation features (for example, FDG-PET in NEC or ^{68}Gallium-PET in NET) [16].

All discrepancies should always be commented on pathology reports to provide a plausible and meaningful interpretation to tumor behavior for the clinical handling of patients [50]. As a matter of fact, the basic question is not whether to grade or not to grade abdominal neuroendocrine neoplasms but rather how to reliably grade them in the decision-making process within individual tumor patients [16]. The clinical compliance of this grading system in abdominal neuroendocrine tumors is further endorsed by its inclusion into the International Union for Cancer Control (UICC), the WHO 2010 on digestive tract tumors, and the American Joint Cancer Committee (AJCC) classification schemes and the European Neuroendocrine Tumor Society (ENETS) guidelines.

2.3 Staging

Tumor stage is also fundamental information, which never should be missing in the pathology report of abdominal neuroendocrine tumors, because it complements the prognostic information and the clinical handling of these patients. The assessment schemes on neuroendocrine tumor extent are anatomical site-specific for the definition of tumor (T) parameters based on size and invasion because of the significant differences in clinical behavior of these tumors according to the organs of origin. By contrast, for the nodal (N) and distant metastasis (M) definition, current clinical experience and biological knowledge about neuroen-

docrine neoplasms is insufficient for further subdividing them, beyond a mere dichotomous separation based on the occurrence or absence of tumor deposits. The current staging systems of abdominal neuroendocrine neoplasms are in keeping with the 8th edition of the UICC and AJCC classification for the single organs (stomach, duodenum/ampulla/proximal jejunum, pancreas, lower jejunum and ileum, appendix, large bowel and rectum). The ENETS staging system overlaps largely with these proposals, with some differences and conflicting results in the prognostic stratification of neoplasms arising in the stomach, pancreas and appendix [26, 27]. In cases of discrepancy, both staging classifications should be mentioned and the worst stage used for classification [35]. For example, the ENETS staging system of pancreatic NEN is better suited than the UICC and AJCC proposal to forecasting patient survival, while the opposite holds true for appendix NEN. Lastly, in the stomach, both the TNM/AJCC and ENETS systems predict survival especially in NEC, while the TNM/AJCC seems to be superior in the NET subgroups [51]. An integration of staging and grading (the so-called TGM system) has been proposed in keeping with other malignancies as a tool for better predicting survival in pancreatic NEN as compared with singly used stage and grade schemes [52], but further confirmation is still needed before accepting this innovative approach into the clinical practice of abdominal neuroendocrine neoplasms.

2.4 Conclusive Remarks

Pathology reports of abdominal neuroendocrine tumors should always contain information useful for the best clinical handling of patients. In particular, anatomical location, appropriate classification (tumor vs. carcinoma), differentiation levels (well vs. poor differentiated), 2010 WHO/ENETS tumor grading (G1 to G3) with details on mitotic count and Ki-67 LI, and the TNM/AJCC/ENETS pathological stage should not be missing in any diagnosis of abdominal neuroendocrine neoplasms, especially resection specimens. Small biopsy samples in turn are most often the only available material in the setting of metastatic NEN, where grading assessment is clinically warranted. The time-honored and conceptually surpassed term “carcinoid” should be avoided, and only confined to carcinoid syndrome-associated NET. On surgical specimens, additional histologic features such as microscopic multicentric disease, presence and extent of non-ischemic necrosis, vascular and perineural invasion, definition of resection margins and evaluation of the functional status may help better complement the overall clinical assessment. Upon request or inside specific trials, information about predictive markers, such as somatostatin receptors, m-TOR pathway molecules or thymidylate synthase expression, may be integrated into the pathology reports to best manage these patients on clinical grounds. All pathology diagnoses should be discussed within multidisciplinary teams.

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Hereditary Syndromes and Abdominal Neuroendocrine Tumors

3

Francesco Tonelli, Francesca Giusti, Francesca Marini, and Maria Luisa Brandi

In recent decades, several hereditary diseases predisposing to abdominal neuroendocrine tumors (NETs) have been identified, and clinically and genetically characterized: multiple endocrine neoplasias type 1 (MEN1), multiple endocrine neoplasias type 4 (MEN4), von Hippel-Lindau syndrome (VHL), neurofibromatosis 1 (NF1; von Recklinghausen's disease), tuberous sclerosis (TSC; Bourneville disease), and Mahvash disease (MD). These syndromes are characterized by the onset of multiple tumors of different types, arising in different organs at a relatively early age. Furthermore, malformative or hyperplastic lesions are frequently concomitant. One of the easily assessable stigmata is the presence of cutaneous manifestations (Table 3.1) presenting an age of onset that can be earlier than that of NETs. Recognition of specific cutaneous lesions can favor the diagnosis [1]. The diagnosis of a hereditary syndrome can be suspected in the presence of two or more tumors or malformative lesions or when a patient, affected by only one characteristic associated tumor, has a first-degree relative with an ascertained hereditary syndrome [2]. A search for the genetic mutation can confirm unequivocally the diagnosis or provide the diagnosis when the carrier of the germline genetic mutation is a propositus.

The penetrance of abdominal NETs is largely different: it is very high for MEN1, low for VHL, and rare for MEN4, NF1 and TSC (Table 3.2). The genetically predisposed abdominal NETs represent 10% of all abdominal NETs, thus the search for germline mutations in an early onset apparently sporadic abdominal NET is strongly suggested to determine if it is really part of a familial syndrome. The identification of the hereditary syndrome and the mutational study of relatives enable the early detection of still asymptomatic gene carriers, and the subsequent surveillance of syndrome-associated malignancies, in particular for non-functioning tumors and asymptomatic pancreatic NETs (P-NETs). Therefore, it is not surprising that patients with hereditary P-NETs show a lower risk of

M.L. Brandi (✉)
Department of Surgery and Translational Medicine, University of Florence
Florence, Italy
e-mail: marialuisa.brandi@unifi.it

M. Carlini (Ed), *Abdominal Neuroendocrine Tumors*,
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Table 3.1 Characteristic skin lesions in hereditary syndromes with abdominal NETs that can help in the clinical diagnosis

Syndrome	Cutaneous lesion	Localization	Frequency	Age of onset
MEN1	Multiple angiofibromas	Facial	Over 85% of MEN1 patients	Before 40 years of age
	Multiple collagenomas	Trunk, neck and upper limbs	Over 70% of MEN1 patients	Before 40 years of age
TSC	Angiofibromas (called also adenoma sebaceum)	Facial	80-90%	3-4 years of age
	Fibromas	Ungual	40-50%	During childhood to early adolescence
	"Confetti" skin lesions	Total body	40%	During childhood to early adolescence
	Hypomelanotic macules	Total body	80-90%	Within the first year of life
	Fibrous plaques (shagreen patch)	Facial and lumbar	25-40%	During childhood to early adolescence
NF1	"Cafe au lait" macules	Total body	Over 99% of NF1 patients	At birth and within the first year of age
	Neurofibromas of the skin	Total body	Over 99% of NF1 patients	Develop in adulthood and continue to increase in number and size with age
	Skin-fold freckling	Total body	About 85% of NF1 patients	5 years of age
VHL	Capillary malformations	Total body	Less than 5% of VHL patients	Before 40 years of age
	"Cafe au lait" macules	Total body	Less than 5% of VHL patients	Before 40 years of age

MEN1, multiple endocrine neoplasia type 1; *TSC*, tuberous sclerosis; *NF1*, neurofibromatosis 1; *VHL*, von Hippel-Lindau.

Table 3.2 Main characteristics and frequency of abdominal NETs in hereditary syndromes

Syndrome	Prevalence	Gene	Main clinical features	Pancreatic NET types and frequency	Other abdominal NET types and frequency
MEN1	1/30,000	*MEN1*	Multiple adenomas of parathyroid glands (PHPT), adenomas of anterior pituitary, GEP-NETs	Gastrinoma 40% Insulinoma 10% Pancreatic insulinoma <5% NFTs and PPoma (20–55%) Glucagonoma (<1%) VIPoma (<1%) Somatostatinoma (<1%)	Adrenal cortical tumors (40%) PHEOs (<1%) Gastric NETs (10%)
MEN4	Unknown	*CDKN1B*	Multiple adenomas of parathyroid glands (PHPT), adenomas of anterior pituitary, tumors of adrenal glands, kidneys and reproductive organs	Gastrinoma (undetermined frequency)	Not reported
VHL	1/53,000	*VHL*	Retinal and CNS hemangioblastomas, PHEOs, epididymal cystadenomas, multiple cysts of pancreas and kidneys, RCC, pancreatic cystadenomas and cystcarcinomas	NFTs (10-17%)	PHEOs (20-35%)
TSC	1/6,000	*TSC1* and *TSC2*	Hamartomas of skin, brain, kidney, lung and heart, hypomelanotic macules, ''confetti" skin lesions, subependymal nodules, SEGAs	Gastrinomas Insulinomas (undetermined frequency)	PHEOs (undetermined frequency)
NF1	1/3,000	*NF1*	Cafe-au-lait spots, cutaneous and subcutaneous neurofibromas, hamartomas of the iris (Lisch nodules), skin-fold freckling, optic pathway gliomas	Duodenal somatostatinomas (0-10%) Pancreatic somatostatinomas Gastrinoma Insulinoma NFTs	PHEOs (2%)

MEN1, multiple endocrine neoplasia type 1; *MEN4*, multiple endocrine neoplasia type 4; *VHL*, von Hippel-Lindau; *TSC*, tuberous sclerosis; *NF1*, neurofibromatosis 1; *PHPT*, primary hyperparathyroidism; *GEP-NETs*, gastroenteropancreatic neuroendocrine tumors; *PHEO*, pheochromocytoma; *CNS*, central nervous system; *RCC*, renal cell carcinoma; *SEGAs*, subependymal giant cell astrocytomas; *NFTs*, non-functioning tumors.

malignancy and cancer-related death compared to patients with sporadic P-NETs in whom the tumors are diagnosed when symptoms have already manifested. No genotype-phenotype correlation for P-NETs or other gastroenteropancreatic NETs (GEP-NETs) has been clearly found until now. More rarely, gastroenteric carcinoids can be observed in these diseases, essentially in MEN1 [3–7].

The clinical management of hereditary abdominal NETs is similar to that followed for their sporadic counterparts, and surgery remains the treatment of choice.

3.1 Multiple Endocrine Neoplasia Type 1 (MEN1)

3.1.1 General Overview

MEN1 is an autosomal dominant hereditary multiple endocrine neoplasia syndrome with a complete penetrance by the age of 50. The main clinical manifestations of the MEN1 syndrome are multiple adenomas of the parathyroid glands, GEP-NETs, and tumors of the anterior pituitary. Primary hyperparathyroidism (PHPT) is the most common clinical sign, affecting more than 95% of MENl patients, with age of onset between 20 and 25 years. PHPT remains benign and may be asymptomatic throughout the patient's life. GEP-NETs are the second most common tumors in MEN1, mainly occurring in the pancreatic islets and duodenum, usually as multiple tumors. Pituitary tumors are the third most common clinical manifestation of MEN1, including prolactinoma, somatotropinoma, and ACTHomas and non-functioning adenomas. Also thymic, bronchial and gastric carcinoids often develop in MEN1 patients, rarely secreting amine or peptide hormones. Typical multiple cutaneous lesions, facial angiofibromas and collagenomas, have been reported in over 85% and 70% of MEN1 patients, respectively (Fig. 3.1) [8]. The estimated prevalence of MEN1 is about 1 in 30,000 births. Gender distribution appears to be equal.

3.1.2 Clinical Characteristics of MEN1-Associated Abdominal NETs

Clinical characteristics of MEN1-associated abdominal NETs depend on the specific site of tumor development and on the secreted hormone. GEP-NETs in MEN1 patients can be non-functioning or functioning tumors (Table 3.2). The most common GEP-NETs in MEN1 are P-NETs. When endoscopic ultrasonography (EUS) is utilized, P-NETs are found in approximately 40–54% of asymptomatic MEN1 patients [9, 10]; about 50% of 16–25-year-old MEN1 patients harbor P-NETs [10]. With time, almost all MEN1 patients develop multiple non-functioning P-NETs, which are a common hallmark of the syndrome. MEN1 predisposes to multiple P-NETs: the majority of them are microadenomas (less than 5 mm in diameter). Lesions over 5 mm in diameter (macroadenomas) are a minority but they are potentially malignant.

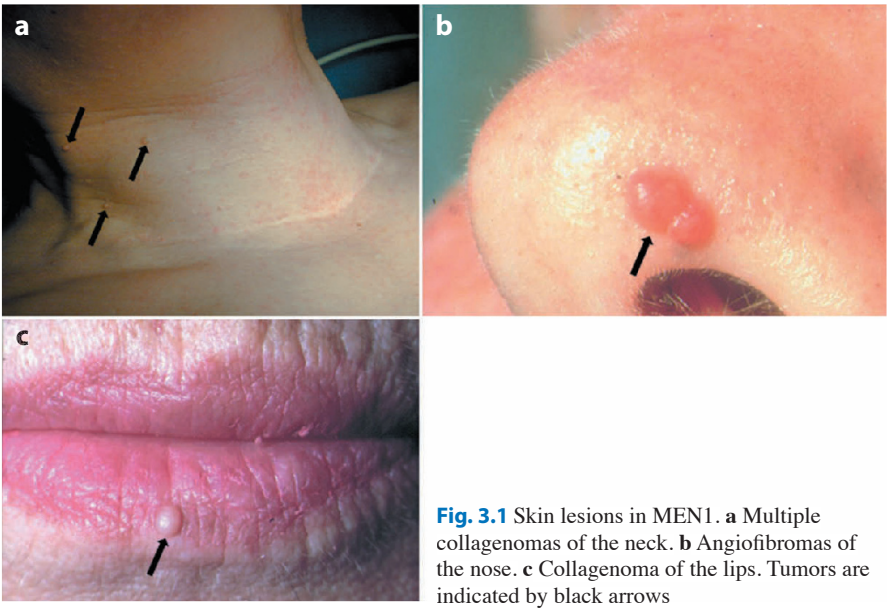

Fig. 3.1 Skin lesions in MEN1. **a** Multiple collagenomas of the neck. **b** Angiofibromas of the nose. **c** Collagenoma of the lips. Tumors are indicated by black arrows

Non-functioning tumors are the most frequent P-NETs in MEN1, usually characterized by a later onset. They are usually asymptomatic and discovered incidentally either by imaging examinations or by assessing the increase of pancreatic polypeptide (PP), which is secreted by approximately half of them.

Insulinoma is the most frequent functioning P-NET. It is a prerogative of the MEN1 syndrome, affecting about 15% of MEN1 patients harboring a *MEN1* mutation, and generally develops at a young age (Fig. 3.2b). In some patients, neuroglycopenic symptoms, due to the over-secretion of insulin, may be the first manifestation of MEN1 syndrome. The presence of multiple insulinomas and the

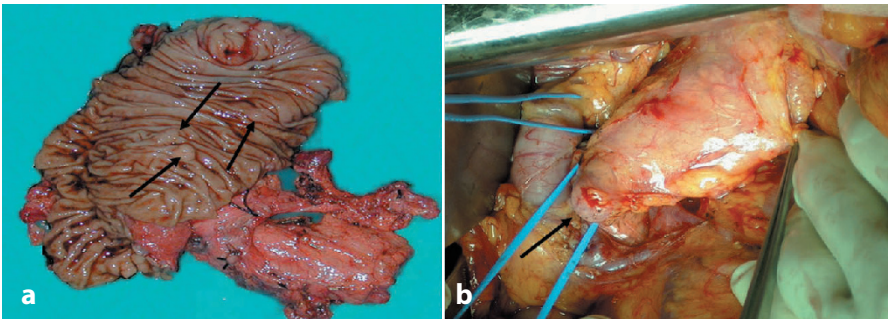

Fig. 3.2 Surgical resection of a NET in MEN1. **a** Surgical specimen of duodenopancreatectomy performed for multiple duodenogastrinomas and non-functioning tumors. **b** Surgical intervention for resection of a pancreatic insulinoma associated with multiple non-functioning tumors. Tumors are indicated by black arrows

association with other non-functioning P-NETs or with duodenal gastrinomas characterize the MEN1 organic hyperinsulinism.

The majority of gastrinomas develop in the deep part of the duodenal mucosa (Fig. 3.2a) and are usually multiple.

Glucagonomas, somatostatinomas, vasoactive intestinal polypeptide (VIP)-secreting tumors (VIPomas) and growth hormone-releasing factor tumors (GRFomas) manifest more rarely in MEN1 patients. Glucagonomas are accompanied by the typical necrolytic syndrome. Conversely, somatostatinomas are usually asymptomatic.

Gastric carcinoids may also develop in MEN1 through a sequence of hyperplasia-dysplasia-neoplasia. They are usually benign, but the biallelic inactivation of the *MEN1* gene can induce tumor progression and aggressive behavior.

3.1.3 Diagnosis

3.1.3.1 Clinical Diagnosis

Clinical diagnosis of MEN1 syndrome usually includes the presence of at least two NETs affecting the parathyroids, GEP tract or pituitary gland. Other less frequent MEN1-related tumors, such as carcinoid tumors, adrenocortical tumors, lipomas, visceral leiomyomas, truncal and facial collagenomas, facial angiofibromas and breast carcinoma can help in the diagnosis. In particular, typical skin lesions, such as angiofibromas and collagenomas, have been proposed as possible clinical markers in the diagnosis of MEN1 (Table 3.1). Biochemical testing for MEN1 includes the evaluation of serum concentrations of: 1) parathyroid hormone (PTH) and calcium to assess the PHPT; 2) gastrin, glucagon, VIP, PP, chromogranin A, and insulin with an associated fasting glucose level to identify the presence of a functioning GEP-NET; 3) prolactin and insulin-like growth factor-1 (IGF1) to identify the presence of a pituitary tumor (Table 3.3).

3.1.3.2 Genetic Diagnosis

Since the identification of the causative gene, the *MEN1* tumor suppressor gene, in 1997, the genetic diagnosis of MEN1 syndrome is chiefly made by PCR-based sequencing analysis of coding region (exons 2-10) and intron-exon junction of the gene. Inactivating germinal mutations of the *MEN1* gene are found in 78–93% of MEN1 patients and families [11, 12]. Over 1,500 different loss-of-function germinal and somatic mutations have been reported to date [11, 12]. In a negative sequencing test (in a MEN1 family or case with a clear clinical diagnosis of the syndrome), the application of multiplex ligation-dependent probe amplification (MLPA), a quantitative PCR-based method to detect DNA copy number changes, is suggested.

Patients with MEN1-like clinical phenotypes but negative to *MEN1* sequencing and MPLA tests may represent clinical phenocopies, and carry a mutation in members of the cyclin-dependent kinase inhibitor (*CDKN*) family, such as *CDKN1B* (see Section 3.2), *CDKN1A*, *CDKN2B*, or *CDKN2C* genes encoding,

Table 3.3 Diagnosis and clinical surveillance of P-NETs in hereditary syndromes

Syndrome		Biochemical tests		Imaging tests	
		Test	Annual surveillance starting age	Test	Annual surveillance starting age
MEN1	Gastrinoma	Fasting serum gastrin (±gastric pH; diagnostic values <2)	20 years	–	
	Insulinoma	Fasting plasma insulin and glucose	5 years	–	
	Other pancreatic NETs *	Plasma glucagon, VIP, PP, CgA	10 years	CT or MRI or EUS	10 years
MEN4	Gastrinoma	Fasting serum gastrin (±gastric pH; diagnostic values <2)	20 years	–	
	Insulinoma	Fasting plasma insulin and glucose	5 years	–	
	Other pancreatic NETs *	Plasma glucagon, VIP, PP, CgA	10 years	CT or MRI or EUS	10 years
VHL	NFTs	Plasma PP, CgA	10 years	CT or MRI or EUS	20 years
NF1	Duodenal somatostatinomas	Plasma somatostatin	10 years	CT or MRI or EUS	20 years
	Pancreatic somatostatinomas	Plasma somatostatin	10 years	CT or MRI or EUS	20 years
	Gastrinoma	Fasting serum gastrin (±gastric pH; diagnostic values <2)	20 years	–	
	Insulinoma	Fasting plasma insulin and glucose	5 years	–	
	NFTs	Plasma PP, CgA	10 years	CT or MRI or EUS	20 years
TSC	Gastrinoma	Fasting serum gastrin (±gastric pH; diagnostic values <2)	20 years	–	
	Insulinoma	Fasting plasma insulin and glucose	5 years	–	

* Glucagonoma, VIPoma, NFTs

MEN1, multiple endocrine neoplasia type 1; *MEN4*, multiple endocrine neoplasia type 4; *VHL*, von Hippel-Lindau; *NF1*, neurofibromatosis 1; *TSC*, tuberous sclerosis; *VIP*, vasoactive intestinal polypeptide; *NFTs*, non-functioning tumors; *PP*, pancreatic polypeptide; *CgA*, chromogranin A; *CT*, computed tomography; *MRI*, magnetic resonance imaging; *EUS*, endoscopic ultrasound.

respectively, the $p27^{kip1}$ $p21^{cip1}$, $p15^{Ink4b}$, $p15^{Ink4c}$ negative regulators of cell cycle progression [2]. No specific correlation between a single *MEN1* mutation or a mutated gene region and the MEN1 clinical phenotype has been confirmed.

In adult patients with MEN1, genetic analysis is mainly useful for confirmation of the clinical diagnosis or for the correct diagnosis of the syndrome in cases of controversial and/or equivocal clinical data. In young subjects, from a MEN1 pedigree with an identified *MEN1* mutation, the genetic test is fundamental for the early identification of asymptomatic mutation carriers. This grants the possibility to direct them into a specific routine program of biochemical and instrumental diagnostic surveillance for the early identification of tumors, and the subsequent early therapies.

3.1.4 Molecular Tumorigenesis

The *MEN1* gene (OMIM *613733, cytogenetic location 11q13.1) encodes a 610 amino acid nuclear protein, called menin, which is ubiquitously expressed in endocrine and non-endocrine tissues. Menin is directly and indirectly involved in gene transcription regulation, is responsible for positive control of genome stability and apoptosis and for negative regulation of cell growth [13].

Studies on MEN1 pancreatic and duodenal NET tissues evidenced that the inactivation of a single *MEN1* allele is associated with endocrine cell hyperplasia, but not neoplasia [14, 15]. Conversely, the adjunctive loss of the second *MEN1* wild type copy, at somatic level, results in endocrine cell dysplasia and development of micro and/or macro abdominal NETs [14, 15]. The complete loss of wild type menin, results in a severe reduction of $p27^{kip1}$ and $p18^{INK4c}$ expression, and it is responsible for increase of cell proliferation due to the lost inhibition of S-phase cell cycle progression. The observation of distinct somatic patterns of loss of the second copy of *MEN1* gene suggests that, in multifocal abdominal NETs, each tumor presumably arises separately from a single cell [15].

Somatic mutations of the *MEN1* gene have been described also in 27–39% of sporadic P-NETs, and loss of heterozygosity (LOH) at the *MEN1* locus (11q13.1) have been reported in 5–93% of sporadic P-NETs [16], confirming the importance of wild type menin in preventing neuroendocrine tumorigenesis of the pancreas, and presumably of other GEP-NETs.

3.1.5 Pharmacological Therapy of MEN1-associated Abdominal NETs

Gastric acid hypersecretion in gastrinomas and Zollinger–Ellison syndrome is well controlled by proton pump inhibitors (PPI). Somatostatin analogues (SSAs) are synthetic molecules (octreotide and lanreotide) that retain the binding affinity for somatostatin receptors (SSTRs), at least with high affinity for SSTR2 and moderate affinity for SSTR5. SSAs are successfully employed for control-

ling clinical symptoms, inhibiting both pancreatic and gastrointestinal hormone secretion (i.e., insulin, glucagon, gastrin, secretin and VIP). SSAs are effective only in few patients with insulinomas because of the low expression of SSTR2 in tumor cells [17].

Preliminary data on peptide receptor radionuclide therapy (PRRT) suggest a potential therapeutic role of this therapy in the treatment of advanced P-NETs in MEN1 patients [18]. PRRT with radio labeled SSAs takes advantage of the SSA specificity for somatostatin receptors to deliver cytotoxic doses of a radioactive isotope (i.e., yttrium-90 or lutetium-177) selectively to GEP-NETs cells.

Everolimus is an oral inhibitor of the signal transduction mTOR pathway, used in patients with advanced, low-grade or intermediate-grade P-NETs [19]. Sunitinib, an oral tyrosine-kinase inhibitor, targets the vascular endothelial growth factor receptor (VEGFR) and is used for the treatment of advanced P-NETs, since they are highly vascular tumors expressing high levels of VEGFR [20].

3.1.6 Surgical Therapy of MEN1-associated Abdominal NETs

In MEN1, the presence at surgery of multiple pancreatic NETs suggests pancreatic resection instead of enucleation for avoiding persistence or early recurrence of the clinical symptoms.

Insulinoma requires a prompt surgery since the risk of neuroglycopenic damage and the inefficacy of the medical treatment (Fig. 3.2b). Differently from the surgical procedure adopted for sporadic insulinoma for which simple tumor enucleation is recommended, the best surgical approach for MEN1 insulinoma is to resect the most affected part of the pancreas and remove by enucleation any additional lesions in the preserved pancreas [21].

In MEN1, other functioning P-NETs (gastrinoma, glucagonoma, somatostatinoma, VIPoma) are very rare, but they require surgery at the time of the diagnosis either to resolve the hormone-associated syndrome or for the risk of malignancy that can be present in at least half of these tumors.

Surgery is the treatment of choice for MEN1 non-functioning P-NETs, to prevent malignant progression and metastases, which are one of the most common cause of death in MEN1. Periodic EUS surveillance of the pancreas and surgical resection of all macroadenomas (if their size substantially increases over 6-12 months or reaches >2 cm), are recommended.

3.2 Multiple Endocrine Neoplasia Type 4 (MEN4)

3.2.1 General Overview

MEN4 syndrome is a MEN1-like very rare autosomal dominant inherited cancer syndrome, characterized by the development of parathyroid and anterior

pituitary adenomas, possibly associated with adrenal, renal, and reproductive organ tumors. Other associated tumors have been reported, such as gastrinomas and gastric and bronchial carcinoids.

Due to the extreme rarity of the disease and the fact that it is often misdiagnosed as MEN1, the exact prevalence of MEN4 is unknown, but it is estimated to be less than 1/million. To date, only twelve index cases have been reported worldwide.

3.2.2 Clinical Characteristics of MEN4-associated Abdominal NETs

Tonelli et al. [22] reported a case of a MEN4 female patient with two gastrin-secreting NETs of the pancreas and suspected liver metastases. None of the other MEN4 cases described to date reported the presence of abdominal NETs.

3.2.3 Diagnosis

3.2.3.1 Clinical Diagnosis

Clinical diagnostic approaches to MEN4 syndrome are the same as those used for MEN1, both for biochemical dosages and imaging screenings. The differential diagnosis with respect to MEN1 is made by specific genetic testing.

3.2.3.2 Genetic Diagnosis

MEN4 syndrome represents a phenocopy of MEN1, caused by inactivating mutations in the *CDKN1B* tumor suppressor gene. Mutation screening of the *CDKN1B* gene is recommended for all these patients presenting a MEN1-like clinical phenotype but with a negative genetic test for the *MEN1* gene. This test is predicted to detect a causative mutation in about 3% of MEN1 patients without a detectable mutation in the *MEN1* gene [23]. To date, nine different mutations have been identified in MEN4 patients and families. However, any possible association between a mutation and a peculiar clinical phenotype is still not well defined. Specific MEN4 guidelines are not yet available, and currently MEN4 patients are referred to the MEN1 guidelines [8].

3.2.4 Molecular Tumorigenesis

The *CDKN1B* gene (OMIM *600778, genomic location 12p13.1) encodes for the cyclin-dependent kinase inhibitor 1B, called $p27^{kip}$, that blocks cell cycle progression at the G0/G1 checkpoint [22]. During MEN4 tumorigenesis, the main mechanism responsible for reduced $p27^{kip1}$ expression seems to be the post-translational ubiquitin-proteasome-mediated increased degradation of the protein.

The sequence analysis of exome and genome in small bowel NETs identified recurrent somatic frameshift mutations of the *CDKN1B* gene and detected hemizygous deletions encompassing the *CDKN1B* locus, suggesting that loss and/or reduction of wild type $p27^{kip1}$ is responsible for cell cycle dysregulation in the etiology of small bowel NETs [24].

3.2.5 Pharmacological and Surgical Therapy of MEN4-associated Abdominal NETs

Pharmacological and surgical therapies of MEN4-associated abdominal NETs are the same as for the MEN1 counterparts.

3.3 Von Hippel-Lindau Syndrome (VHL)

3.3.1 General Overview

VHL syndrome is a rare genetic disorder characterized by visceral cysts and benign tumors in multiple organ systems that have subsequent potential for malignant change.

Clinical hallmarks of VHL include retinal and central nervous system hemangioblastomas (blood vessel tumors), pheochromocytomas (PHEOs), epididymal cystadenomas, multiple cysts or tumors of pancreas and kidneys, and an increased risk for malignant transformation of renal cysts into renal cell carcinoma (RCC) [25]. Retinal hemangioblastomas are the most common lesion, multiple and bilateral in about 50% of cases, and usually asymptomatic. They can cause retinal detachment, macular edema, glaucoma, and vision loss. CNS hemangioblastomas are most often located in the cerebellum, but also in the brainstem and spinal cord. These lesions are benign but cause symptoms by compressing adjacent nervous tissue. In the cerebellum they are most often associated with increased intracranial pressure, headaches, vomiting, and limb or truncal ataxia. Epididymal cysts may occur in 60% of male patients. Multiple renal cysts are very common and the lifetime risk of RCC is very high (70%). Less than 5% of VHL patients develop “Cafe au lait” macules and capillary malformations before the age of 40 (Table 3.1) [25].

VHL is classified in type 1 (without PHEO), type 2A (with PHEO), type 2B (with PHEO and RCC), and type 2C (with isolated PHEO without hemangioblastoma or RCC) [26].

Prevalence is estimated at 1/53,000 and incidence has been estimated as 1/36,000 live births. Men and women are equally affected. Mean age at diagnosis is 26 years [27].

3.3.2 Clinical Characteristics of VHL-associated Abdominal NETs

Pancreatic lesions are detected in more than 75% of the VHL patients submitted to abdominal imaging. These lesions are usually multiple and cystic with the macroscopic and histological features more frequently of true cyst, more rarely of the serous cystadenoma. The patient can remain asymptomatic and their finding can be incidental. P-NETs develop in about 10–17% of VHL patients in association or not with pancreatic cysts (Table 3.2). They are usually non-functioning and asymptomatic, although they may cause occasional pancreatitis or mass-derived pain. They can be associated with microscopic alterations of the endocrine pancreas: nesidioblastosis, islet dysplasia, peliosis or microadenomatosis [28]. In VHL patients P-NETs are usually discovered in the fourth decade of life. Generally, patients with VHL present a single P-NET, with a mean size of about 2.6–4.3 cm in diameter. VHL-associated P-NETs can be malignant in 8–50% of cases, presenting metastases to the liver in 9–37% of patients [29]. Pancreatic gastrinoma has been described. The malignancy of these tumors is frequent: more than 60% of P-NETs have signs of angioinvasion, perineural invasion, peritumoral adipose tissue infiltration and/or poor differentiation.

3.3.3 Diagnosis

3.3.3.1 Clinical Diagnosis

The clinical diagnosis of VHL is based on the presence of one single typical tumor (i.e., retinal or CNS hemangioblastoma or RCC) in association with a positive family history of VHL. In the case of a negative family history (about 20% of cases) the presence of multiple tumors (i.e., two hemangioblastomas, or a hemangioblastoma and an RCC) is required for diagnosis.

Conventional biochemical testing includes complete blood count, measurement of urinary catecholamine metabolites and urinalysis. Urine cytology may be indicative of polycythemia, PHEOs, renal anomalies, and RCC. Histological criteria distinguish VHL PHEOs from those occurring in MEN2. Magnetic resonance imaging (MRI), computed tomography (CT), EUS and a metaiodobenzylguanidine (MIBG) scan can be used [26].

3.3.3.2 Genetic Diagnosis

VHL syndrome is caused by inactivating germline mutations of the *VHL* tumor suppressor gene. More than 370 different inherited mutations have been identified in VHL patients. Genetic screening should include both the sequencing of the coding regions (exons 1-3) and intron-exon junctions, and the search for large germline deletions, as this latter kind of mutations is estimated to affect about 20–30% of VHL patients [30]. VHL type 1 clinical variant is often associated with truncating mutations [30]. A positive genetic test for a heterozygote *VHL* germline mutation establishes the diagnosis and enables genetic diagnosis for relatives.

3.3.4 Molecular Tumorigenesis

The *VHL* (OMIM *608537, genomic location 3p25.3) gene regulates important biological processes, such as cell-cycle control, mRNA stability, stability and orientation of microtubules, and, mainly, post-translational regulation of hypoxia-inducible protein expression. The VHL protein functions as part of a complex named VCB-CUL2, involved in targeting proteins to induce their degradation. In normal conditions, VCB-CUL2 targets and favors the degradation of the hypoxia-inducible factor 2-alpha (HIF-2α), a key protein of the HIF complex (which plays a critical role in cell ability to adapt to hypoxia). When the VHL protein is mutated or lost, the VCB-CUL2 complex cannot target HIF-2α to be broken down. The subsequent excess of HIF-2α stimulates cells to divide abnormally and activates the abnormal production of novel blood vessels favoring cyst and tumor development and maintenance in *VHL* mutation carriers.

3.3.5 Pharmacological and Surgical Therapy of VHL-associated Abdominal NETs

The treatment of choice for VHL syndrome is surgery with a multidisciplinary approach. Recommendations for the surgical management of P-NETs in VHL syndrome are derived from the review of the clinical experience of a large series [31]. Only in the presence of at least two risk factors (pancreatic tumor size >3 cm, tumor doubling time less than 500 days and *VHL* mutation in exon 3) is surgery indicated. Pancreatic resection is more frequently performed than simple enucleation [31]. However, the timing of the pancreatic surgery must be well chosen considering various factors: the presence of endocrine symptoms, the failure of medical control and the association with other potentially malignant tumors.

3.4 Tuberous Sclerosis (TSC)

3.4.1 General Overview

Tuberous sclerosis (TSC) is an autosomal dominant genetic chronic, life-long multisystem disorder. TSC is characterized by multisystem hamartomas, developed principally at the skin, brain, kidney, lung and heart, and appearing at different ages.

Skin involvement includes hypomelanotic macules (ash leaf) present within the first years of life, angiofibromas that appear at age of 3–4 as erythematous and papulonodular lesions, ungual fibromas, “confetti” skin lesions appearing during childhood to early adolescence, and cephalic and lumbar fibrous plaques (shagreen patch).

Brain lesions include cortico/subcortical tubers, radial migration lines, subependymal nodules, subependymal giant cell astrocytomas (SEGAs). SEGAs can cause hydrocephalus (growth risk higher in the first three decades of life). Early-onset epilepsy (infantile spasms and/or focal seizures) is present in 85% of patients. Disabling neurologic disorders have also been reported.

Renal angiomyolipomas (AMLs) develop during childhood with a higher risk of growth during adolescence and adulthood and manifest with pain, hematuria/retroperitoneal hemorrhage, abdominal masses, hypertension and renal failure.

Pulmonary manifestations include lymphangioleiomyomatosis (LAM), multifocal micronodular pneumocyte hyperplasia (MMPH) and pulmonary cysts that develop during adulthood and manifest with dyspnea, pneumothorax, or chylothorax.

Cardiac rhabdomyomas (CRs) are very common and often multiple, appear during the fetal period and may become symptomatic (outflow tract obstruction or by interfering with valvular function) during infancy and early childhood. Additional features include dental enamel pitting, intraoral fibromas and skeletal dysplasias [32–34].

The reported birth rate of TSC is 1 in 6,000. The estimated frequency in children under age of 10 ranges from 1 in 12,000 to 1 in 14,000 [34].

3.4.2 Clinical Characteristics of TSC-associated Abdominal NETs

Functioning and non-functioning P-NETs are both reported in a small percentage of patients with TSC. The largest clinical series of TSC has been recently published, including 2093 patients included in the TOSCA registry: five P-NETs were observed in five patients [35].

3.4.3 Diagnosis

3.4.3.1 Clinical Diagnosis

Diagnosis is based on the presence of major and minor clinical features, as recently updated in 2012 [36]. Major features include cortical dysplasias, subependymal nodules, SEGAs, hypomelanotic macules (at least three with a diameter ≥5 mm), shagreen patch, angiofibromas (at least three) or fibrous cephalic plaque, multiple retinal hamartomas, ungual fibromas (at least two), CRs, LAM and AMLs (at least two,). Minor features include dental enamel pits (at least three), intraoral fibromas, “confetti” skin lesions, non-renal hamartomas, multiple renal cysts, retinal achromatic patches.

A clear TSC diagnosis is defined in the presence of at least two major features, or one major feature with two minor features, or positive genetic testing. Possible TSC is considered in the presence of only one major feature, or one major and one minor feature, or two or more minor features [37]. Typical skin lesions are important in helping an early diagnosis (Table 3.1).

3.4.3.2 Genetic Diagnosis

TSC is caused by dominant germinal loss-of-function mutations in *TSC1* or *TSC2* genes. Somatic loss of the second wild type copy of the gene in target tissues is necessary for the development of tumors. The diagnosis of the disease is usually clinical. Genetic testing can help when the diagnosis is not clear by clinical criteria and to establish an early diagnosis, even in the absence of any signs or symptoms of the disease, in individuals from a *TSC1* or *TSC2* mutated pedigree. No genotype-phenotype correlation exists and therefore genetic testing cannot help to foresee the clinical presentation of the disease during lifetime. Nevertheless, genetic testing is necessary to determine the familial or sporadic occurrence of the disease [38].

3.4.4 Molecular Tumorigenesis

TSC1 gene (OMIM # 605284, genomic location 9q34.13) encodes hamartin, and TSC2 (OMIM # 191092, genomic location 16p13.3) gene encodes tuberin. These two proteins interact with each other to form a protein complex that inhibits signal transduction to the mammalian target of rapamycin (mTOR). Complete loss of wild type hamartin or wild type tuberin in cells is responsible for the constitutive activation of mTOR effector and other signaling elements, resulting in tumor development.

3.4.5 Pharmacological Therapy of TSC-associated Abdominal NETs

Inhibition of mTOR effector, constitutively activated by mutated *TSC1* or *TSC2*, can be achieved pharmacologically by rapamycin (sirolimus). This molecule proved to be effective to treat SEGAs not suitable for surgery in adults and children, and for the treatment of AMLs in adults. Everolimus, a derivative of sirolimus, has been successfully used to treat SEGAs [31, 32]. No data are available about the specific effect of sirolimus and everolimus in abdominal NETs in TSC.

3.4.6 Surgical Therapy of TSC-associated Abdominal NETs

The surgical approaches to TSC-associated abdominal NETs are similar to those followed for patients with abdominal NETs in MEN1 syndrome.

3.5 Neurofibromatosis Type 1 (NF1)

3.5.1 General Overview

NF1 is an autosomal dominant disorder characterized by cafe-au-lait spots (over 99%), cutaneous and subcutaneous neurofibromas (over 99%), hamartomas of

the iris (Lisch nodules; over 95%), skin-fold freckling (85%), optic pathway gliomas (15%), and an overall cancer risk higher than the general population. Osteopenia, osteoporosis, bone overgrowth, short stature, macrocephaly, scoliosis, skeletal dysplasia (sphenoid wing, vertebral), and pseudarthrosis may be present. Other clinical characteristics include hypertension, vasculopathy, intracranial tumors, malignant peripheral nerve sheath tumors, and, occasionally, seizures or hydrocephalus [39].

NF1 prevalence is reported to be 1 every 3,000 live births. Distribution in males and females is reported to be equal.

3.5.2 Clinical Characteristics of NF1-associated Abdominal NETs

Two types of abdominal NET can be observed in patients with NF1: duodenal somatostatinoma and P-NETs. The first tumors are the most frequent, they arise preferentially in the periampullary zone and cause jaundice, bleeding, anemia, weight loss. Duodenal somatostatinoma develops usually as single tumor and can reach several centimeters in size. They are almost always hormonally silent and do not cause a functioning somatostatinoma syndrome [29]. Also P-NETs occur in NF1, but they are extremely rare: only seven cases are described. Three of them were insulinomas, two non-functioning tumors, and two somatostatinomas that provoked the typical syndrome. Malignancy was present in the majority of these P-NETs.

3.5.3 Diagnosis

3.5.3.1 Clinical Diagnosis

The diagnosis of NF1 is defined by the presence of two or more of the following signs: six or more “cafe au lait” macules of significant size (Table 3.1), two or more skin neurofibromas and/or one plexiform neurofibroma, auxiliary or inguinal freckling, optic glioma, two or more iris Lisch nodules, osseous lesions such as sphenoidal dysplasia or pseudarthrosis, a first-degree relative with one or more of the previous lesions [39, 40]. CT scan, MRI, radiographs and ultrasound are essential in the diagnosis and follow-up of the disease (Table 3.3).

3.5.3.2 Genetic Diagnosis

Inactivating dominant germline mutations in the tumor suppressor *NF1* gene have been found in 20–60% of patients and families diagnosed as NF1 [41]. More than 1,000 *NF1* mutations associated with NF1 syndrome have been identified, and most of them are unique to a particular family. Nevertheless, mutation screening of the *NF1* gene remains difficult, time-consuming and expensive. In addition, mutations are distributed all along the entire gene and about 50% of NF1 cases result from neo-mutations [30]. The diagnosis of NF1 is almost always made by clinical findings, particularly after the age of 8. Genetic testing is useful

for confirmation of the clinical diagnosis and for reproductive counseling. No genotype-phenotype is described and the clinical characteristics are highly variable even within the same family.

3.5.4 Molecular Tumorigenesis

The *NF1* (OMIM #613113, genomic location 17q11.2) gene encodes a protein named neurofibromin, a cytoplasmic protein that is predominantly expressed in neurons, Schwann cells, oligodendrocytes, and leukocytes. In normal conditions, neurofibromin acts as tumor suppressor protein, preventing cell overgrowth by acting as a GTPase, and turning off RAS protein from the active Ras-GTP form to the inactive Ras-GDP form. Many *NF1* mutations produce a short version of neurofibromin that is not able to perform its normal activity and inhibit cell division.

3.5.5 Pharmacological Therapy of NF1-associated Abdominal NETs

Treatment of abdominal NETs associated with NF1 is principally surgical. Due to the rarity of these clinical manifestations in this syndrome, no specific trials are reported and pharmacological therapy, when used, is the same as approved for the sporadic tumors.

3.5.6 Surgical Therapy of NF1-associated Abdominal NETs

Duodenal somatostatinomas in NF1 can be treated either by a local excision if the size is small or by duodenopancreatectomy in the case of large dimension, or if local invasion or suspected malignancy are present. The P-NETs must be treated by pancreatic resection and regional lymphadenectomy considering the great potential for malignancy of the tumors [42].

3.6 Mahvash disease

3.6.1 General Overview

Mahvash disease (MD) is a newly discovered [43] very rare autosomal recessive hereditary pancreatic neuroendocrine syndrome characterized by pancreatic alpha cell hyperplasia, P-NETs, severe hyperglucagonemia not associated with glucagonoma syndrome, and mild hypoglycemia.

Due to extreme rarity of MD, data about its prevalence are not available. Only six cases have been reported thus far [43].

3.6.2 Clinical Characteristics of MD-associated Abdominal NETs

P-NETs represent the most important feature of MD, usually with a malignant potential. Patients develop gross P-NETs in their middle age, and the initial symptoms are usually abdominal pain and discomfort. Post-surgical recurrence is not evident in 2–9 years [43]. Patients need life-long surveillance for P-NETs.

3.6.3 Diagnosis

3.6.3.1 Clinical Diagnosis

MD is very rare but it should be suspected in patients with P-NETs associated with extremely high glucagon level without glucagonoma syndrome and with normal glycemia or even mild hypoglycemia. The absence of glucagonoma syndrome, even with very high glucagon level, is a distinctive diagnostic marker of the syndrome.

3.6.3.2 Genetic Diagnosis

MD is caused by homozygous, inactivating mutation of the glucagon receptor gene (*GCGR*). A subject has to bear a mutation on both GCGR alleles to develop the disease. Genetic screening is strongly recommended in individuals with P-NETs, hyperglucagonemia but not glucagonoma syndrome for the diagnosis and the correct therapy.

3.6.4 Molecular Tumorigenesis

GCGR (OMIM *138033; 17q25.3) gene encodes for the glucagon receptor protein (GCGR). Loss-of-function homozygote mutations render the receptor unable to work properly, resulting in a constitutive signal for the pancreatic alpha cells to produce and secrete more glucagon, even when not necessary. Conversely to other genetic syndromes, such as MEN1 and VHL, pancreatic cells are “normal” and they are not necessarily induced to proliferate; hyperplasia can be the result of hyperglucagonemia-induced neogenesis rather than proliferation [43]. The stochastic occurrence of secondary mutations in these constantly hyperplastic cells can act as a trigger to initiate pancreatic tumorigenesis.

3.6.5 Therapy

Untreated MD may cause severe hypoglycemia, cachexia, and early death. MD patients should undergo surgery to remove P-NETs. Pharmacological treatment consists of SSAs to suppress glucagon over-production, and synthetic chaperones contrasting the abnormal glucagon receptor trafficking.

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Sporadic Gastroenteropancreatic Neuroendocrine Tumors

4

Marialuisa Appetecchia, Rosa Lauretta, Francesca Rota, and Massimo Carlini

More than 90% of gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are sporadic. They are usually classified into *functioning* and *non-functioning*.

4.1 Functioning GEP-NETs

Functioning GEP-NETs synthesize and secrete peptides and amines [1, 2]. Clinical presentation has specific features. The main clinical aspects of each tumor are reported in Table 4.1.

4.1.1 Carcinoid

Carcinoid tumors secrete vasoactive peptides and amines (such as serotonin and tachykinins) into the systemic circulation resulting in carcinoid syndrome (CS). CS is usually present in patients with GEP-NETs arising in the midgut (i.e., small intestine, appendix, and proximal colon), and less commonly in the pancreas and lung [3, 4]. CS rarely occurs in the absence of hepatic metastases, except when the tumor products drain directly into the systemic circulation, as in the case of lung NETs or patent foramen ovale [5].

CS is classified as *typical*, characterized by diarrhea, abdominal pain and flushing (95% of cases) [6, 7], due to the release of serotonin, and *atypical* (5%), in which the clinical picture is variable depending on the bioactive substances secreted (serotonin, tachykinins, prostaglandins and kallikrein).

M. Appetecchia (✉)
Endocrinology Unit, Regina Elena National Cancer Institute
Rome, Italy
e-mail: marialuisa.appetecchia@ifo.gov.it

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Table 4.1 Main clinical aspects of functioning GEP-NETs

Tumor	Biologically active peptide	Tumor sites (abdominal)	Main symptoms/signs
Carcinoid syndrome	Serotonin, tachykinins	Midgut, pancreas	Diarrhea, abdominal pain, flushing
Gastrinoma	Gastrin	Duodenum, pancreas	Pain, diarrhea
Insulinoma	Insulin	Pancreas	Hypoglycemic symptoms (100%)
Glucagonoma	Glucagon	Pancreas	Specific dermatitis, glucose intolerance, weight loss
Somatostatinoma	Somatostatin	Pancreas, duodenum/jejunum	Diabetes mellitus, cholelithiasis, diarrhea
VIPoma	VIP	Pancreas	Diarrhea, hypokalemia, dehydration
PPoma	PP	Pancreas	Asymptomatic or diarrhea
GRFoma	GHRH	Pancreas, jejunum	Acromegaly
ACTHoma	ACTH	Pancreas	Cushing's syndrome
PTHrpoma	PTHrp	Pancreas	Diarrhea
RFT-CT	CT	Pancreas	Hypercalcemia
RFT-LH	LH	Pancreas	Anovulation, virilization (female); reduced libido (male)
RFT-renin	Renin	Pancreas	Hypertension
RFT-GLP1/IFG2	GLP1/IFG2	Pancreas	Hypoglycemia
RFT-erythropoietin	Erythropoietin	Pancreas	Polycythemia
RFT-CCK	CCK	Pancreas	Diarrhea, weight loss, peptic ulcer

GEP-NET, gastroenteropancreatic neuroendocrine tumor; *RFT*, rare functional tumor; *VIP*, vasoactive intestinal peptide; *PP*, pancreatic polypeptide; *GHRH*, growth hormone releasing hormone; *ACTH*, adrenocorticotropic hormone; *PTHrp*, parathyroid hormone-related protein; *CT*, calcitonin; *LH*, luteinizing hormone; *GLP1*, glucagon like peptide 1; *IGF2*, insulin-like growth factor II; *CCK*, cholecystokinin.

Diarrhea has a chronic course, is mainly secretory, does not improve with fasting and it is associated with electrolyte imbalances. The stools are usually watery, due to an increased peristalsis and hypersecretion [8, 9].

Abdominal pain occurs in about half of patients with CS, it may be intermittent, crampy or dull and it is not relieved by defecation. Flushing is the most frequent symptom, accentuated by food and alcohol intake, physical exercise and emotional states.

The characteristics of the flushing are particular: face, neck and upper chest take on a red color with typically dry skin [10]. The flushing may be associated with transient hypotension and bronchospasm. The overproduction of serotonin, a feature of CS, if not treated effectively, can lead to complications [11] such as muscle wasting and proximal myopathy [12, 13], niacin deficiency [14] and cognitive disorders [15–18].

Carcinoid heart disease (Hedinger's syndrome) is one of the most common and critical aspects of CS, present in 10–20% of patients at diagnosis. Hedinger's syndrome causes thickening of the heart valves, altering cardiac function, cardiac fibrosis and consequent right heart failure [19–21]. Up to 50% of deaths related to CS are due to heart failure.

Atypical CS is characterized by prolonged flushing, bronchospasm, headache, watery eyes, wheezing, and hypotension. Atypical CS is due to the production of 5-hydroxytryptophan and histamine instead of serotonin [22]. The carcinoid crisis is the extreme manifestation of CS, it is life-threatening and is in fact considered an oncological emergency [23]. It is caused by the massive release into the circulation of amine after anesthesia, interventional procedures or intake of drugs [24]. The main features are hypotension (rarely hypertension), tachycardia, dyspnea and dysfunction of the central nervous system [25].

4.1.2 Gastrinoma

Gastrinoma occurs as a result of hypersecretion of gastrin, generally by a GEP-NET of the duodenum or pancreas and rarely by other NETs (i.e., thymus) [26]. Gastrinoma is the most common functioning pancreatic NET (P-NET) [27–30]. This tumor is sporadic, with duodenal localization, in 50–88% of cases, or occurs in the context of multiple endocrine neoplasias type 1 (MEN1) in 25–30% of the cases, with duodenal localization in 70–100% [1, 31]. In rare cases gastrinoma occurs in other abdominal non-pancreatic, non-duodenal (stomach, liver, bile duct, ovary) (5–15%) and extra-abdominal (heart, small cell lung cancer) sites [28, 32–34]. The syndrome is suspected in patients with peptic ulcers refractory to proton pump inhibitors and who complain of diarrhea. In fact, stomach acid hypersecretion, due to the production of gastrin by the neoplasia, causes the Zollinger-Ellison syndrome [27, 35–37]. The main symptoms are due to peptic ulcer disease or severe gastroesophageal reflux disease with abdominal pain (75–98% of cases), nausea/vomiting (12–30% of cases), heartburn (44–56% of

cases), diarrhea (30–73% of cases) and weight loss (7–53% of cases) [27, 28, 35–38]. Only 33% of patients present with bleeding, obstruction, penetration, perforation, all complications of peptic ulcer at diagnosis [35, 36, 39]. Only in the presence of advanced disease, found in a small percentage of patients, are the symptoms and signs due to the tumor burden itself (i.e., pain, jaundice, bleeding) [27, 35, 36, 39].

4.1.3 Insulinoma

Insulinoma is the most common functioning NET of the pancreas. In less than 10% of cases it is malignant, in approximately 10% of cases it is multiple, and in about 5% of cases it is associated with the MEN1 syndrome [27, 30, 40–43]. Tumor-induced hyperinsulinemia causes hypoglycemia. This leads to neuroglycopenia of the central nervous system (90%) which results in confusion, forgetfulness, coma, visual changes, altered consciousness or coma. Most patients also have symptoms due to the adrenergic stimulation secondary to the hypoglycemia (60–70%), such as sweating, tremors, palpitations, weakness, hyperphagia [27, 44–46].

These symptoms usually occur during fasting, delay in meals or during exercise. The presence of one of the above symptoms, especially associated with fasting or exercise must arouse a suspicion of hypoglycemia. Insulinoma should be suspected if Whipple's triad is present: symptoms of hypoglycemia, documented hypoglycemia (plasma glucose ≤40 mg/dL) and relief of symptoms with glucose administration [31, 47].

4.1.4 Established Rare Functioning P-NETs (RFTs)

Other well-described and established functioning P-NETs are a group called rare functioning tumors (RFTs) [27, 30, 48, 49]. RFTs represent less than 10% of all P-NETs [27, 50]. They can occur in the pancreas or in other sites such as the small intestine, lung or paraganglia [27, 29, 48–51]. Each of the established RFTs is associated with a distinct clinical syndrome reflecting the actions of the ectopically secreted hormone. The majority of patients with RFTs of the pancreas presents with metastatic disease (40–90%) to the liver [32].

4.1.4.1 Glucagonoma

The most frequent familial condition associated with RFT is MEN1, where glucagonoma is present in 3% of patients [40, 52]. The excess of circulating glucagon levels generally causes a specific dermatitis (necrolytic migratory erythema, NME) (55–90%), weight loss (60–90%), diabetes mellitus or glucose intolerance (30–90%), mucosal abnormalities (glossitis, cheilitis, stomatitis) (30–40%), diarrhea (10–15%) [44, 53–55].

4.1.4.2 Somatostatinoma (SSoma)

Somatostatinoma (SSoma) can occur in the pancreas (55%) or proximal small intestine (44%). However, the duodenal tumor secreting somatostatin is rarely associated with a functional clinical syndrome [27, 56, 57]. Patients with MEN1 present SSoma in <1% of cases [40, 52] while, especially in the duodenal periampullary localization, SSoma is seen in up to 10% of patients with von Recklinghausen's disease (neurofibromatosis 1, NF1), but in almost all cases it is not associated with a functional syndrome. The somatostatinoma syndrome is characterized by diabetes mellitus, gallbladder disease, diarrhea, weight loss and steatorrhea [27, 44, 58].

4.1.4.3 Vasoactive Intestinal Peptide Tumor (VIPoma)

Vasoactive intestinal peptide tumor (VIPoma) is present in 3% of patients with MEN1 [40, 52]. This tumor is located in the pancreas in 90% of cases. VIP can also be rarely secreted by tumors of neural crest (10%) with paravertebral and adrenal location. VIPoma characteristically presents with Verner-Morrison syndrome, characterized by large volume watery secretory diarrhea (10–15 motions per day, up to a volume of 10 liters) that leads to the development of dehydration and hypokalemia [27, 44, 59]. Initially, the diarrhea may be intermittent, then progressively severe and uncontrollable. The excess of VIP secretion may also lead to hyperglycemia (20–50%), hypercalcemia (25–50%), hypochlorhydria (20–50%) and flushing (15–30%) [27, 44, 59].

4.1.4.4 Pancreatic Polypeptide Tumor (PPoma)

Increasing pancreatic polypeptide (PP) cells are found in 20–67% of functioning and non-functioning tumors of the pancreas [60]. The number of cells and hormone levels are not related, because subnormal, normal or supernormal concentrations of circulating PP can be found in insular tumors and in healthy pancreatic tissue. The only recognized physiologic effects in humans are inhibition of gallbladder contraction and pancreatic enzyme secretion [61]. PPoma is generally asymptomatic and when symptomatic it causes a diarrheal syndrome [62].

4.1.4.5 Growth Hormone-releasing Factor Tumor (GRFoma)

Growth hormone-releasing factor tumor (GRFoma) is localized in the pancreas (30%), lung (54%), jejunum (7%) and other sites (13%). This tumor, given the secretion of growth hormone-releasing hormone (GHRH), clinically resembles acromegaly. Like acromegaly, it often occurs primarily with alteration of the menstrual cycle in women and erectile dysfunction in men, macroglossia, dental malocclusion, prognathism and stiffness of the joints, as well as the increase in size of the hands and feet [27, 44, 63].

4.1.4.6 Adrenocorticotropic Hormone Tumor (ACTHoma)

Adrenocorticotropic hormone tumor (ACTHoma) represents 4–16% of all cases of ectopic Cushing's disease and is localized in the pancreas [32]. Patients present

the clinical features of Cushing's disease, such as visceral obesity, hypertension, diabetes, osteoporosis and psychiatric disorders.

4.1.4.7 Parathyroid Hormone-related Protein Tumor (PTHrPoma)

Parathyroid hormone-related protein tumor (PTHrPoma) is a very rare tumor caused by the production by the pancreas of the parathyroid hormone-related protein (PTHrp) that causes symptoms related to hypercalcemia, such as constipation, nausea, gastric hyperacidity (hypercalcemia increases gastrin secretion), abdominal pain, vomiting, psychological disorders (depression, confusion, apathy, lethargy to coma), and weakness. Severe hypercalcemia is associated with severe symptoms that may constitute a real medical emergency (serious arrhythmias, coma, renal failure) [64, 65].

4.1.5 Possible Rare Functioning P-NET Syndromes

There are other RFTs that can cause a specific syndrome, but the series reported in the literature are small. In addition, there is disagreement as to whether the characteristics described actually correspond to a real syndrome. Five possible RFTs are reported in P-NETs: secreting calcitonin, renin, luteinizing hormone (LH), erythropoietin and insulin-like growth factor II (IGF2) [27, 30, 48–51].

Calcitonin-secreting pancreatic endocrine tumors have been described as extremely rare (66, 67). Most are asymptomatic. When symptomatic, the most frequent symptoms are watery diarrhea (51.4%) and abdominal pain (35.1%). Most patients (59.5%) present with metastatic spread at the time of diagnosis [68].

In the literature very rare cases of LH-secreting pancreatic neuroendocrine tumors are reported. These cause libido alterations, menstrual abnormalities, hirsutism and infertility [67, 69]. Other cases of ectopic secretion of renin with hypertension [70], glucagon-like peptide 1 (GLP-1) [71] and IGF-2 [72] with hypoglycemia symptoms, and erythropoietin with polycythemia have also been reported [73]. Recently, a case of cholecystokinin-secreting P-NET with metastatic disease has been described, which presented with non-watery diarrhea, severe weight loss, gallbladder and peptic ulcer disease, but normal gastrin level [74].

4.2 Non-Functioning GEP-NETs

Non-functioning NETs are not associated with the presence of circulating specific hormonal substances and, for this reason, do not manifest with characteristic symptoms. Non-functioning NETs give clinical signs when they are large enough to cause compression or invasion of adjacent organs or when they metastasize [75]. These tumors may still cause non-specific symptoms attributable to the

tumor burden, such as abdominal pain (35–78%), anorexia and nausea (45%), weight loss (20–35%).

Less frequent signs are intra-abdominal hemorrhage (4–20%), jaundice (17–50%) or palpable mass (7–40%). Non-functioning NETs may secrete bioactive hormones or amines at subclinical levels [76–78] such as pancreatic polypeptide, chromogranin A, neuron-specific enolase, human chorionic gonadotrophin subunits, calcitonin, neurotensin or other peptides, but their serum concentrations are insufficient to induce specific symptoms [25, 27, 48, 50, 51, 79]. For these reasons, they are often diagnosed late or incidentally in the context of routine imaging studies performed for other conditions. In these cases the neuroendocrine origin of the tumor can be confirmed only at immunohistochemistry.

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Part II

Diagnosis

Neuroendocrine Tumors Biomarkers

5

Marialuisa Appetecchia, Rosa Lauretta, Francesca Rota, and Massimo Carlini

The diagnosis of neuroendocrine tumors (NETs) is challenging due to the non-specificity of the symptoms. Since NETs produce and secrete a large variety of bioactive substances, it is important to identify those that can be used in clinical practice as biomarkers with a diagnostic, prognostic or predictive role. An ideal diagnostic biomarker should have high sensitivity and specificity, as well as the ability to discriminate the tumor site and the stage of disease; in addition, it should have prognostic significance and be able to be used in the follow-up to evaluate the effectiveness of therapy and the progression or relapse of the disease.

Neuroendocrine markers are divided into *non-specific*, present in all NETs, and *specific*.

5.1 Non-Specific Biomarkers

The most important non-specific NET marker is chromogranin A (CgA). CgA is part of the granin family, which includes eight proteins: CgA, CgB or secretogranin I (SgI), CgC or secretogranin II (SgII), SgIII, SgIV, SgV or 7B2, SgVI or NESP55 (neuroendocrine secretory peptide), and VGF [1–4]. These proteins are the principal components of dense-core secretory vesicles in neuroendocrine cells, probably play an important role in regulating the function of secretory granules and are precursors of biologically active peptides. In clinical practice only CgA is used, an acidic protein of 439 amino acids with a molecular mass of 48 kDa. CgA contains a disulfide bridge in the N-terminal region, which is used for some CgA-related activities, and 10 pairs of basic amino acids, which, along with other sites in the molecule, can be subjected to cleavage by endogenous

M. Appetecchia (✉)
Endocrinology Unit, Regina Elena National Cancer Institute
Rome, Italy
e-mail: marialuisa.appetecchia@ifo.gov.it

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proteases. Several biologically active peptides derive from proteolysis of CgA, such as pancreastatin, catestatin and vasostatins I and II. CgA and CgA-related peptide perform many biological activities. In fact, even though their role is not definitively clarified, they act on endocrine organs such as parathyroid glands, adrenal glands and endocrine pancreas, and on the cardiovascular system and adipose tissue.

CgA is a pan-neuroendocrine marker and it is the best available biomarker for NETs. However, several problems are related to its use in clinical practice and so it is far from the ideal marker. CgA levels are abnormal when they exceed by two-threefold the upper normal range. As CgA levels increase after food intake, they should be tested in fasting patients [2]. The determination of CgA may be done on plasma or serum, since a strong positive linear relationship has been reported between both types of tests ($r = 0.9858$, $P < 0.0001$) [5]. There are several commercial assays for the measurement of circulating CgA concentrations. Stridsberg et al. compared three methods of assay of CgA: CgA RIA-CT (CIS Bio International, Gif-sur-Yvette Cedex, France), Dako CgA ELISA kit (Dako A/S, Glostrup, Denmark) and CgA EuroDiagnostica (ED) (Malmö, Sweden). CgA was measured with the three methods in 77 patients. Forty-six patients had NETs, 31 patients were considered not to have NET or to be tumor-free after radical surgery.

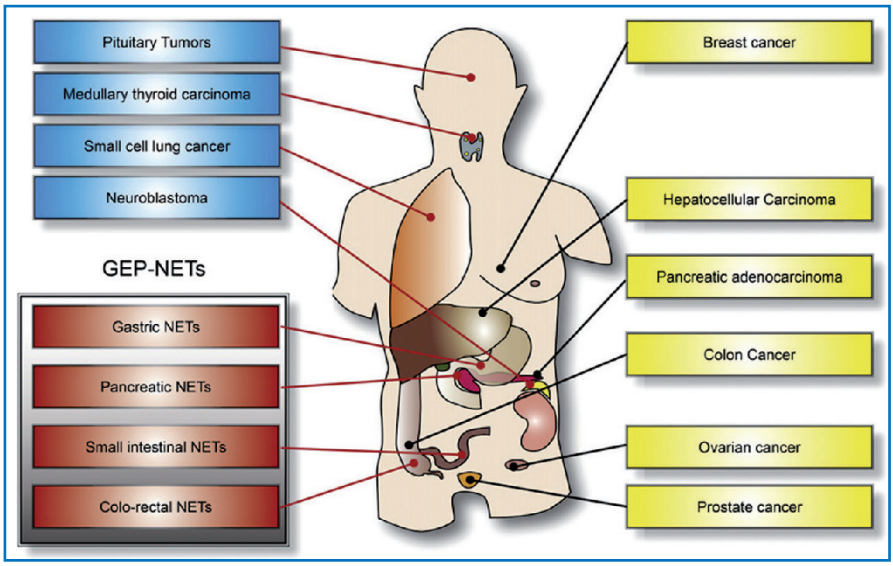

Fig. 5.1 Neoplastic causes of elevated Chromogranin A (CgA). CgA elevations occur in different types of NETs but are usually more pronounced in GEP-NETs (small intestinal, gastric, and pancreatic NETs). CgA elevations may occur in carcinomas with a complete or a partial neuroendocrine phenotype (left and right box stacks, respectively). In HCC, the cause of CgA elevation may reflect impaired metabolism of CgA fragments due to concurrent liver failure (Reproduced with permission from [7])

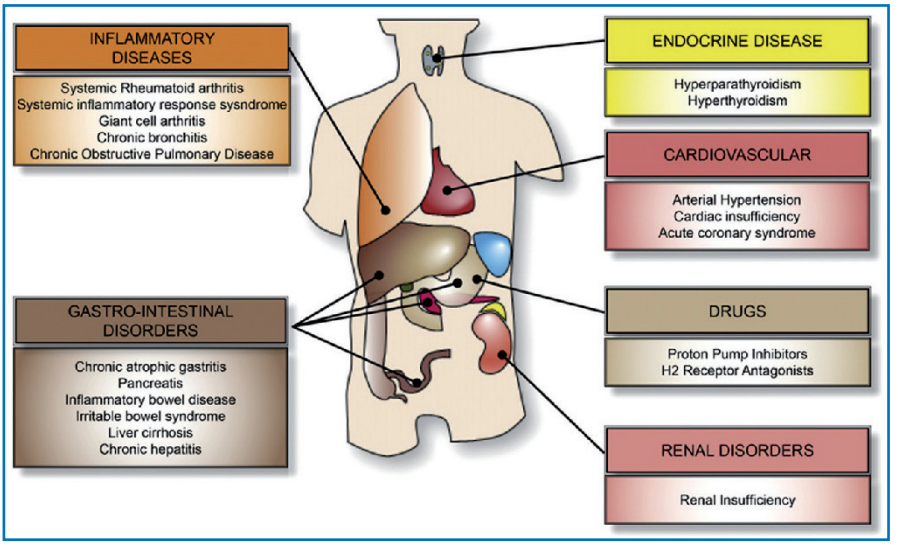

Fig. 5.2 Non-neoplastic causes of Chromogranin A (CgA) elevation. CgA is elevated in endocrine diseases, chronic and acute inflammation, and cardiac insufficiency. Acid-suppressive medications result in hypergastrinemia (G cell and ECL cell hyperplasia) and a concomitant increase in cosecreted CgA. Renal failure increases detectable plasma CgA (p-CgA) by reducing glomerular filtration of CgA-related peptides. P-CgA alone cannot discriminate between GEP-NETs, pancreatitis, inflammatory bowel disease, irritable bowel syndrome, or hepatitis (Reproduced with permission from [7])

The results obtained with different methods are not comparable so, in the same patient, CgA should always be measured always with the same method [6]. Although, among all markers, CgA presents the best combination of sensitivity and specificity, there are numerous cases of false negatives and especially false positives. In fact CgA levels increase in several neoplastic and non-neoplastic conditions [2, 7] (Figs. 5.1 and 5.2). Some non-neuroendocrine carcinomas, such as prostate cancer, small-cell lung cancer, breast cancer, colorectal cancer, may have a neuroendocrine differentiation and be associated with increased levels of CgA [8–11]. Higher CgA values were even detected in pancreatic adenocarcinoma and hepatocellular cancer, but the pathophysiological meaning is unknown [2, 7, 12]. Increased CgA levels were also found in endocrine disease such as pheochromocytoma, hyperthyroidism, hyperparathyroidism, pituitary tumors, medullary thyroid carcinoma, and in non-neoplastic conditions, i.e., gastrointestinal disorders, cardiovascular and inflammatory diseases [2, 7]. However, the most common causes of false positive results in clinical practice are renal failure and drugs. Decreased CgA clearance can increase circulating CgA levels in proportion to the degree of renal failure, up to values similar to those found in NETs.

Proton pump inhibitors (PPIs) and, to a lesser extent, H2-receptor antagonists, are a frequent cause of CgA elevation. PPIs inhibit gastric acid secretion, enhancing gastrin release by the antral G cells. The resultant hypergastrinemia

causes hyperplasia of enterochromaffin-like neuroendocrine cells with consequent increase in CgA. CgA levels increase more than tenfold with PPIs use. The PPI effects are early, presenting within the first six days after the start of therapy, and persist for 1–2 weeks after discontinuation of the drug [13].

CgA, however, lacks specificity and is not to be used for population screening in the absence of strong clinical or radiological evidence of tumor presence [14]. The guidelines recommend CgA serum determination for the diagnosis and follow-up of all NETs [15–19]. A recent meta-analysis demonstrated that circulating CgA was an efficient biomarker for the diagnosis of NETs with high sensitivity and specificity (73% and 95%, respectively) [20]. Many studies investigated the diagnostic accuracy of CgA compared to other general biomarkers. Bajetta et al. studied the role of CgA, neuron-specific enolase (NSE), carcinoembryonic antigen (CEA), and urinary 5-hydroxyindole-3-acetic acid (5-HIAA) in 127 patients with NETs, including gastroenteropancreatic NETs (GEP-NETs). CgA was the best marker (a specificity of 85.7% and sensitivity of 67.9%) compared to NSE, CEA and 5-HIAA. CEA had a sensitivity of only 15.4%; NSE and 5-HIAA showed a very high specificity (100%) but a lower sensitivity (32.9% and 35.1%, respectively) [21].

Other studies demonstrated a better diagnostic accuracy of CgA than NSE, 5-HIAA, α-subunit of glycoprotein hormones and pancreatic polypeptide. There is scientific evidence that the simultaneous detection of CgA and pancreatic polypeptide significantly improves the sensitivity of CgA (96% vs. 84%) in patients with GEP-NETs [22]. Some studies suggested an association between type of NET and increased CgA levels. The highest values were found in ileal NET and GEP-NET associated with MEN1, whereas intermediate values were detected in functioning and non-functioning pancreatic NET, type II and III gastric NET and Zollinger-Ellison syndrome in MEN1. Type I gastric NET, pituitary, and parathyroid tumors have lower levels [2].

The diagnostic accuracy of CgA measurement also varies with the degree of differentiation, being more frequently elevated in well-differentiated tumors than in poorly differentiated ones [2]. This may be due to the greater functional integrity of the secretory system in more differentiated neuroendocrine cells. In a study on 63 NET patients including 35 patients with GEP-NETs, the sensitivity of CgA levels for detecting well-differentiated carcinoids, well-differentiated neuroendocrine carcinomas and poorly differentiated neuroendocrine carcinomas was 58%, 68% and 37%, respectively. The specificity was 100% for patients with well-differentiated carcinoids and neuroendocrine carcinomas, but only 67% for well-differentiated neuroendocrine carcinomas [23].

In well-differentiated NETs, there was indirect evidence for a prognostic role of CgA. In fact, the CgA levels were related to tumor stage and advanced stages were associated with reduced survival. CgA concentrations were higher in patients with extensive metastases than in those with localized disease or with limited hepatic metastases [2]. Gastrinomas may be an exception because they are associated with high circulating CgA levels even in the absence of hepatic

involvement [24]. In GEP-NETs the diagnostic accuracy of CgA is higher in functioning versus non-functioning tumors [25], as well as in metastatic versus loco-regional NETs and in well-differentiated versus poorly differentiated tumors [2].

It has been suggested that, due to the CgA-related tumor burden, a change in CgA concentrations may indicate a response to treatment. To assess whether CgA is a predictive marker of response to treatment with somatostatin analogs (SSAs), the octreotide test was developed. CgA levels are measured 0, 3 and 6 hours after the i.v. injection of 200 mcg octreotide. Massironi et al. evaluated whether plasma CgA levels in response to the octreotide test predicted the clinical response to SSAs in GEP-NET patients. They concluded that a decrease of CgA greater than 30% after the octreotide test identifies the patients most likely to be responsive to SSA therapy [26]. It should be noted that SSAs reduce CgA as a result of its effect not only on tumor burden, but also on the secretory activity of neuroendocrine cells [27]. Therefore, the reduction of disease burden can be evaluated through CgA only if the dose of SSAs does not vary over time [28]. In pancreatic NET patients treated with everolimus in the RADIANT-1 study, increased baseline CgA and NSE circulating levels were predictive markers of survival and progression-free survival [29, 30]. Jensen et al. showed that a reduction in CgA circulating levels greater than or equal to 80% following cytoreductive surgery for a carcinoid tumor with hepatic metastases was predictive of subsequent complete symptom resolution and disease control. Substantial reduction in CgA was associated with improved patient outcomes, even after incomplete cytoreduction [31]. Recently, it was confirmed that CgA could have a role in identifying disease recurrence. In 152 patients with jejunal, ileal and pancreatic NETs, CgA proved to be a predictor of disease recurrence 6 months before radiological progression, according to RECIST 1.1 criteria [32]. Massironi et al. reported increased CgA levels 9–12 months prior to clinical and radiological relapse in 15 GEP-NET patients who recurred after radical surgery [25].

Another unspecific marker used in NETs is NSE, which is the neuron-specific isomer of the glycolytic enzyme 2-phospho-D-glycerate hydrolyase or enolase. NSE is found in neurons and neuroendocrine cells. Circulating NSE levels have been reported to be increased in patients with thyroid cancer, prostate carcinoma, neuroblastoma, small cell lung carcinoma (SCLC), and pheochromocytoma. NSE has a very good sensitivity in SCLC and a good discriminatory power between SCLC and non-small cell lung cancer [33]. NSE levels are increased in 30-50% of NET patients, particularly in those with a poorly differentiated tumor [1, 34, 35]. However, because of its low diagnostic accuracy, NSE is inadequate for diagnostic and prognostic use [36–38].

Pancreatic polypeptide (PP), secreted by the PP cells of the pancreatic islet cells, is a marker with low diagnostic accuracy (63%sensitivity and 81%specificity) [39], but in the diagnosis of GEP-NETs, a combined assessment of PP and CgA leads to a significant increase in sensitivity, particularly in non-functioning pancreatic NETs [22].

Other unspecific markers, such as alpha and beta subunits of human chorionic gonadotropin (hCG) have a limited clinical usefulness [40].

A recent consensus agreed that current general blood biomarkers, including CgA, are inadequate [36-38], and new biomarkers have been proposed, including circulating tumor cell and multianalyte biomarkers, such as microRNA and mRNA [36–38, 41–43]. An mRNA-based, specific multianalyte assay with algorithmic analyses has been shown to have better sensitivity and specificity than CgA in initial clinical studies. [42, 43].

5.2 Specific Biomarkers

The specific markers are typical of the functioning NETs and vary according to the tumor hormone production, which causes a specific clinical syndrome.

5.2.1 Serotonin

Serotonin (5-HT) is a biogenic amine derived from tryptophan. It is stored and secreted by enterochromaffin cells of the gastrointestinal tract (80%), platelets (storage only) and serotoninergic neurons of the central nervous system. 5-HT is a potent vasoconstrictor and acts as a regulator of gastrointestinal motility, mood, appetite and sleep. The urinary metabolite of serotonin is 5-hydroxyindole acetic acid (5-HIAA) which is particularly useful in the diagnosis and follow-up of patients with carcinoid syndrome [44]. High-performance liquid chromatography (HPLC) is the currently recommended assay for the measurement of urinary 5-HIAA.

As the determination of this metabolite is sensitive, the sample should be kept away from direct light and refrigerated [28]. Written instructions should

Table 5.1 Confounding agents of urinary 5-HIAA levels

Increase 5-HIAA levels		Decrease 5-HIAA levels
Medication	**Food**	**Medication**
Acetaminophen	Avocado	Aspirin
Antihypertensive	Banana	Ethyl alcohol
Caffeine	Eggplant	Heparin
Diazepam	Pineapple	Imipramine
Ephedrine	Plantain	Isoniazid
Glyceryl guaiacolate	Plum	Levodopa
Nicotine	Tomato	MAO inhibitors
Phenobarbital	Walnut	Methyldopa, tricyclic antidepressants

From: Albertelli M, Campana D, Pelosi G (2016) Marker tumorali. Select 2, 2016 (reprinted with permission of Thenewway Srl).

be handed out to patients including food and medications that could falsely increase urinary 5-HIAA levels [45]. A diet free of these confounding agents should be carried out within three days before the urine collection [45–47] (Table 5.1). Certain co-morbidities or associated disorders may have effects on the concentration of 5-HIAA. Falsely low 5-HIAA levels may be encountered in patients with renal impairment and those on hemodialysis. 5-HIAA may increase in untreated patients with malabsorption [48–50].

Overall sensitivity and specificity of urinary 5-HIAA is 70% and 90%, respectively [48]. Monitoring levels of 5-HIAA allows checking of the secretory activity of carcinoid tumors and serves as an objective marker of biochemical response to treatment with antisecretory agents such as somatostatin analogs [1]. As an intra-individual variability in 5-HIAA values exists, especially in the diagnostic phase, it is recommended to carry out the examination twice so as to obtain an average value of 5-HIAA [28].

5.2.2 Gastrin

Gastrin is a peptide hormone that stimulates secretion of gastric acid (HCl) by the parietal cells of the stomach and acts in gastric motility. It is released by G cells in the pyloric antrum of the stomach, in the duodenum and pancreas. Zollinger-Ellison syndrome (ZES) is caused by gastrin producing tumors (gastrinomas) and is characterized by recurrent peptic ulcers and secretory diarrhea. The diagnostic marker of this condition is fasting serum gastrin (FSG), which is usually elevated (more than tenfold the upper limit of normal (ULN) in the presence of a low gastric pH [24, 51, 53, 56, 57]. FSG alone is not adequate to make the diagnosis of ZES because hypergastrinemia may be present in achlorhydric patients with chronic atrophic fundus gastritis and in other conditions associated with hyperchlorhydria (i.e., *Helicobacter pylori* infection, gastric outlet obstruction, renal failure, antral G-cell syndromes, short bowel syndrome, retained antrum) [51–53].

The chronic use of proton pump inhibitors (PPIs) [52–55] leads to high FSG levels and a gastrin provocative test is needed to establish the diagnosis of ZES [24, 51, 52, 56, 57] as well as the assessment of the gastric pH [58].

The current recommended criterion for the diagnosis of ZES depends on FSG elevation (24, 51, 53, 58). In the presence of hypergastrinemia (FSG: two- to tenfold the ULN or greater than tenfold the ULN) with gastric pH less than 2, a complete gastric analysis is recommended prior to performing a secretin test [24, 51, 58, 59].

The patient must undergo esophagogastroduodenoscopy (EGDS) with gastric antral and fundus biopsies ± a serum test for antiparietal and intrinsic factor antibodies to exclude an atrophic fundus gastritis, *Helicobacter pylori* testing and 24-hour pH-metry (basal acid output pre and post secretin is recommended) [28].

After establishing that the patient has no active peptic disease, PPIs should be interrupted 10 days to 2 weeks prior to the test and replaced with high doses of H2 blockers (ranitidine 600 mg every 4–6 hours) for 5–7 days. Ranitidine

should then be stopped and a liberal use of antacids can be started. The secretin test should be done in 12–24 hours [53, 59]. However, the interruption of all antisecretory medications should be individually adapted. Patients should be warned about the acute exacerbation of symptoms. In these cases, antisecretory drugs can be restored.

The secretin test should be performed with the patient fasting (12–14 h). Secretin (2 U/kg body weight) is given by i.v. bolus and gastrin serum is measured at baseline, at −15 and −1 min before the test and at 2, 5, 10, 15, 20 and 30 min after secretin. Possible side effects of the secretin test include flushing and allergic reaction. Blood samples must be collected in heparinized vacutainers and placed in ice. An increase in circulating gastrin levels compared to baseline data (delta) of at least 200 pg/mL at any time during the test is considered diagnostic of autonomous secretion [56]. The National Institutes of Health (NIH) reduced the gastrin delta to ≥120 pg/mL with a sensitivity of 94% and specificity of 100% [24]. If the secretin test is negative but the suspicion of ZES remains high, a calcium stimulation test may be helpful, as it may be positive in 5–10% of such cases [60, 61]. Calcium gluconate 255 mg/3 mL is injected intravenously in 30 seconds. As done in the secretin test, venous blood sampling is performed before and at 2-min intervals up to 10 min after calcium injection [61]. When diagnostic, serum gastrin gradients show increased values greater than 20% above baseline at any time point (2, 4, or 6 min after i.v. calcium injection) usually with gastrin values higher than 300 pg/mL [61].

An alternative diagnostic test is the glucagon stimulation test, during which glucagon is infused at 20 μg/kg/hours in 30 min [62]. A gastrinoma is suggested when the percentage increase over the basal value of circulating gastrin reaches the peak within 10 min after glucagon administration, with circulating gastrin levels greater than 200 pg/mL [62]. The diagnosis of ZES is supported by the monitoring of the basal acid output (BAO). When BAO is greater than 15 mmol/hours, it is highly suggestive for this diagnosis [28].

5.2.3 Insulin

Proinsulin is the biosynthetic precursor of insulin that is synthesized in pancreatic islet cells. Proinsulin derives from a preproinsulin that acts as a signal for its transport to the Golgi apparatus, where it reaches the correct conformation. Insulin consists of two polypeptide chains linked by disulfide bridges. It is produced by the proteolytic cleavage of proinsulin through a connecting peptide of 33 amino acids. This peptide is called C-peptide, while the enzyme responsible for the proteolytic cleavage is an endopeptidase.

Insulin regulates the metabolism of carbohydrates, fats and protein by promoting the absorption of glucose from the blood into fat tissue, liver and skeletal muscle cells. Excessive insulin secretion leading to hypoglycemia usually results in neurologic and autonomic symptoms.

The diagnosis of insulinoma is suggested in the presence of symptoms of hypoglycemia with glucose values lower than 2.2 mmol/L (40 mg/dL) and relief of symptoms with the administration of glucose [63]. Fasting allows one to check an autonomous insulin secretion because it causes the lowering of glycemia, and in this circumstance insulin secretion should be suppressed. The 72-hour fast test is the gold standard for a biochemical diagnosis of insulinoma [64–66].

Factitious hypoglycemia, due to exogenous insulin, should be suspected in the presence of high (often very high) insulin serum levels, in combination with a suppressed C-peptide. Intake of sulphonylureas and related insulin secretagogues may be diagnosed by a urinary drug test [67]. For the 72-hour fast test the patient should be monitored in a secure inpatient setting. Blood samples for insulin, glucose and C-peptide assay should be obtained at least 2–4 times per day, even when the patient becomes symptomatic. β hydroxybutyrate (or urinary ketones), a metabolite of the oxidation of fatty acids is produced during fasting. It should be measured at the end of the test in order to confirm the validity of the fasting. Symptoms appear within 12 hours in one-third of patients, within 24 hours in 80%, within 48 hours in 90% and within 72 hours approaching 100% [64]. The endpoint of the test is a documented hypoglycemia with blood levels equal to or less than 2.2 mmol/L and concomitant insulin levels greater than 6 μU/L and β hydroxybutyrate levels equal to or less than 2.7 mmol/L. If the results are still equivocal, a glucagon stimulation test after the 72-hour fast test is suggested. Patients with insulinoma respond to glucagon administration (1 mg i.v. push) with a rise in blood serum glucose levels, indicating adequate glycogen stores [68].

Occasionally, insulinoma patients have been reported to exhibit postprandial hypoglycemic symptoms rather than in the fasting state. In such cases, hypoglycemia may be erroneously considered reactive. When performed, the oral glucose tolerance test may provide misleading results, since insulinoma cells may retain their glucose reactivity [69].

5.2.4 Glucagon

Glucagon is a peptide hormone produced by alpha cells of the pancreas. Its effect is opposite to that of insulin as it raises the concentration of glucose in the bloodstream. The diagnosis of functioning NETs producing glucagon (glucagonomas) is established by glucagon circulating levels generally greater than 500 pg/mL (normal <120 pg/mL). However, other diseases can cause hyperglucagonemia, including cirrhosis, pancreatitis, diabetes mellitus, prolonged fasting, sepsis, burns, renal failure, acromegaly and familial hyperglucagonemia [55].

In the majority of cases the diagnosis is done by the finding of hyperglucagonemia combined with clinical symptoms and signs often in the presence of hepatic metastatic disease with a large pancreatic mass, both of which are usually positive at whole-body somatostatin receptor scintigraphy.

5.2.5 Somatostatin (SS)

Somatostatin (SS) is a polypeptide hormone produced by the hypothalamus, the pancreatic delta cells, the gastric antral D cells and the APUD cells. It inhibits the release of pancreatic insulin and glucagon hormones and exocrine enzymes. In addition, it inhibits gastric hydrochloric acid secretion. It also acts as a neurotransmitter and inhibits the secretion of some pituitary hormones (GH, TSH, PRL).

Plasma SS levels are assessed in the suspicion of a SS-secreting tumor (SSoma) which is characterized by glucose intolerance, cholelithiasis and steatorrhea [70]. As there is no specific reagent for the determination of circulating SS levels, SS-like immunoreactivity (SLI) and SS-28, the main molecular form of circulating SLI, are assessed. Other conditions associated with elevated plasma SS levels are medullary thyroid cancer, lung cancer, pheochromocytoma and paraganglioma [5, 71].

5.2.6 Vasoactive Intestinal Peptide (VIP)

Vasoactive intestinal peptide (VIP) is a peptide hormone part of the glucagon/secretin superfamily. VIP is produced in many tissues such as gut, pancreas, and suprachiasmatic nuclei of the hypothalamus. The diagnosis of a VIP-secreting tumor (VIPoma) is suspected in patients with the Verner-Morrison syndrome characterized by severe watery diarrhea, documenting the presence of large volume secretory diarrhea (usually greater than 3 liters per day, with no osmolar gap in the stool fluid) accompanied by dehydration and electrolyte disorders with an increase plasma VIP level, usually greater than 500 pg/mL (normal, less than 190 pg/mL) [55, 71, 72].

Other diseases that can give large volume diarrhea and can mimic VIPomas, but are not associated with increased plasma VIP levels, include Zollinger-Ellison syndrome, chronic laxative abuse, sprue, AIDS, and rarely secretory diarrhea of unknown origin [4, 55, 71].

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Endoscopic Diagnosis of Gastrointestinal and Pancreatic Neuroendocrine Tumors

6

Guido Costamagna, Ivo Boškoski, and Fabia Attili

6.1 Introduction

The incidence of gastrointestinal and pancreatic neuroendocrine tumors (NETs) has increased in the past two decades mostly due to the widespread use of endoscopic screening programs and radiological imaging modalities [1, 2]. Gastrointestinal and pancreatic NETs today are considered rare neoplasms and the clinical presentation is generally indolent. Up to 60% of the patients present with distant metastases at the time of diagnosis and more than two-thirds of them are alive at 5 years [3].

The prognosis of gastrointestinal and pancreatic NETs is strictly related to their site, histology, proliferation activity and staging [4]. Using these parameters, the risk of progression, the most accurate treatments, the risk of recurrence and the type of follow-up can be estimated. Prompt diagnosis and correct staging of these lesions are therefore of paramount importance.

Nowadays endoscopists have many different instruments for sophisticated diagnoses, even though gastrointestinal and pancreatic NETs are still clinically challenging mostly due to their usual small size. Besides incidental finding of gastrointestinal and pancreatic NETs, the role of endoscopy is important in localization of the primary tumor site in metastatic disease, endoscopic resection and follow-up.

6.2 Neuroendocrine Tumors of the Gastrointestinal Wall

NETs of the gastrointestinal wall are the ones most commonly incidentally diagnosed during upper and lower endoscopic examinations [5], including capsule

I. Boškoski (✉)
Digestive Endoscopy Unit, Fondazione Policlinico Gemelli
Rome, Italy
e-mail: ivo.boskoski@policlinicogemelli.it

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endoscopy. Among all NETs, the most commonly found are gastric and rectal NETs [6, 7]. NETs of the duodenum are rare (1–2%), are generally associated with genetic syndromes (i.e., MEN1, von Recklinghausen's disease), and may be functioning [6].

The most important characteristics that should be evaluated and that will lead the choice of treatment are size, layer of origin, degree of infiltration of the gastrointestinal wall and lymph node involvement. Endoscopic ultrasound (EUS) is the best "all-in-one" tool for this purpose.

6.2.1 Esophagus

Esophageal NETs are rare and usually located in the distal esophagus, corresponding to the distribution of the neuroendocrine cells. Esophageal NETs arise from the lamina propria or the submucosa. The diagnosis is mostly incidental during routine endoscopy as they present as polypoid lesions [8, 9].

Esophageal NETs can rapidly invade the muscularis propria and give lymph node metastases [8–10]. Esophageal NETs are generally covered by normal mucosa, and EUS is the best diagnostic tool for staging. Endoscopic treatment modalities are endoscopic mucosal resection (EMR and cap-assisted EMR) and endoscopic submucosal dissection (ESD). Peroral endoscopic tumor resection with submucosal tunneling could be an alternative, but no papers have been published on resection of esophageal NETs with this technique.

6.2.2 Stomach

Gastric NETs are divided in four types according to their clinicopathological characteristics (Table 6.1).

Type 1 lesions represent up to 80% of gastric NETs, appear multifocal on endoscopy, develop in the gastric corpus and fundus, and are small (<10 mm) [11, 12]. These lesions are often associated with fundic chronic atrophic gastritis. In up to 90% of cases they are limited to the mucosa or submucosa and infiltration of the muscularis propria can be observed only when their size is greater than 10–20 mm [12, 13]. Metastases to loco-regional lymph nodes can be found in up to 9%, and this usually occurs in lesions >20 mm that often infiltrate the muscularis propria and also become angioinvasive [11, 14, 15].

Type 2 gastric NETs are similar to type 1 (Fig. 6.1). These NETs usually present in up to 30% of patients after a long course (up to 20 years) of MEN1 or Zollinger-Ellison syndrome (ZES) [16–19]. As in type 1, lymph node metastases in type 2 are found in tumors >20 mm, which infiltrate the muscularis propria and are angioinvasive [11, 14, 15].

Type 3 gastric NETs endoscopically can be found in any part of the stomach as solitary polypoid lesions, and they are not associated with other diseases

Table 6.1 Characteristics of gastric neuroendocrine tumors

	Type 1	Type 2	Type 3	Type 4
Frequency (%)	70–80	5–6	14–25	6–8
Age of occurrence	40–60	45	50	>60
Dimensions (mm)	<10	<15	>20	>20
Features	Multiple	Multiple	Single	Single
Histology	Well-differentiated	Well-differentiated	Well-differentiated	Poorly differentiated
Proliferation rate (%)	<2	<2	>2	20–30
Gastrinemia	High	High	Normal	Mostly normal
pH	Anacidic	Hyperacidic	Normal	Mostly normal
Metastases (%)	<10	10–30	50–100	80–100
Associated diseases	CAG	MEN1, ZES	No	No

CAG, chronic atrophic gastritis; *MEN1*, multiple endocrine neoplasia type1; *ZES*, Zollinger-Ellison syndrome.

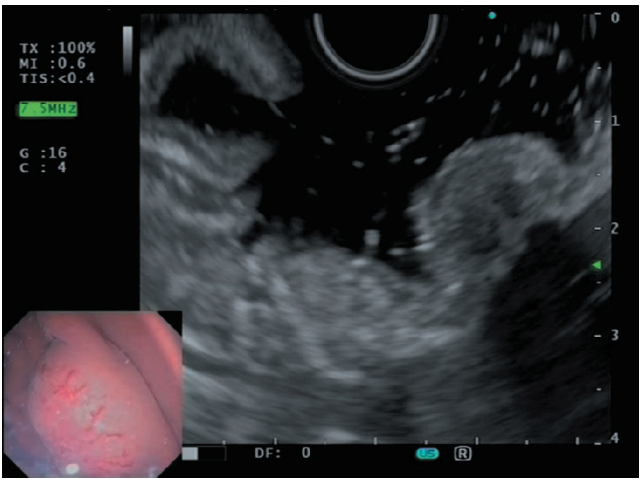

Fig. 6.1 Endoscopic image and endoscopic ultrasound image of a type 2 gastric neuroendocrine tumor

or conditions (Table 6.1). When diagnosed, these lesions are often >20 mm in dimensions, infiltrate the muscularis propria and are angioinvasive, and have a 75% rate of metastases at presentation [20].

Type 4 gastric NETs are poorly differentiated solitary carcinomas that can be found anywhere in the stomach. These lesions on endoscopy are usually ulcerated, big and adenocarcinoma-like. The prognosis is very bad since more than 50% of the patients die within 12 months [7, 21–23].

Type 1 and 2 NETs that are confined to the mucosa can be resected *en bloc* by EMR [11]. EUS can be useful in these lesions for staging. ESD is the treatment of choice for bigger NETs confined to the mucosa or infiltrating the submucosa, but without lymph node involvement confirmed at EUS [24, 25].

Type 3 gastric NETs confirmed by EUS-guided fine-needle aspiration (FNA) or EUS-guided fine-needle biopsy (FNB) should be evaluated for loco-regional lymph node metastases and distal metastases, even when these lesions are small [20].

A follow-up, with repeat gastroscopy every 1 to 2 years is recommended, especially for type 1 gastric NETs, due to their tendency to recur.

Multiple random biopsies and biopsies of the antrum, fundus and gastric body and of any suspected lesion and/or polyp is recommended in this setting.

Surgery with or without neoadjuvant chemotherapy is indicated for all types of NETs that are associated with high rates of lymph node metastases.

6.2.3 Duodenum

Primary duodenal NETs represent about 2% of all gastrointestinal NETs [26] (Fig. 6.2). Duodenal NETs are classified into five types based on their pathohistological characteristics: gastrinomas, somatostatinomas, non-functioning NETs, poorly differentiated neuroendocrine carcinomas, and duodenal gangliocytic paragangliomas (predominantly located in the ampulla of Vater).
The diagnosis is often incidental during upper endoscopy for unrelated symptoms. Jaundice is the more frequent presentation of ampullary NETs.

Some duodenal NETs can present in the setting of Zollinger-Ellison or Cushing syndrome and, rarely, acromegaly. Duodenal NETs generally arise in the submucosa (third layer) and on EUS appear as hypoechoic, rounded and with

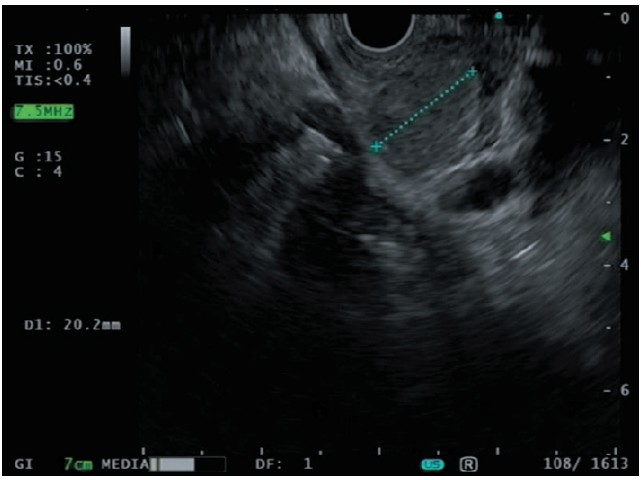

Fig. 6.2 Large duodenal neuroendocrine tumor

a characteristic salt-and-pepper appearance. These lesions are usually small (<10 mm), but when their size is >10 mm they have a very high metastatic potential.

When less than 10 mm in diameter, these lesions can be removed by EMR, after careful EUS evaluation for adjacent lymph node metastasis [27, 28]. Independent factors for metastases are invasion of the muscularis propria, tumor size (>20 mm) and the presence of mitotic figures on histology [29].

According to the ENETS guidelines for the management of duodenal NETs, endoscopic resection can be done for lesions smaller than 10 mm, located outside the ampulla of Vater, without signs of invasion of the muscularis propria and with no evidence of lymph node metastases on EUS [20]. Several studies have reported no correlation between tumor size and the presence of malignancy and/or metastases in ampullary and periampullary NETs; therefore, surgical resection with lymphadenectomy is the best treatment of choice of these lesions [30–34].

Endoscopic papillectomy is an option in high-risk surgical candidates and in the case of small ampullary NETs without local angioinvasion and lymph node metastases confirmed by EUS, and EUS-FNA or EUS-FNB showing high differentiation [31].

6.2.4 Small Intestine

The small intestine should be investigated in all patients with neuroendocrine metastases of unknown origin. Endoscopic options for investigation of the small intestine are videocapsule endoscopy (VCE) and enteroscopy. Fig. 6.3 shows a small NET of the small intestine found on VCE.

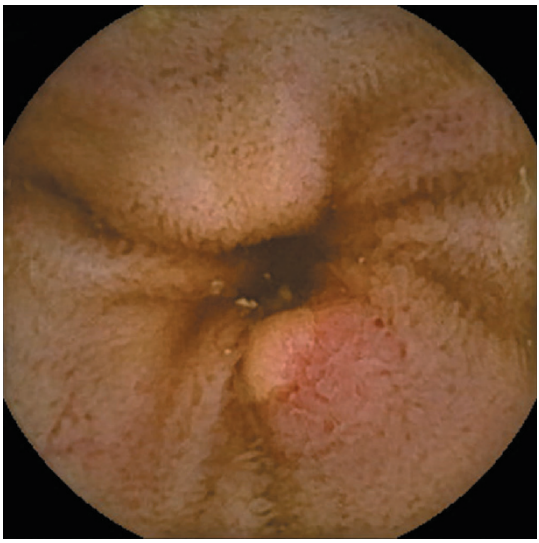

Fig. 6.3 Neuroendocrine tumor found in the small intestine during videocapsule endoscopy (Courtesy of Dr. Cristiano Spada)

Computed tomography enteroclysis seems to be superior to VCE in the detection of the primary neoplasm and also in the evaluation of extraluminal invasion [35, 36]. Furthermore, in cases of mesenteric involvement, almost always there is a significant stricture in the intestine that can block the capsule progression [37].

Enteroscopy (single or double balloon) has low diagnostic potential, and should be performed in selected cases after positive previous non-invasive investigations [38]. Other indications for enteroscopy are preoperative tattooing of operable NET lesions and endoscopic removal of small incidental NETs.

6.2.5 Colon and Rectum

NETs of the colon originate from the enterochromaffin cells in the crypts of Lieberkühn. Clinically they can present with abdominal pain, change in bowel habits, anorexia, bleeding, weight loss and weakness, but can also be asymptomatic. The most frequent localizations are the cecum and the ascending colon. Generally, colonic NETs tend to present later especially if located in the cecum and ascending colon, due to the bigger diameter of this part of the colon, which is probably the reason why more than 60% are metastatic at the time of diagnosis [39, 40]. The 5-year survival rate is about 60% [41].

It is important to underline that colonic NETs bigger than 20 mm have almost always regional lymph node metastases; therefore the recommended treatment in these patients is surgery combined or not with chemotherapy [42, 43].

EMR is the best treatment modality for lesions <20 mm, involving the mucosa and/or submucosa, without distant metastases, while ESD bears major perforation risks in the colon.

As far as NETs of the rectum are concerned, these lesions are often small (<10 mm) and with polypoid appearance. As a result, in clinical practice, most of these lesions are removed routinely by snare polypectomy and only after histological examination are they reclassified as NETs [44]. The need for additional investigations in these lesions is driven by the status of the resection margins and tumor grading. However, complete colonoscopy should always be performed in order to exclude other synchronous lesions [45]. In Fig. 6.4 a small NET of the rectum involving the muscularis mucosae is shown.

EUS for staging is indicated for rectal lesions larger than 20 mm. Rectal NETs bigger than 20 mm with invasion of the muscularis propria or aggressive histological features should undergo surgery.

The ENETS guidelines treatment algorithm for rectal NETs is based on EUS findings and suggests exclusion of pararectal lymph node metastases before endoscopic removal [20].

Standard polypectomy compared to EMR or ESD for rectal NETs has shown to be less effective in terms of complete resection (31% vs. 72%) [46]. A combination of EUS and ESD for rectal NETs larger than 10 mm is the best approach in this setting [47].

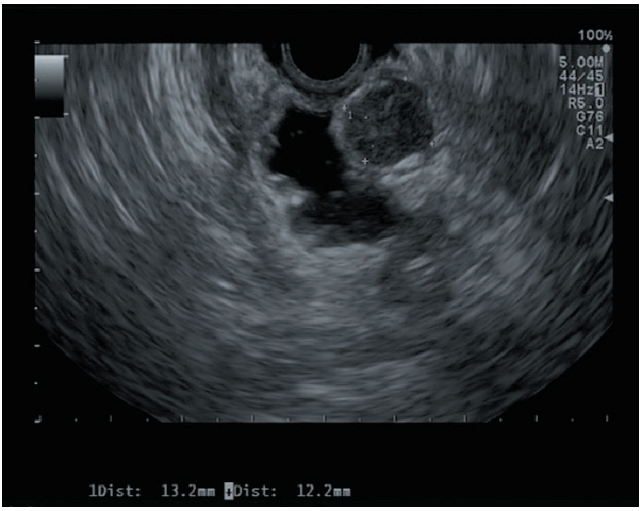

Fig. 6.4 Small neuroendocrine tumor of the rectum involving the muscularis mucosae

6.3 Pancreatic NETs

The pancreas can be entirely studied with EUS through the stomach and the duodenum obtaining high-resolution images. EUS can precisely visualize small anatomical structures that cannot be studied by other imaging modalities. These features, together with the possibility to perform tissue sampling (EUS-FNA and/or EUS-FNB) makes EUS the best diagnostic tool for pancreatic NETs [48, 49]. In Fig. 6.5 a small NET of the pancreatic isthmus and in Fig. 6.6 a NET in the pancreatic tail are shown.

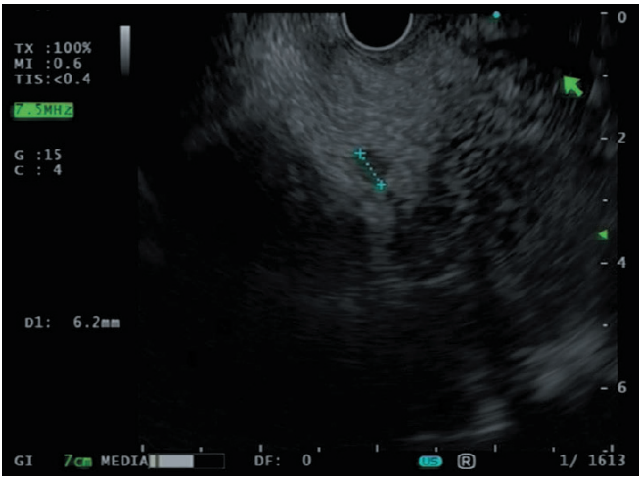

Fig. 6.5 Small neuroendocrine tumor in the pancreatic isthmus

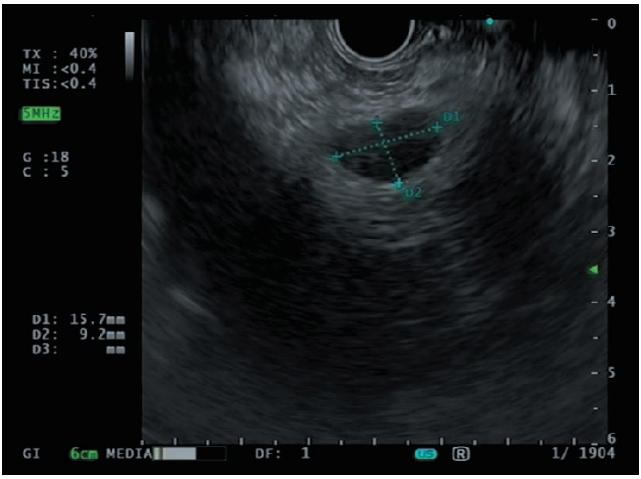

Fig. 6.6 Neuroendocrine tumor in the pancreatic tail

Pancreatic NETs appear on EUS as rounded, hypoechoic lesions with clear and regular margins and a homogeneous pattern. In cases of advanced lesions, pancreatic NETs can also appear irregular, with the same features as pancreatic adenocarcinoma. EUS can also establish the localization and the distance from the tumor of the main pancreatic duct, which can indicate the best surgical approach: enucleation versus resection [50]. Furthermore, EUS-guided fine needle tattooing (EUS-FNT) is a valid technique that helps surgeons in the precise localization of small pancreatic NETs, especially when laparoscopic surgery is planned [51].

NETs of the pancreas are rare tumors and represent about 1% of all pancreatic neoplasms [7, 52]. As for other gastrointestinal NETs, the incidence of pancreatic NETs has significantly increased in recent decades, mostly due to the wide availability of EUS. The clinical course of pancreatic NETs is generally indolent [7, 52]. As mentioned in previous chapters, pancreatic NETs are classified into functioning and non-functioning lesions based on the presence or absence of a clinical hormonal hypersecretion syndrome [53]. Pancreatic functioning NETs are mainly insulinomas, which in 50% of the cases are less than 1 cm in diameter, and therefore sometimes very difficult to detect [53].

The sensitivity of EUS in pancreatic NETs is up to 94% [54–60]. The ENETS consensus guidelines classify EUS as the imaging study of choice for these patients. [20]. EUS combined with FNA can predict the 5-year survival of these patients through determination of malignant potential [61]. Today, determining the tumor proliferation index Ki-67 of pancreatic NETs is an essential step in the choice of the best patient-tailored treatment [62]. This is important both for operable and non-operable patients. For instance, the degree of proliferation index can lead to the choice of different targeted therapies, such as everolimus and sunitinib, peptide receptors targeted therapy, etc. [62–64].

The role of EUS in pancreatic NETs is not only diagnostic, but also therapeutic. EUS-guided fine-needle injections and tumor ablation therapies are both possible [65, 66]**.** This minimally invasive approach can be done in selected, mostly non-operable or high-risk surgical patients, and consists in radiofrequency and cryotherapy tumor ablation, fine-needle injection of ethanol, antitumor agents [66]. The long-term efficacy of these treatments is currently not known. Serious adverse events such as portal vein thrombosis and large pancreatic tissue necrosis have been reported [67, 68].

6.4 Conclusions

Upper and lower endoscopy have an essential role in the diagnosis and treatment of gastrointestinal NETs. VCE and enteroscopy are suboptimal modalities for the study of the small bowel, and other diagnostic modalities should be preferred in this setting. EUS has a critical role in the diagnosis of NETs of the gastrointestinal wall and gives precise information about localization within the intestinal wall, size, depth of invasion, and it is essential for staging (alone or in combination with other diagnostic modalities). EUS is also essential in the selection of the best endoscopic resection approach: EMR versus ESD versus surgery. EUS combined with FNA and/or FNB has a pivotal role in the setting of diagnosis and treatment of pancreatic NETs. EUS-guided tattooing of pancreatic NETs is another important approach that helps surgeons to avoid destructive surgery. Finally, EUS-guided tumor ablation and fine-needle injections in selected patients are becoming promising treatment modalities, but more investigation is required in this field.

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Role of Non-Functional Imaging in the Diagnosis of Abdominal Neuroendocrine Tumors

7

Marta Zerunian, Davide Bellini, and Andrea Laghi

7.1 Introduction

Over the last two decades, imaging detection and characterization of abdominal neuroendocrine tumors (NETs) has improved significantly thanks to technological improvements [1]. Several non-functional imaging techniques are available for the study of these lesions: ultrasound (US), multidetector computed tomography (MDCT), magnetic resonance imaging (MRI) and endoscopic ultrasound (EUS).

Non-functional imaging has a crucial role in the initial detection and evaluation of the extent of primary tumor, providing useful information to plan patient management. Imaging can also be used to guide biopsy (mainly CT and US), to plan surgical resection and to evaluate response to therapy. Sometimes gastroenteropancreatic NETs (GEP-NETs) are discovered incidentally during an abdominal US examination or during other high-resolution imaging modalities performed for different reasons. On the other hand, small subtle lesions, hardly detectable by imaging [2], always require specific acquisition protocols, especially for CT and MRI (Table 7.1).

The challenge of radiologists during recent years has been to meet these needs by tailoring protocols for cross-sectional imaging according to the specific clinical scenario and the anatomical location of the tumor. Clinicians should be aware that a proper imaging protocol, especially for CT and MRI, is necessary to improve detection and evaluation of abdominal NETs [3].

M. Zerunian (✉)
Department of Radiological Sciences, Oncological and Pathological Sciences, Sapienza University of Rome – ICOT
Latina, Italy
e-mail: marta.zerunian@gmail.com

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Table 7.1 Acquisition protocols and imaging features of gastroenteric neuroendocrine tumors (GE-NETs) and pancreatic neuroendocrine tumors (P-NETs)

	US	CT	MRI	EUS
Stomach	Hypoechoic mass (often hyperechoic rim)	Stomach distension with 500 mL of water administered orally before the scan Types I and II: enhancing polypoid or submucosal lesions (<2 cm); larger lesions may ulcerate the mucosa Type III: (>2 cm) infiltrative morphology and ulceration (malignant behavior)	–	Assessment of depth of tumor invasion Useful for performing a biopsy
Duodenum	Hypoechoic mass (often hyperechoic rim)	Bowel distension with 500 mL of water administrated orally before the scan Arterial enhancement of lesions (ulceration 50%; intraluminal polyps 40%; intramural mass with possible obstructive biliary dilatation 10%)	Hyperintense on T2-w images Hypointense on T2-w images Hyperintense on T1-w fat-suppressed images after i.v. administration of gadolinium-based CM	Detection of small lesions (<2 cm) with possibility to perform guided fine-needle aspiration for histopathologic evaluation
Small bowel	–	CT enteroclysis: bowel distension with 1500-2000 mL of water + osmotic solution administered orally 40 min before the scan Hypervascular focal lesion or bowel thickening. Additional findings: desmoplastic mesenteric reaction, mesenteric masses with/without calcification. Complications: bowel obstruction, loop ischemia/infarction	MR enterography: bowel distension with 1500-2000 mL of water + osmotic solution administered orally 40 min before the scan Hyperintense on T2-w images Hypointense on T2-w images Hyperintense on T1-w fat-suppressed images after i.v. administration of gadolinium-based CM	Assessment of terminal ileum (miniprobes through biopsy channel of colonoscope)
Appendix	–	Enhancing lesions (1–2 cm); diffuse circumferential mural thickening; differential diagnosis with appendicitis (primary tumor may not be readily seen)	Hyperintense on T2-w images Hypointense on T2-w images Hyperintense on T1-w fat-suppressed images after i.v. administration of gadolinium-based CM	–

Colon	–	Ulcerating lesions or necrotic lesions (more common in ascending colon) Complications: intussusception, bowel obstruction	Hyperintense on T2-w images Hypointense on T2-w images Hyperintense on T1-w fat-suppressed images after i.v. administration of gadolinium-based CM	–
Rectum	–	Usually, small submucosal lesions	Hyperintense on T2-w images Hypointense on T2-w images Hyperintense on T1-w fat-suppressed images after i.v. administration of gadolinium-based CM	Assessment of depth of tumor invasion (small solid nodule or polypoid mass) and possibility to perform a biopsy
Functioning P-NETs	Hypoechoic nodular lesion with regular margins, hypoanechoic appearance of cystic areas may be present Hyperechoic after i.v. administration of CM during CEUS	Isodense unenhanced phase (calcification, cystic degeneration) Hypervascular, small smooth, masses (arterial phase) Isodense during portal phase	Hyperintense on T2-w images Hypointense on T2-w images Hyperintense on T1-w fat-suppressed images after i.v. administration of gadolinium-based CM	Detection of small lesions (<2 cm) with possibility to perform guided fine-needle aspiration for histopathologic evaluation
Non-functioning P-NETs	Hypoechoic nodular lesion with regular margins, hypoanechoic appearance of cystic areas may be present Larger masses show heterogeneous echogenicity (hyalinized stroma, cystic degeneration) Hyperechoic after i.v. administration of CM during CEUS	Heterogeneous enhancement (large masses); necrotic, cystic changes or calcification in malignant tumors; local invasion and metastases	Hyperintense on T2-w images Hypointense on T2-w images Heterogeneous enhancement (cystic lesions with thin rim enhancement, fibrous tissue present delayed enhancement)	Usually incidentally detected Guided fine-needle aspiration for histopathologic evaluation can be performed

US, ultrasound; *CT*, computed tomography; *MRI*, magnetic resonance imaging; *EUS*, endoscopic US; *i.v.*, intravenous; *CM*, contrast media; *CEUS*, contrast-enhanced US.

7.2 Pancreatic Neuroendocrine Tumors

In non-functioning NETs the priority is to identify correctly the lesions, avoiding misdiagnosis. For these reasons the choice of a proper imaging method and acquisition protocol is of utmost importance.

7.2.1 Transabdominal Ultrasound

Transabdominal US represents the first diagnostic approach in most cases. It is a non-invasive, radiation-free, readily available and inexpensive imaging technique enabling the study the entire abdomen, including pancreatic lesions. However, it is considered extremely dependent on operator expertise and limited by patient characteristics such as bowel gas and obesity (for instance, most patients with insulinoma tend to gain weight, and this limits the value of US [4]).

Usually pancreatic NETs (P-NETs) appear as a homogenous hypoechoic nodular lesion with regular margins. Larger lesions may appear with a more heterogeneous echogenicity, probably due to different internal composition such as greater amounts of hyalinized stroma, cystic degeneration or bleeding due to the fragility of new blood vessels; any cystic areas within the lesion will have a hypo-anechoic appearance [5]. After the administration of contrast medium, during contrast-enhanced US (CEUS) examination, P-NETs show a hypervascular behavior, enhancing avidly in comparison with the surrounding parenchyma. Particular attention should be paid to enlarged and rounded locoregional lymph nodes and to the presence of liver metastases, considered indirect signs of malignant transformation.

Transabdominal US has a sensitivity ranging between 20% and 80% for the detection of P-NETs [6, 7]. This wide range could be explained by the great sensitivity in detecting rare large histotypes (85–95%) and intermediate-to-low sensitivity for the most common histotypes (around 50%), with a variable range of 19–40% for gastrinomas and 25–64% for insulinomas [8].

7.2.2 Computed Tomography

MDCT is the first-line non-functional imaging technique when a P-NET is clinically suspected. The CT protocol generally includes an unenhanced phase, a delayed arterial phase (specific to assess pancreatic parenchyma, about 40 s after i.v. injection of contrast medium), a portal venous phase (55–70 s after i.v. injection of contrast medium) and a delayed phase (120 s after i.v. injection of contrast medium) [9, 10].

Functioning P-NETs during the unenhanced phase are usually isodense to the parenchyma and this can be useful to detect hemorrhage or calcifications.

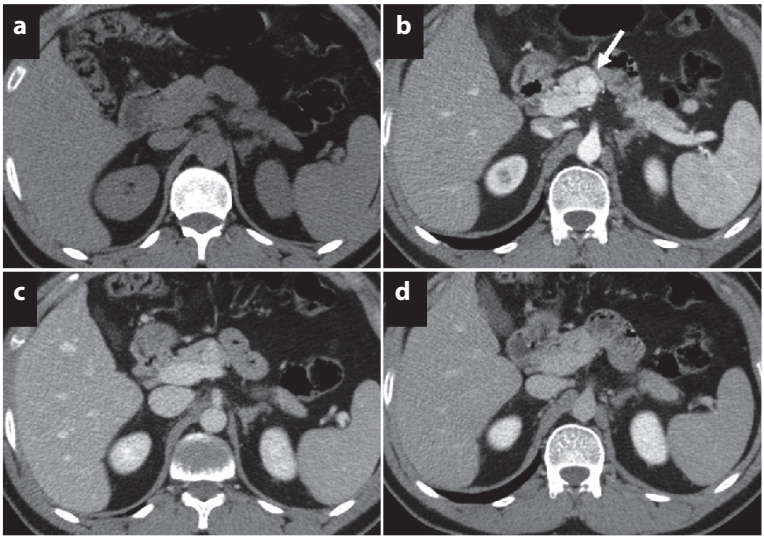

Fig. 7.1 CT features of a functioning P-NET. Axial contrast-enhanced CT images acquired in **a** 36-year-old man show pancreatic insulinoma (*arrow* in **b**) with typical imaging features: small size (12 mm) and avid enhancement (brighter than normal surrounding pancreatic parenchyma in the arterial phase). During the portal (**c**) and equilibrium phase (**d**) the lesion appears isodense compared to the surrounding parenchyma

The arterial phase is considered the most sensitive acquisition phase. In this phase, the hypervascular feature of P-NETs makes even a rounded smooth lesion hyperattenuating with the best attenuation difference compared to the surrounding pancreatic parenchyma (Fig. 7.1). During the venous phase the attenuation of both the tumor and the normal parenchyma generally decreases, so that the lesion is less detectable [8]. However, in larger tumors (e.g., glucagonomas) it is possible to observe increased tissue degeneration and consequent heterogeneity of density during both phases [1].

Moreover the portal venous phase can be, in a minority of cases, more useful for identifying liver metastases, evaluating locoregional lymphadenopathy and assessing the relationship between the tumor and the surrounding structures. For this reason, biphasic imaging after i.v. injection of contrast medium is currently recommended to improve detection and characterization [11].

Regarding non-functioning tumors, they are larger in size (average diameter, 4 cm) at the time of detection, due to the absence of symptoms, and well defined, encapsulated and with heterogeneous enhancement. Heterogeneity can be related to degenerative cystic areas, necrotic regions and, rarely, fibrosis. Even in the remote case that they are completely cystic, a hypervascular rim is detectable in up to 90% of cases. Moreover, tumor local aggression is evincible through retroperitoneal invasion or metastases (up to 80% of cases) to regional lymph

nodes or to the liver. It is very uncommon to find pancreatic or biliary duct obstruction [1].

In recent studies, the diagnostic accuracy and sensitivity of MDCT in the assessment of P-NETs has improved from a range of 14–30% (reported in older studies) to 69–94% [1]. Moreover, the wide range in sensitivity can also be interpreted observing that many small P-NETs are missed on CT [12]. Recently, sensitivity has improved considerably. In a recent study comparing MDCT and dual-energy CT, MDCT had a sensitivity of 68.8%, while dual-energy CT reached 95.7% [13]. An important limitation to this technique is radiation exposure, in particular for repeated follow-up examinations. By applying new dose reduction techniques, such as iterative reconstruction, it would be possible to reach a low dose exposure with a good diagnostic image quality [14, 15].

7.2.3 Magnetic Resonance Imaging

MRI plays an important role in the characterization of P-NETs. Indications include localization of suspected lesions not clearly characterized by US or CT and avoidance of radiation dose in young patients, especially during follow-up [8].

An appropriate protocol for the study of pancreatic parenchyma and specifically of P-NETs includes T1- and T2-weighted sequences, both with and without fat suppression, diffusion-weighted imaging (DWI) and postcontrast T1-weighted fat-suppressed dynamic imaging after the injection of gadolinium contrast medium. In the assessment of P-NETs, unenhanced T1-weighted fat-suppressed MRI has marked sensitivity, reaching 75% [6]. P-NETs usually show a decreased signal intensity on T1-w sequences and high signal intensity on T2-w sequences relatively to the normal pancreatic gland; after i.v. injection of gadolinium-based contrast media they usually show homogeneous enhancement. However, due to the wide histological heterogeneity of P-NETs, the signal intensity and enhancement patterns might be varied: cystic lesions may show a thin rim enhancement and appear bright on T2-w images, tumors rich in collagen or fibrous tissue could present low signal on T2-w images (Fig. 7.2). MRI, because of the higher soft tissue contrast compared with US and CT, usually allows a better evaluation in the case of multiple NET lesions [16]. Regarding sensitivity and specificity, MRI has improved its accuracy reaching a range of 74–94% and 78–100%, respectively [1].

A relatively new tool to be implemented in all MRI acquisition protocols, is DWI. This is a novel bioimaging technique that allows one to evaluate quantitatively the hypercellularity of neoplastic lesions. DWI has a diagnostic role in the case of negative or doubtful imaging findings to support the diagnostic process especially in non-hypervascular lesions [8].

Moreover, a cholangiopancreatography (MRCP) sequence should be included in the protocol to assess the relation between the tumor and the pancreatic and main bile ducts [16].

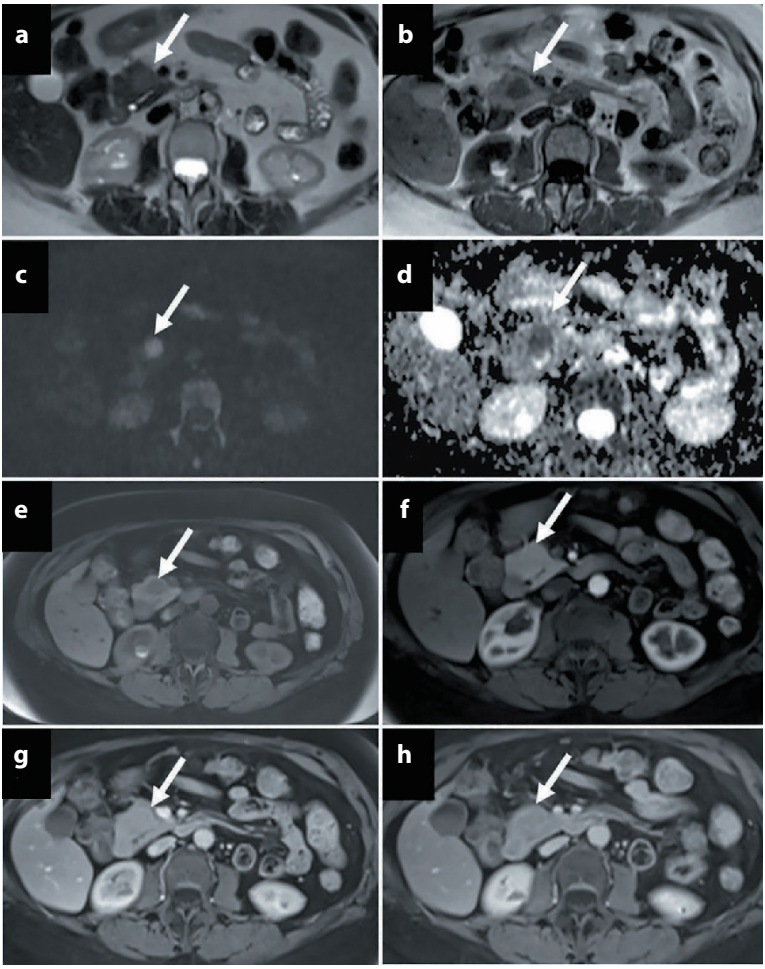

Fig. 7.2 Malignant non-functioning P-NET in a 70-year-old woman presenting with vague abdominal pain. Lesion located in the pancreatic head (17 mm) appears slightly hyperintense on axial T2-weighted MRI (**a**) and hypointense on axial T1-weighted imaging (**b**) (*arrow*). On the diffusion-weighted image (**c**) and ADC map (**d**) the tumor shows hypercellularity with appreciable restriction of the movement of the water molecules. On dynamic contrast-enhanced fat-suppressed T1-weighted images, acquired before contrast media administration (**e**), during the arterial (**f**), portal (**g**) and equilibrium (**h**) phases, the tumor appears isointense to the surrounding parenchyma, without showing any hypervascular behavior

7.2.4 Endoscopic Ultrasound

Endoscopic US (EUS) has been increasingly used in the localization of P-NETs, particularly for the detection of small insulinomas [8]. Furthermore, EUS plays an important role in early detection and follow-up of multifocal lesions that are common among patients with MEN1 and von Hippel-Lindau syndromes [17]. The

close proximity of the pancreas to the stomach and the duodenum allows a detailed examination of small lesions, especially those located at the pancreatic head. EUS has the best spatial resolution compared to other different imaging modalities: it can detect lesions smaller than 0.2 cm as well as adjacent celiac, peripancreatic, para-aortic, and periportal lymphadenopathies, and it can assess vascular invasion [18]. Typically, small P-NETs appear as rounded, homogeneous, hypoechoic lesions. If calcifications are present, a diagnosis of somatostatinoma can be made.

Rösch et al. [18] in 1992 reported an EUS sensitivity of 82% and specificity of 92% in patients whose tumors had previously remained undetected by other different methods. More recent studies reported a diagnostic accuracy ranging from 79% to 94% [19-22].

Another very important advantage of EUS is the possibility to perform fine-needle aspiration (FNA) during the procedure. Using a 22- or 25-gauge needle, a tissue sample can be obtained for cytological evaluation and immunohistochemistry. With this combined approach, sensitivity and specificity improve up to 84% and 92.5%, respectively [23].

7.2.5 Angiography

An accurate preoperative vascular assessment prior to pancreatic surgery is of utmost importance. Angiography, performed through selective intra-arterial injection of contrast medium, is considered the best method to investigate vascular anatomy and it improves detection of duodenal and pancreatic gastrinomas [24]. Neuroendocrine tumors are seen on arteriography as diffusely enhancing masses without tumor vessels and without arteriovenous shunting.

Furthermore angiography can lead to several interventional procedures. Preoperative angioembolization of hypervascular tumors was proven safe and may result in a decreased risk of bleeding during surgery. Embolization of primary pancreatic cancer was described by Hirose et al. [25] and Ben-Ishay et al. [26] who reported on preoperative angioembolization of a hypervascular P-NET located at the head of the pancreas. Preoperative angioembolization of the hepatic artery prior to *en bloc* celiac axis resection for pancreatic body cancer was also reported [27].

7.3 Extrapancreatic Neuroendocrine Tumors

Detection of a primary tumor in the gastrointestinal tract is challenging because of the small tumor size (functioning lesions), the length of the tract and its tortuous course. Non-functioning NETs, usually larger than functioning lesions, can be incidentally discovered on routine CT and MRI, especially if locally advanced or metastasized [28, 1].

7.3.1 Transabdominal Ultrasound

Transabdominal US has the great advantage of not using ionizing radiation, and it is extremely useful especially in young patients. On the other hand, its role in gastrointestinal primary NETs is generally limited due to the presence of bowel gas artifacts that usually interfere with an accurate bowel wall evaluation [29]. For those reasons, a very large range of sensitivity (15–80%) in the detection of gastroenteric NETs (GE-NETs) has been reported, not only due to small tumor size, but also due to anatomical location and operator experience [29].

The procedure is quite simple: the probe (3–5 MHz) is placed directly on the abdomen; the bowel loops can be distended before the examination through oral administration of polyethylene glycol [1, 29]. Usually GE-NETs appear as hypoechoic nodules, often with a hyperechoic rim [1, 29]. CEUS improves the sensitivity and specificity by highlighting the hypervascular behavior of these lesions, especially if small. US can also been used during interventional procedures to guide biopsy [1].

7.3.2 Computed Tomography

GE-NETs are most commonly studied by using CT for tumor localization, staging, and follow-up during and after therapies [29, 30]. MDCT scanners have optimal spatial resolution (<0.6 mm for most modern CT scanners) and allow acquisition of the entire abdomen in a single breath-hold and reconstruction of images on multiple planes (coronal, axial, sagittal), extremely useful to improve lesion detection and to evaluate relationships with adjacent abdominal organs. Moreover, by applying advanced reformatting techniques (curved reformats, three-dimensional volume rendering and maximum intensity projection) it is possible to obtain an accurate evaluation of vascular structures, contributing to plan properly surgical procedures [1, 29, 31].

A specific CT acquisition protocol is crucial to improve diagnostic accuracy. A good evaluation of the small bowel requires a combination of two important conditions: a fast imaging technique and good luminal distension through the administration of enteric contrast agents (water, methylcellulose, solutions containing locust bean gum, mannitol, barium sulfate, and polyethylene glycol). The most accepted filling strategy involves a 6-hour fast prior to the examination and a large volume of enteral contrast material (1500–2000 mL) administered orally during the 40 min before the examination. Spasmolytics are useful for reducing bowel peristalsis and motion artifacts. Furthermore, the use of i.v. injection of contrast medium is mandatory for the assessment of bowel walls, lesion enhancement, and mesenteric vessels [32]. The CT protocol generally includes an unenhanced phase, an arterial phase (the most sensitive phase to assess lesion enhancement, about 20 s after i.v. injection of contrast medium), a portal venous phase (55–70 s after i.v. injection of contrast medium) and a delayed

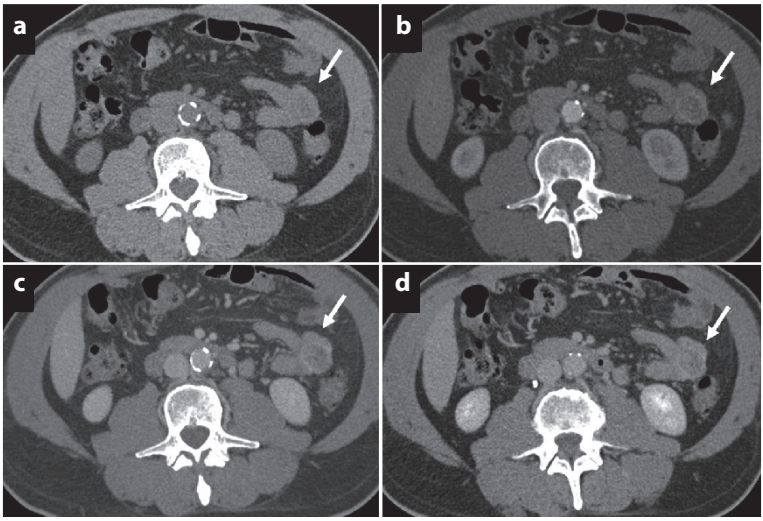

Fig. 7.3 A 54-year-old man with a history of abdominal pain. (**a**) Axial unenhanced CT scan shows a rounded isoattenuating mass in the ileum (*arrow*), not easily detectable. The axial arterial (**b**), portal (**c**) and delayed (**d**) phases show ring enhancement (*arrows*) due to the presence of necrosis within the lesion

phase (120 s after i.v. injection of contrast medium) [29, 33]. It is important to underline that differences in time delays after i.v. injection of contrast media, especially for the arterial phase, could substantially affect image quality, risking potentially both false-positive or false-negative findings [29].

On unenhanced CT images, GE-NETs appear as hypoattenuating rounded lesions (Fig. 7.3), and may present calcifications [34] or internal hemorrhage; small lesions can appear isodense to the normal surrounding tissue and detection can be very difficult [1, 29]. GE-NETs have typically hypervascularization in the late arterial acquisition phases [29, 35] (Fig. 7.4). Sometimes, pathological involvement of mesentery induces desmoplastic reaction (fibrotic-like tissue) with vessel retraction, which may be the only appreciable CT features [29, 34].

A very promising technique, especially for the detection of small hypervascular lesions, is dual-energy CT (DECT). This technique is based on performing scans at two different energy levels (e.g., 80 and 140 kVp). It allows material decomposition and provides iodine images that map the iodine content of the tissue and thus provide a more reliable measurement of tissue enhancement [29, 36].

Moreover, virtual monoenergetic level imaging can be obtained which provides valuable information regarding the attenuation of different tissues over a wide range of discrete energies (from 40 up to 190 keV), improving conspicuity of hypervascular lesions at low keV (40–70 keV), reducing artifacts and, potentially, the amount of i.v. contrast media.

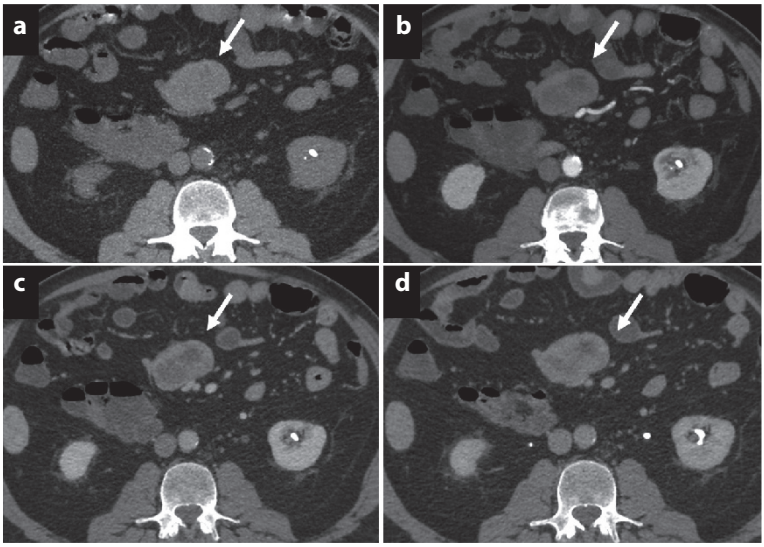

Fig. 7.4 A 63-year-old man with a history of subocclusion. (**a**) Axial unenhanced CT scan shows a rounded isoattenuating mass in the ileum (*arrow*). The axial arterial (**b**), portal (**c**) and delayed (**d**) phases show progressive centripetal enhancement (*arrows*), an atypical presentation of GE-NETs

7.3.3 Magnetic Resonance Imaging

In the last decade MRI has grown in the locoregional assessment of GE-NET tumors. MRI has several advantages over CT: higher contrast resolution, greater interobserver agreement and, above all, it is an ionizing radiation-free imaging method. Moreover, MRI combines anatomical, functional and molecular imaging techniques, allowing a multiparametric evaluation of lesions [16, 29, 37].

A 1.5T scanner equipped with a phased-array body coil is required, but 3T scanners offer better spatial and contrast resolution, and a shorter breath-hold time [29, 38]. The standard protocol includes T2-weighed fat-suppressed turbo spin echo and T1-weighted fat suppressed spoiled gradient echo sequences, before and after injection of a gadolinium-based contrast agent. A triphasic postcontrast assessment (arterial, portal and equilibrium phases) provides high diagnostic performance, while DWI sequences with apparent diffusion coefficient (ADC) maps may be additionally acquired in order to assess hypercellularity of the lesion.

Most GE-NETs show low-signal intensity on T1-weighted images and hyperintensity on T2-weighted images. However, their appearance on MRI may vary depending on cellular composition and tumor biology [16] (Fig. 7.5).

Small bowel NETs may appear with two different morphologies: nodular mass or regional bowel wall thickening. Given the small dimensions, GE-NETs can be undetected in 20–50% of the cases, in particular gastrinomas and carcinoids of

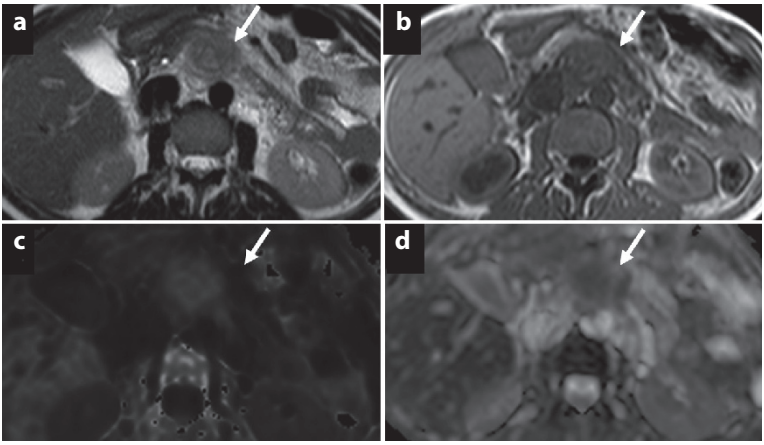

Fig. 7.5 A 54-year-old man with NET of the jejunum. A rounded mass (*arrow*) of around 30 mm located within the mesentery, showing a mildly hyperintense signal on the axial T2-weighted image (**a**), appears hypointense on the axial T1-weighted image (**b**); the lesion shows appreciable restriction (*arrows*) on diffusion-weighted imaging (**c**) and appears hypointense on the ADC map (**d**)

the small bowel [37, 39], representing a diagnostic challenge in clinical practice. In such a scenario, when a small bowel NET is suspected, it is possible to perform bowel distention with biphasic contrast media, administered orally (MR enterography) or with the use of a naso-jejunal catheter (MR enteroclysis) [40].

MRI sensitivity is strictly dependent on good image quality and a particular effort should be made to avoid factors responsible for image degradation such as bowel motion artifacts and patient intolerance. Therefore, the use of spasmolytic agents and detailed instructions for patients are of paramount importance to obtain a diagnostic and effective examination [41].

7.3.4 Endoscopic Ultrasound

EUS plays an important role in the preliminary staging of GE-NETs in the colon and in the upper gastrointestinal tract, giving useful information about depth of intramural invasion, tumor extension beyond the wall and evaluation of regional lymphadenopathy [1, 16]. With a high frequency US probe (7.5–10 MHz) is it possible to assess the depth of gastric, duodenal and colonic wall invasion, guiding the choice of the appropriate endoscopic or surgical procedure (in most patients endoscopic mucosal resection, EMR).

EUS has a relatively high sensitivity, ranging from 79% to 92% [11, 29]. Moreover concerning the appropriate selection of candidates for endoscopic resection, EUS has a very high diagnostic accuracy (sensitivity, 94%) [42]. The most relevant limitation of such a promising method is related to the short portions of the intestinal tract that can be evaluated.

7.4 Imaging of Metastatic Disease

The liver is the second most common organ affected by metastasis from GEP-NETs (44%), preceded by the lymph nodes (90%) [1, 11, 43, 44] and followed by the lungs, bones (7–15%), peritoneum and mesentery (6%, mainly from ileal carcinoids), soft tissue, brain (1.5%), and breast [45–47]. Liver metastasis is an important prognostic factor that negatively influences survival [1, 41].
Liver NET lesions often appear hyperechoic on US, especially those derived from gastrinomas; the specificity is 92–100%, while the sensitivity of this technique has a broad range, from 14% to 63%, mainly related to the patients' characteristics such as fatty liver (brighter than normal) or obese habitus [1, 39, 41].

CT and MRI are the best method to evaluate liver metastases, even though small lesions (3–5 mm) are difficult to detect [1, 16, 39, 43]. On both contrast-enhanced CT and MRI, liver metastases show hypervascular behavior in the arterial phase and washout in the late phases (portal or equilibrium phases) [1, 16]. However, about 15% of GEP-NET metastases appear hypovascular or show a progressive hemangioma-like enhancement (10%) [48] (Fig. 7.6). MDCT has a sensitivity and specificity of 82% (range, 78–100%) and 92% (range, 83–100%) respectively [10]. A triple phase CT protocol with i.v. contrast administration and the "bolus tracking" technique is necessary for an accurate evaluation of

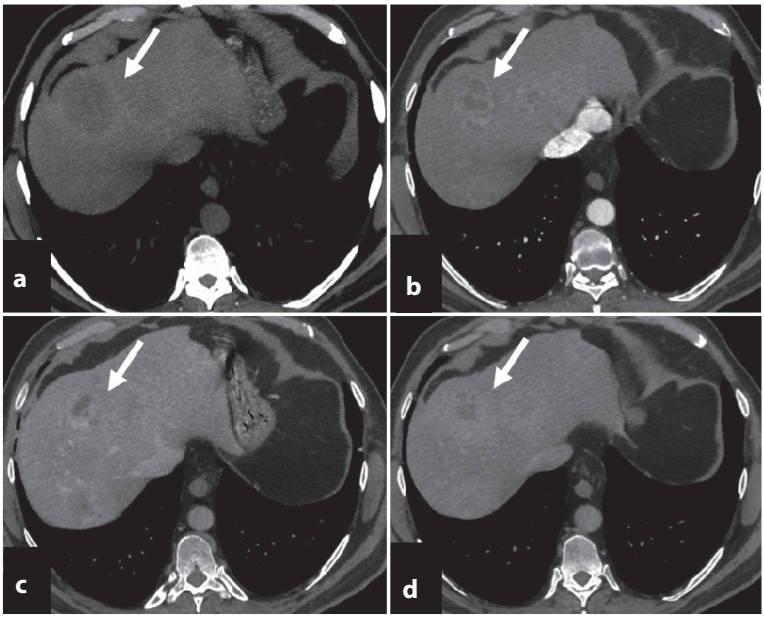

Fig. 7.6 Liver metastasis from NET of the ileum in a 54-year-old man. (**a**) Axial unenhanced CT shows a hypodense lesion (*arrow*) in segment VIII (50 mm). (**b**) Axial early arterial phase contrast-enhanced CT shows hypervascular ring enhancement (*arrow*). The portal (**c**) and delayed phase (**d**) show a progressive hemangioma-like enhancement (*arrows*)

liver lesions. A late arterial phase improves visualization of lesions fed by the hepatic artery, which appear brighter than the surrounding liver. On the other hand, hypervascular lesions are better appreciated during the portal phase, when the healthy liver parenchyma is enhanced. In the case of lesions with internal necrosis, a central hypodense area can be seen with a peripheral contrast-enhanced viable rim [16], so-called ring enhancement.

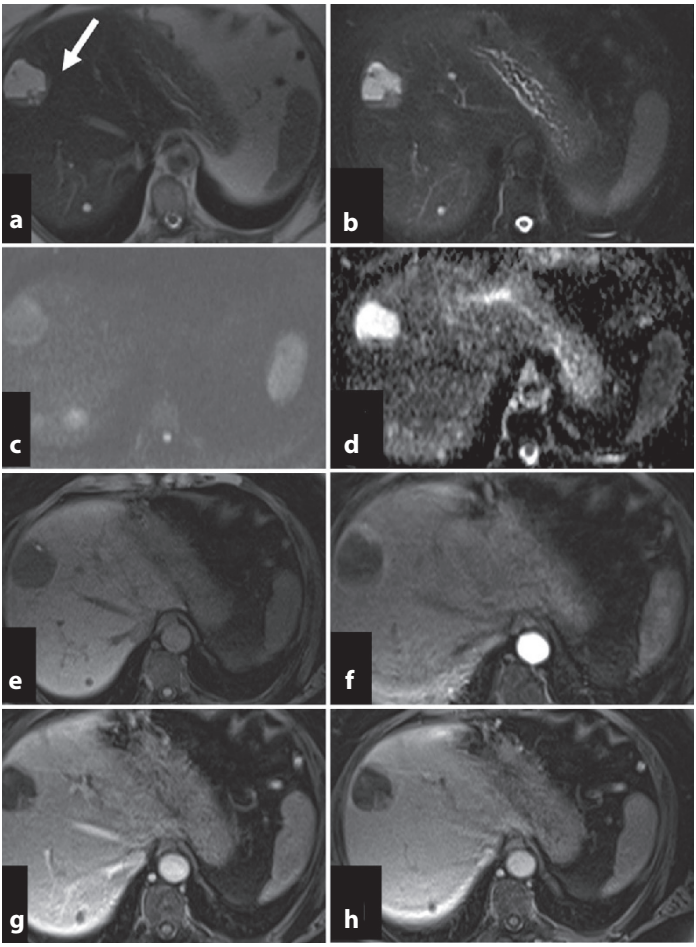

Fig. 7.7 MR imaging of liver metastasis from a malignant non-functioning P-NET in a 70-year-old woman. A rounded well-defined hepatic lesion in segment VIII (30 mm) appears hyperintense on axial T2-weighted MR imaging (**a**) and axial T2-weighted fat-suppressed imaging (**b**) due to the internal cystic component. On the diffusion-weighted image with high b value (**c**) and the ADC map (**d**) the tumor shows hypercellularity at the outer border with appreciable restriction of the movement of the water molecules. On dynamic contrast-enhanced fat-saturated T1-weighted images, acquired before contrast media administration (**e**), during the arterial (**f**) portal (**g**) and equilibrium (**h**) phases, the tumor appears hypointense to the surrounding parenchyma, showing a hypervascular ring enhancement

MRI has sensitivity and specificity ranging from 55% to 79% and from 88% to 100%, respectively [1, 39, 49]. Liver NET metastases appear iso-hypointense on T1-weighted unenhanced images, and slightly hyperintense on T2-weighted images [48, 50]. DWI with ADC maps improves the detection and characterization of malignant liver lesions (Fig. 7.7) [51]. Moreover, liver metastases from NETs had significantly lower ADC values compared to normal liver. This can be considered a truly quantitative parameter potentially able to monitor the progression of disease [52, 1].

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Role of Functional Imaging in the Diagnosis of Neuroendocrine Tumors

8

Stefano Severi and Giovanni Paganelli

8.1 Background

The classification of neuroendocrine tumors (NETs) is complex and constantly changing, with a greater focus placed on their proliferative index [1]. Although NETs are characterized by a low incidence, they have a relatively high prevalence because of their aggressiveness [2].

The term neuroendocrine characterizes the ability of these tumors to secrete neurohormones, and in about 40% of cases they cause a syndrome that can be more or less pronounced [3].

NETs are also characterized by the expression of somatostatin receptors, i.e., biologically active peptides present in the hypothalamus, brain stem, gastrointestinal tract and pancreas, on the cell membrane. Somatostatin exists in isoforms composed of 14 or 28 amino acids but has a very short half-life which makes it unusable for diagnostic purposes. The receptor profile consists of five different subtypes (sst1-5) capable of binding the native somatostatin and synthetic analogs characterized by longer half-life. The most important of these synthetic analogs, octreotide, binds with high affinity to the sst2 subtype, has moderate affinity for sst5 and intermediate affinity for sst3. sst2 is widely expressed in NETs and is therefore an excellent target for the scintigraphic localization of primary and metastatic lesions [4].

Morphological imaging is extremely useful for the characterization of primary and secondary disease, but scintigraphic functional imaging adds important information on specific features such as the expression of the somatostatin receptor – using ^{111}In-Octreoscan and ^{68}Ga-peptide positron emission tomography (PET) [5, 6], metabolic activity FDG PET, and the production of specific amines

S. Severi (✉)
Nuclear Medicine Unit, Cancer Center Institute Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST)
Meldola, Italy
e-mail: stefano.severi@irst.emr.it

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(^{131}I-MIBG, ^{18}F-DOPA PET). In addition, functional imaging provides a clear picture of the extent of disease, which facilitates accurate staging and orients the choice of specific therapies.

8.2 Acquisition Techniques

Nuclear medicine functional investigations have been improved over time and have been further enhanced with the implementation of PET diagnostics. Traditional scintigraphic imaging reconstructs images using single photon emitters, while PET imaging uses positron-emitting radiopharmaceuticals.

Radiopharmaceuticals must be handled by qualified, trained personnel to ensure that labeling procedures and quality controls are performed in accordance with standards of good preparation. Scintigraphic acquisition and analysis are carried out according to Nuclear Medicine Association guidelines.

In the area of traditional scintigraphic imaging, NETs are predominantly studied with ^{111}In-Octreotide (^{111}In-Octreoscan) and $^{123/131}$I-MIBG (metaiodobenzylguanidine). The physical properties of these radionuclides require a gamma camera detection system equipped with parallel-hole medium-energy collimators for indium-111 and iodine-123, and parallel-hole high-energy collimators for iodine-131. Photopeaks are set at 172 and 245 KeV for indium-111, 159 KeV for iodine-123, and 346 KeV for iodine-131. The width of the window is set at 20%, while image matrices, duration of the acquisition, and timing of the same are fixed in a characteristic manner for each radiopharmaceutical. Static, total body and tomographic (SPET – single photon emission tomography) images can all be acquired. The use of hybrid gamma cameras also makes it possible to combine the scintigraphic images with a low-dose CT acquisition to better orient the functional studies.

The technological advances have greatly increased image sensitivity, which has led to more accurate diagnoses. In nuclear medicine, imaging must be carefully evaluated to differentiate the areas of physiological uptake from the pathological ones. Acquisition guidelines have been published by the European Association of Nuclear Medicine (EANM)/Society of Nuclear Medicine (SNM), and the Italian Nuclear Medicine Association (AIMN) [7, 8].

PET is specifically designed to register with appropriate detectors the position of the emission of a positron, which indirectly emits pairs of gamma rays. For both ^{68}Ga-DOTA-peptides [9] and ^{18}F-FDG PET [10], images are acquired one hour after radiopharmaceutical administration. Nowadays acquisition can be continuous and takes about 20 minutes to cover the entire body. Modern scanners are all hybrid with either computed tomography (CT) or magnetic resonance imaging (MRI).

High-performance instruments coupled with different radiopharmaceuticals enable us to explore a wide variety of functions such as local consumption of glucose,

number of somatostatin receptors and local concentration of neurotransmitters such as dopamine and norepinephrine. In addition to producing images, PET imaging also allows us to evaluate semiquantitative data to define the extent of the radiopharmaceutical uptake, which takes into consideration the instrument used, the injected dose, the patient's weight, and the time elapsed between the administration of the radiopharmaceutical and the acquisition. These data are very useful for assessing differences in uptake registered over time. The information acquired through imaging and uptake studies provides a series of bodily function parameters that can be used to evaluate the tumor metabolic burden.

The quantification parameters used for PET imaging are: 1) standardized uptake value (SUV), which is a semiquantitative measurement of fluorodeoxyglucose (FDG) uptake in tumors; 2) metabolic tumor volume (MTB), which measures the total volume of the tumor calculated using a three-dimensional volumetric method; 3) total lesion glycolysis (TLG), which represents the product of the average SUV by the MTV [11, 12].

8.3 ^{18}F-DOPA

^{18}F-DOPA, one of the first radiopharmaceuticals used in NETs, was originally developed to study patients with movement disorders. This aromatic amino acid marked with fluorine-18 is captured, stored and decarboxylated by neuroendocrine cells in the same way as the native amino acid. Once inside neuroendocrine cells, it undergoes catecholamine synthesis by following a specific enzymatic pathway that converts the amino acid tyrosine into L-DOPA (L-3,4 dihydroxyphenylalanine), subsequently decarboxylated to dopamine. ^{18}F-dopamine is then stored in secretory granules where the radioactivity remains trapped.

The decarboxylation to dopamine (aromatic amino acid decarboxylase) is the basis of premedication with carbidopa, a peripheral aromatic amino acid that inhibits decarboxylation and reduces background. The procedure, used by many authors, increases the tumor/background ratio increasing the sensitivity of the scan [13]. ^{18}F-DOPA, registered since the early 2000s, is commercially available but its use is limited by a complex and expensive process of synthesis.

In a prospective single center study, Koopmans et al. compared the sensitivity of ^{18}F-DOPA, somatostatin receptor scintigraphy (SRS) and CT in 53 patients with metastatic carcinoid tumors. ^{18}F-DOPA showed a sensitivity of 100% versus 92% of SRS and 87% of CT. The combination of SRS and CT showed a sensitivity of 96% [13]. ^{18}F-DOPA PET also identified a higher number of positive lesions and the lesions with the highest positivity in each region than SRS. This technique has proven particularly useful for the management of cases not clearly diagnosed with other methods (ultrasound, CT, SRS, MRI) [14].

Although ^{18}F-DOPA PET can be used for all types of well-differentiated NETs and is indicated for staging, restaging after therapy and follow-up, and for the

identification of the primary tumor in patients with metastatic disease, it is currently only used in specific cases. This is because ^{18}F-DOPA PET is expensive, technically difficult to produce and, unlike ^{68}Ga-DOTA-peptide PET and $^{123/131}$I-MIBG, does not provide indications for a subsequent therapy. Nonetheless there are specific settings in which ^{18}F-DOPA PET has shown diagnostic superiority, e.g., medullary thyroid cancer, neuroblastoma and pheochromocytoma. In a meta-analysis comprising 11 studies and 275 patients with suspected paraganglioma, ^{18}F-DOPA PET showed 91% sensitivity and 95% specificity [15]. However, a more recent study including a limited number of cases (n = 20) reported that ^{68}Ga-DOTA-TOC PET documented a higher number of lesions than ^{18}F-DOPA PET or CT (45 vs. 43 vs. 32, respectively) [16].

Images are acquired 60–90 minutes after the administration of 5–6 MBq/kg of ^{18}F-DOPA. Physiological uptake areas include the striatum and pancreas, with possible minimum adrenal uptake. As the radiopharmaceutical is eliminated via the biliary tract, intestines and kidneys, these organs are normally visualized.

8.4 $^{123/131}$I-MIBG

The enzymatic pathway of L-dopamine leads to dopamine that can be subsequently hydroxylated to norepinephrine. MIBG is an analog of norepinephrine that follow the same metabolic pathway and can be concentrates within secretory granules of catecholamine-producing cells.

MIBG labeled with either iodine-131 or iodine-123 is used in nuclear medicine. The latter is the more sensitive of the two agents and has a better dosimetry [17–19]. The scan is also widely used in traditional scintigraphic imaging. The acquired images have a good quality and provide indications also for the potential benefit from treatment with ^{131}I-MIBG. ^{123}I-MIBG scintigraphy is more frequently used to confirm the suspicion of pheochromocytoma in an adrenal mass, with a sensitivity and specificity of 90% and 95%, respectively. Taking into consideration an observed increase in catecholamines and positivity scintigraphy with ^{123}I-MIBG, the sensitivity of this diagnostic tool rises to almost 100% [20]. However, it must be remembered that the method is less sensitive for small lesions and extra-adrenal tumors (85% adrenal vs. 65% non-adrenal and familial forms) [21, 22].

Whilst abdominal and chest paragangliomas may be functionally active (catecholamine secretion), those of the head and neck are rarely secretory and in such cases ^{123}I-MIBG imaging is not particularly helpful [23]. Given the overexpression of somatostatin receptors, other scintigraphic imaging techniques have proven useful in this patient setting, with higher sensitivity (93% vs. 44%) and better image quality compared to ^{123}I-MIBG scintigraphy [24]. In addition, ^{68}Ga-DOTA-peptide PET has been reported to show a higher sensitivity than ^{123}I-MIBG in patients with chromaffin cell tumors in (pheochromocytoma and

paraganglioma) [25], with the advantage of providing an indication for peptide receptor radionuclide therapy (PRRT) [26].

MIBG imaging requires the suspension of several drugs that interfere with its uptake, intracellular transport, accumulation of granules and retention. It also requires a thyroid function block with non-radioactive iodine or potassium perchlorate to avoid possible non-specific irradiation of the gland. The administered activity is about 185 MBq for ^{123}I-MIBG and 37/55 MBq for ^{131}I-MIBG. Planar or single-photon emission CT (SPECT) images are usually acquired after 4 and 24/48 hours with ^{123}I-MIBG and also after 72 hours with ^{131}I-MIBG. Both ^{123}I-MIBG and ^{131}I-MIBG normally clearly show the salivary glands, heart, lungs, liver and spleen, whereas the bladder and intestines are poorly visualized. The adrenals are frequently seen with ^{123}I-MIBG and rarely with ^{131}I-MIBG. Areas of uptake outside these sites of physiological accumulation are suspicious for malignancies.

8.4.1 ^{131}I-MIBG Therapy

Therapy with ^{131}I-MIBG is indicated in cases of malignant pheochromocytoma and paraganglioma. Diagnostic ^{123}I-MIBG positivity may also lead to specific therapy in NET patients not expressing somatostatin receptors or with renal impairment [27]. The coupling of ^{123}I-MIBG and ^{131}I-MIBG is a perfect example of the use of the same tracer for diagnosis and therapy, which forms the basis of the modern concept of theranostics.

As already mentioned, the indications for this type of therapy have decreased in recent years in favor of treatment with PRRT, mainly because of the need to block thyroid function and to protect patients against the high gamma emission of iodine-131.

Homogeneous studies are lacking in terms of regimens used and adequate numbers of patients, both of which are needed to understand the real effectiveness of the treatment. A review of 116 patients with malignant pheochromocytoma treated with 131I-MIBG showed that symptomatic improvement was achieved in 76% of patients, hormonal response was present in 45% of cases, and a tumor response was obtained in 45% of cases. Response was more frequent in cases of limited disease and in the presence of soft tissue lesions [28]. Although there are fewer publications on paraganglioma patients treated with 131I-MIBG, findings appear similar [29].

8.5 ^{18}F-FDG PET

^{18}F-FDG is a glucose analog transported into the cell by a specific system and, once inside, the analog is phosphorylated by cytoplasmic enzymes. The

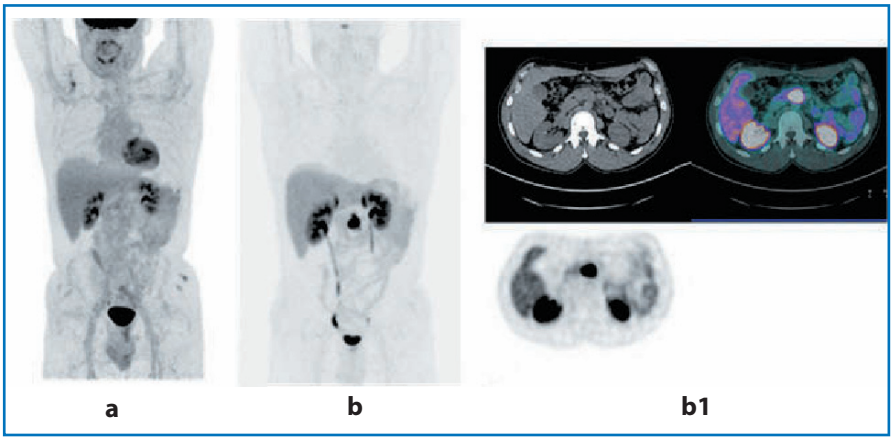

Fig. 8.1 G2 pancreatic NET (Ki-67 12%) in a 48-year-old man. **a** Baseline negative FDG PET. **b** Baseline ^{68}Ga-DOTA-peptide PET with evidence of pancreatic lesion. **b1** Baseline ^{68}Ga-DOTA-peptide PET with evidence of pancreatic lesion not clearly defined by morphologic imaging

compound obtained cannot be further metabolized and accumulates in the cell. A common characteristic of tumors, especially the more aggressive ones, is their high utilization of glucose which is metabolized primarily in an anaerobic manner (Warburg effect). This phenomenon is the basis for the use of ^{18}F-FDG in oncology as a tool for staging, assessing response to treatment and planning external beam radiotherapy.

In general, ^{18}F-FDG PET sensitivity is not high in NETs because of their reduced metabolism, making this procedure unsuitable for staging NET patients. In an important prospective study, 98 NET patients were submitted to surgery and treated with various therapies. After the first year of follow-up, positivity to ^{18}F-FDG PET showed a greater prognostic value than Ki-67, CG-A and the presence of liver metastases [30]. Thirteen (23%) of the 57 patients with a positive ^{18}F-FDG PET died compared to the only ^{18}F-FDG PET-negative patient (hazard ratio, 10.3; 95% CI, 1.3–78.9).

Our group conducted a prospective phase II study of 52 patients with metastatic and progressive NETs treated with ^{177}Lu-DOTATATE, showing that ^{18}F-FDG PET was positive in 57% of patients with G1 disease and in 66% of those with G2 [31] (Fig. 8.1a). In addition, the disease control rate was 95% in G1 patients and 79% in G2, but was 100% in ^{18}F-FDG PET-negative patients and 76% in those with ^{18}F-FDG PET-positive tumors. These findings highlighted the lack of indication for the use of ^{18}F-FDG PET as a diagnostic tool, but confirmed its usefulness as an indicator of a better response to therapy.

The prognostic value of this scan in NETs was confirmed for different tumor histologies [32] and subsequently demonstrated by other authors. Our group also observed the prognostic value of ^{18}F-FDG PET in another prospective phase II study of patients with inoperable or progressive pancreatic NETs [33].

The main limitation of ^{18}F-FDG PET is that non-malignant lesions with a high concentration of inflammatory cells also show increased ^{18}F-FDG PET activity, due to the high concentration of glucose in activated neutrophils and macrophages, leading to a high risk of false positive images.

8.6 5-Hydroxytryptophan (^{11}C-5HTP)

An overactive serotonin pathway is frequent in many NETs, leading to the development of a tracer involved in the metabolism of serotonin.

The immediate serotonin precursor is 5-hydroxytryptophan (5HTP), which is decarboxylated to serotonin. Serotonin is further converted into 5-hydroxyindoleacetic acid by monoamine oxidase and aldehyde dehydrogenase.

5HTP can be labeled with carbon-11 and has considerable potential for use in PET imaging, especially to study patients with midgut NETs. However, the production of ^{11}C-5HTP is a complex process and only a few centers are adequately equipped for it.

^{11}C-5HTP PET is reported to be superior to CT and SRS in NETs. In a study on 38 NET patients (including those with gastroenteropancreatic NETs and pulmonary NETs), lesions were detected in 95% of cases by ^{11}C-5HTP PET, in 84% by SRS and in 79% by CT [34].

Scanning is generally performed 20 minutes after the injection of ^{11}C-5HTP in patients orally pretreated with carbidopa, which is useful to optimize image quality. The interpretation of ^{11}C-5HTP PET images is facilitated by the high tumor-to-background ratio due to the low tracer concentration in normal tissues.

8.7 ^{111}In-Octreoscan

Iodine-123 was the first radiopharmaceutical to permit the visualization of neuroendocrine tumors [35]. Subsequently indium-111, linked to a somatostatin analog through the diethylenetriaminepentaacetic acid chelator (DTPA), was the first radiopharmaceutical to identify the same NETs through the receptor imaging mechanism.

A few years later ^{111}In-DTPA-pentetreotide obtained full approval of the regulatory authority and is commercially known as ^{111}In-Octreoscan.

Despite the development of numerous diagnostic radiopharmaceuticals with different somatostatin analogs, ^{111}In-Octreoscan is currently the only commercially available radiopharmaceutical and has significantly influenced the study of NETs.

Over the years, scan sensitivity has been incremented by using hybrid gamma cameras [36, 37], and is now substantially higher (75%) for pituitary gland tumors, GEP-NETs, paragangliomas, small cell lung cancer, and moderately

high (40–75%) for insulinomas, pheochromocytomas and medullary thyroid carcinomas [36].

^{111}In-Octreoscan was also used as the first therapeutic radioreceptor agent because the peptide receptor complex is transported into tumor cells near the nucleus and its high-energy Auger electron conversion functions as a cytotoxic agent. In a multicenter study, 40 patients positive for somatostatin receptors who received at least 20 GBq of ^{111}In-Octreoscan showed some clinical benefit, but efficacy data were poor, with one partial remission, six minor responses and 14 cases of stable disease [38, 39].

The radiopharmaceutical is administered at a dosage of 185 MBq and the standard set of acquisitions includes static or total body images 4, 24 and 48 hours after injection. The long half-life of ^{111}In (T/2 2.8 days) makes it easier to assess the dynamics of the radiopharmaceutical uptake, which can sometimes be quite slow. After administration of the radiopharmaceutical, plasma clearance is fairly rapid and progressive tissue accumulation is seen. A normal scan shows physiological uptake in the spleen, kidneys and liver, while a variable uptake is present in the pituitary gland, thyroid, bladder and intestines. The images should be interpreted with the integration of clinical information and morphologic images. To define a region as hyperactive, its radiotracer concentration must be at least equal to that of the liver, and the intensity of radiopharmaceutical concentration is graded following a well-defined scale [40]. Uptake indices are also useful for providing indications for PRRT using either lutetium-177 or yttrium-90 DOTA-peptides.

^{111}In-Octreoscan false positive results may be linked to areas of inflammation or post-surgery scars. False negative results must also be evaluated for possible interference with cold somatostatin analog therapies.

8.8 Somatostatin Receptor PET and Post-PRRT Imaging

The fundamental role played by ^{111}In-Octreoscan scintigraphy in the study of NETs prompted research into alternative PET radiopharmaceuticals that could take advantage of the superior technical characteristics of this type of imaging.

Various somatostatin analogs capable of binding with β^+ emitter gallium-68 (t/2 68 minutes, 89% of positron emission with an energy of 830 keV) through the DOTA chelator were developed. Of these, octreotide (TOC) and octreotate (TATE), both endowed with high receptor affinity for SSTR2 and SSTR5, and [Nal3]-octreotide (NOC), with a high affinity for SSTR2, SSTR3 and SSTR5 [41], are the most widely used. In general, different receptor affinities of peptides did not result in a significant difference in sensitivity in their diagnostic use [9]. If anything, the difference is mainly linked to the fact the octreotide and octreotate, used in combination with β-emitting radionuclide yttrium-90 and lutetium-177, respectively, are widely employed in PRRT and therefore more suitable for theranostics (Fig. 8.1b,b1).

In recent years, there has been a substantial increase in publications on peptides labeled with ^{68}Ga-DOTA-peptides, but the most widely used are ^{68}Ga-DOTA-NOC and ^{68}Ga-DOTA-TOC, with a sensitivity of 90-98% and 92–98%, respectively [42]. Gallium-68 is obtained from the elution of a ^{68}Ge/^{68}Ga generator (most Nuclear Medicine Units have this equipment) and used to label a chosen peptide through the DOTA chelator.

The in-house availability of the radionuclide, together with its favorable diagnostic sensitivity, has largely contributed to the successful use of this β-emitter. Images are acquired about one hour after the administration of an average 100 MBq of gallium-68 (range 75–250 MBq). The physiological distribution of the radiopharmaceutical is found in the pituitary gland, spleen, liver, adrenals, head of the pancreas, thyroid, kidney and bladder. Although there are no proven indications for the suspension of an analogous cold therapy, it is advisable to perform the scan at least 20 days after the last injection. Asymmetrical areas of increased activity outside of normal distribution sites are classified as pathological. False positive images may be observed for accessory spleen, lymphoma and sites of inflammation. Uptake in the uncinate process of the head of the pancreas is not considered suspicious if the CT images are negative. In a series of 245 patients who underwent ^{68}Ga-DOTA-peptide PET, non-specific or diffuse uptake of the pancreatic head was found in 23% and 8% of patients, respectively. This phenomenon, if the SUV is comparable with that of the liver, can be considered non-specific [43].

Generally speaking, the significance of variations in SUV must be carefully evaluated because numerous parameters are involved in its definition and data may differ greatly from center to center [44].

An assessment of the sensitivity of ^{68}Ga-DOTA-peptide PET was made in 84 patients with NETs of various origin, comparing PET performance with that of CT and scintigraphy with ^{111}In-Octreoscan. PET showed higher sensitivity (97%) than CT (61%) or scintigraphy (52%) in evaluating lesions, especially in small tumors and lymph node and bone lesions [45, 46] (Fig. 8.1b1).

^{68}Ga-DOTA-peptide is indicated for well-differentiated NETs and is often capable of accurately identifying the unknown site of the primary tumor. At the same time, ^{68}Ga-DOTA-peptide offers the possibility of evaluating the extent of the disease [47] and provides indications for treatment with cold analogs or PRRT.

The indication for ^{68}Ga-DOTA-peptide PET is not limited to NETs as many tumors overexpress somatostatin receptors including medullary thyroid cancer, lymphoma, paraganglioma, glioblastoma, meningioma, breast cancer, sarcoma and Merkel-cell carcinoma. Consequently, patients with ^{68}Ga-DOTA-peptide PET-positive tumors, for whom there is often no effective therapy, may be candidates for PRRT.

Although the role of ^{68}Ga-DOTA-peptide PET in evaluating response to PRRT [47] has been acknowledged, there are still some concerns about the evidence that a potential dedifferentiation of the disease may generate false negative results.

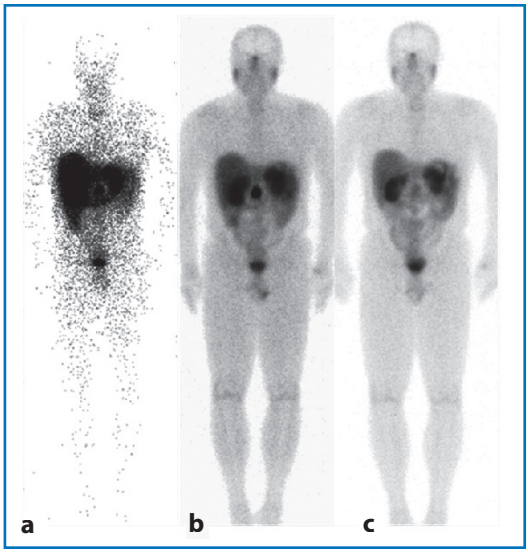

Fig. 8.2 G2 pancreatic NET (Ki-67 12%) in a 48-year-old man. **a** Baseline ^{111}In-Octreoscan 24 hours scan with physiological distribution of the radiopharmaceutical and central pancreatic lesion. **b** ^{177}Lu-DOTATATE total-body acquisition, performed 24 hours after the first cycle of therapy. **c** ^{177}Lu-DOTATATE total-body acquisition, performed 24 hours after the third cycle of therapy

Another interesting diagnostic option in this field is related to ^{177}Lu-DOTATATE PRRT. Lutetium-177 emits both β^- particles and gamma rays (208 KeV at 11% and 113 KeV at 6.5%). Imaging – generally achieved 24 hours after therapy – is of good quality and generally comparable to what can be achieved with ^{111}In-Octreoscan [48]. The administered activity is therapeutic and so fairly high (range 3.7/7.4 GBq each cycle) with respect to ^{111}In-Octreoscan (Fig. 8.2a,b). Consequently, images can be used to evaluate lesion uptake intensity, tumor burden, the presence of non-active lesions and possible tumor response or progression during cycles (Fig. 8.2b,c). This option also reduces the need to perform numerous morphological assessments between cycles.

Despite the success of PET with ^{68}Ga-DOTA-peptide, research has developed new important diagnostic possibilities using ^{64}Cu-DOTATATE. Thanks to the optimal physical characteristics of this radioisotope, which has a longer half-life than that of gallium-68 (t/2 12.7 hours, emission energy β^+ 278 KeV), it is possible to acquire delayed images and to detect lesions with slow uptake, as in the case of ^{111}In-Octreoscan. In a recent study, 59 patients with NETs of various origin underwent PET with ^{68}Ga-DOTATATE and ^{64}Cu-DOTATATE. The latter agent detected 42 lesions not seen with ^{68}Ga-DOTATATE, 33 of which proved to be true positives during follow-up. Conversely, ^{68}Ga-DOTATATE highlighted 26 lesions not detected by PET ^{64}Cu-DOTATATE, but only seven of these were true positives [49].

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Pathological Analysis of Abdominal Neuroendocrine Tumors

9

Erminia Manfrin, Angelica Sonzogni, Aldo Scarpa, and Giuseppe Pelosi

9.1 Introduction

Pathology plays a fundamental role in the proper clinical management of abdominal neuroendocrine neoplasm patients. Essential information regards the precise tumor classification (neuroendocrine vs. non-neuroendocrine) by using morphology and immunohistochemistry, the separation between well-differentiated neuroendocrine tumors (NETs) and poorly differentiated carcinomas (NECs) by means of tumor architecture and neuroendocrine marker immunohistochemistry, the identification of the organ of origin, the attribution of grading (G1 to G3) upon mitotic count and Ki-67 labeling index (LI), and assessment and definition of staging on the basis of tumor size and extent according to existing guidelines [1–4].

Additional information may regard further histologic characterization (non-ischemic necrosis, vascular and perineural invasion, resection margin status) and evaluation of the functional status by means of clinicopathologic correlations [1]. In cases of metastatic tumors or to better substantiate morphologic diagnoses, specific immunohistochemistry or molecular markers may prove to be particularly useful especially in the case of NETs, while NECs especially of the small-cell type may also illegitimately express a wider range of unrelated factors [5–7].

All this information is easily gathered on surgically resected tumors, but fine-needle aspiration cytology (FNAC) or small-sized biopsy samples may be the only available material for rendering an ultimate diagnosis on inoperable neuroendocrine cancer patients to make operative decisions on the clinical treatment. Therefore, the type and extent of information about gastroenteropancreatic NETs (GEP-NETs) is a function of the material under evaluation, as discussed below in some detail.

G. Pelosi (✉)
Department of Oncology and Hemato-Oncology, University of Milan,
and Interhospital Pathology Division, Science & Technology Park, IRCCS MultiMedica
Milan, Italy
e-mail: giuseppe.pelosi@unimi.it

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9.2 Fine-needle Aspiration Cytology

Diagnostic assessment upon FNAC of abdominal NETs is generally performed on space-occupying lesions in parenchymal organs, where primary and secondary lesions can be found by imaging techniques. The prototypical organs where this technique has proved to be particularly useful are the pancreas and the liver, the latter being the most frequent site of metastatic neuroendocrine neoplasms, including those of unknown origin.

The preoperative diagnosis of abdominal NETs or liver metastases may be challenging, requiring an integrated combination of careful examination of the patient history, physical examination, laboratory testing, imaging studies and strategic tissue acquisition [8, 9]. Major development in the diagnosis and management of neuroendocrine cancer patients has stemmed from improvements in strategically sampling lesions for preoperative diagnosis, grading and staging by using a combination of cytology and ultrasound guidance.

FNAC is used worldwide as a cost-effective and highly accurate diagnostic tool for safely performing preoperative diagnoses [10]. The increasing use of FNAC on GEP-NETs has been made possible through the development of minimally invasive techniques of endoscopic ultrasound (EUS)-guided and percutaneous ultrasound (PUS)-guided fine-needle sampling, which have now become the procedures of choice for securing a diagnosis of pancreatic lesions or liver metastases [11, 12]. In a large series of pancreatic NETs, the sensitivity, specificity, positive and negative predictive values and diagnostic accuracy were 98.7%, 100%, 100%, 75.5% and 98.7%, respectively, with a complication rate of only 0.8% [11].

Overall diagnostic accuracy is mainly influenced by the experience of the pathologist who directly performs the aspiration, by the size and site of lesions [13] and by the rapid on-site evaluation for adequacy of FNAC passages on slides [14]. However, improvements in these techniques have made the diagnosis of many abdominal NETs possible even on small lesions measuring 1 cm or less [13].

On cytology, pancreatic NETs or tumors metastatic to the liver are similar to other NETs at any site of the GEP tract or the body. In well-differentiated lesions, smears are rich in single, isolated, monomorphous cells with round and regular nuclei. Nucleoli are inconspicuous and chromatin is fine and evenly distributed. The eccentric polarization of the nucleus confers a plasmacytoid aspect to neoplastic cells and this finding is frequently observed on cytology. Rare giant cells and binucleation are occasionally on record; mitoses are rare and necrosis is uncommon (Fig. 9.1a,b).

Conversely, poorly differentiated NETs show sheets of loosely cohesive and highly atypical cells, irregular and small to large nuclei, even to coarse chromatin pattern and variable cytoplasm. Nuclear molding, stripped nuclei and mitoses are not uncommonly observed (Fig. 9.1c,d). The diagnostic accuracy of cytology is powered by synaptophysin and chromogranin A reactivity by neoplastic cells

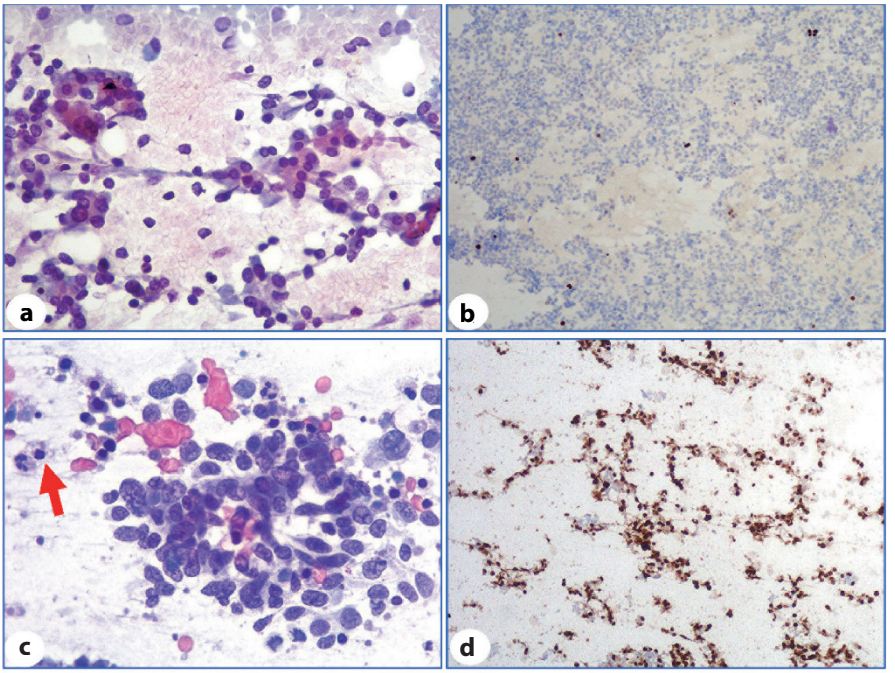

Fig. 9.1 Features of neuroendocrine neoplasms as seen on fine-needle aspiration cytology. A well-differentiated tumor presents with regular cell morphology (**a**) and a low Ki-67 labeling index (**b**), while a poorly differentiated neuroendocrine carcinoma exhibits more evident cell atypia with a recognizable mitotic figure (*red arrow*) (**c**) alongside a very high Ki-67 labeling index (**d**)

and additional immunohistochemistry investigation (TTF-1, CDX-2, PAX-8, Islet 1, serotonin, PSAP) may help identify or confirm the site of origin of the neoplasms [6, 15]. An appropriate and reasonable antibody panel can be applied to cytological preparations for differentiating other primary pancreatic lesions and neoplasms, including chronic pancreatitis with islet aggregation, ductal adenocarcinoma, solid pseudopapillary tumor, acinar cell carcinoma, pancreatoblastoma, whose detailed analysis is beyond the scope of this work.

FNAC findings cannot reliably predict the biological behavior of abdominal NETs, even though the presence of mitoses, irregular nuclear membranes and necrosis appear to be more likely associated with an aggressive behavior [15]. While tumor grading is routinely accomplished on surgical and biopsy specimens according to existing guidelines [1–4], several studies focused on the usefulness of FNAC samples for grading these tumors [16–18]. However, the diagnostic accuracy of this technique ranges from 58–86% and it remains unclear if the Ki-67 labeling index (Ki-67 LI) obtained from EUS-FNA samples accurately reflects that obtained on surgical specimens [16–18]. Results may be influenced by small tumor size (<20 mm) and intratumor heterogeneity in Ki-67 LI measurement, especially in larger tumors, thereby diminishing the cytology-

histology concordance rate [16–18]. Another reason for this relatively lower concordance rate is the unpredictable number of cells obtained by FNAC as demonstrated by an increase of diagnostic accuracy when 2000 tumor cells or more are obtained [19]. The recent introduction of techniques for obtaining core tissue samples and cell blocks will further improve the reliability of diagnostic FNAC techniques in the preoperative phase.

9.3 Small Biopsy

Small biopsies obtained by PUS or EUS investigation allow primary or metastatic GEP-NETs to be preoperatively diagnosed through the adoption of larger needles with cutting edges, which collect adequate tissue fragments. Another source of diagnostic material are endoscopic biopsies from the stomach or large bowel in the case of mass-forming lesions. Core tissue and endoscopic biopsies with different dimensions are optimal materials for histologic interpretation, tissue staining and molecular investigation. Core needle biopsies (CNB) using 25–20-gauge needles, endoscopic sampling and Tru-Cut needle biopsies with 18–19-gauge needles are the technical instruments most often used in clinical practice (Fig. 9.2a–d).

Despite small size, CNB is advantageous because tissue architecture is well preserved for tumor diagnosis and biological assessment in paraffin embedded material [20–22]. Upon histology, tissue fragments lie often intermingled with blood clots due to the high vascularization of most NETs, in which isolated neoplastic cells are preserved enabling a definitive tumor categorization.

There is a great debate in the reported utilization of CNB in NETs patients, with either a preferential use of CNB over FNAC or even limitation of CNB to only indeterminate cases on FNAC being reported on literature [23, 24]. CNB specificity ranges from 96% to 100% for diagnosing primary and secondary NETs, with sensitivity ranging between 60% and 90% [23, 24]. It is accepted that CNB samples provide more accurate subtyping on some tumors than do FNAC samples, with an incremental value of histology over cytology in the specific characterization of pancreatic neoplasms other than adenocarcinoma [25, 26]. On the other hand, it has been proposed to carry out the on-site evaluation of biopsy adequacy by rolling the cores across slides to obtain cytological specimens for immediate assessment [27, 28]. This procedure on CNB can improve the overall diagnostic sensitivity by 8% [29], supporting the view that the combination of cytology and histology is by far superior to either one individually [23, 30, 31]. Overall, tissue fragments of GEP-NETs make up a precious reservoir of material for differential diagnosis, immunohistochemistry characterization and molecular testing by providing the opportunity for multiple tissue sections [20–22]. This is particularly crucial in liver metastases of unproven origin NETs, where a

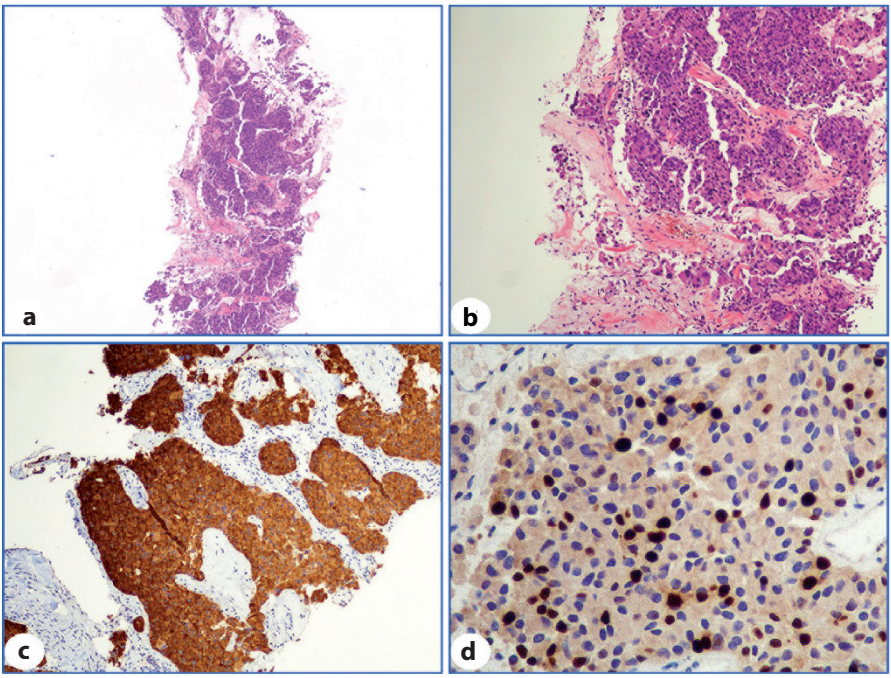

Fig. 9.2 Features of neuroendocrine neoplasms as seen in core biopsy. A well-formed tissue cylinder has been obtained by core biopsy (**a**), containing abundant tissue featuring a well-differentiated neuroendocrine tumor (**b**) with strong synaptophysin immunohistochemistry (**c**) and moderate Ki-67 labeling index (**d**) for the final diagnosis of NET G2

combination of different markers (TTF-1, CDX-2, PAX-8, Islet 1, serotonin, PSAP) may help characterize the cell lineages of tumor growths [5–7, 32].

Whether biopsy samples of primary or metastatic NETs are optimal material also to grade tumors is still a debated but undeniable issue for its important clinical implications. Recent prospective studies have demonstrated that either FNAC [20] or biopsy [33, 34] was adequate for histological diagnosis and Ki-67 LI assessment in patients with non-functioning pancreatic NETs, but the crucial question remains as to whether this recruited material is really representative of the whole lesion due to intratumor heterogeneity [35]. For example, pancreatic NETs larger than 1.8–2.0 cm sampled by CNB showed a lower concordance between Ki-67 LI and tumor grading than smaller tumors [34], indicating that the intratumor heterogeneity accounted for such a discrepancy [20, 33–35].

Some studies have indicated that biopsies from liver metastases of the GEP NETs failed to reliably separate G1 from G2 as compared to the same assessment on the whole tissue sections [35], thus confirming a major role for intratumor heterogeneous distribution of cell clones with different proliferation activity. This phenomenon can even become grueling, when diversely and unpredictably distributed neuroendocrine components with well-differentiated and poorly

differentiated features coexist in the same tumor mass as suggested for NETs arising in the GEP tract [36], lung and thymus [37]. However, a unique study has thus far demonstrated that Ki-67 LI assessment may be concordant between biopsy samples and resection specimens of lung NETs when strict counting guidelines were applied [38]. These included the identification of hot spot regions, which remains a matter of perception, in either type of material and the KI-67 LI assessment on 2,000 cells, 2 mm^2 or the entire biopsy fragments to account for tumor sampling, tissue fragment sizing and intratumor heterogeneous distribution of defining criteria [38]. This innovative approach paves also the way to using Ki-67 LI for better grading of these tumors in close combination with morphologic classification [38].

A crucial issue regards the management of biopsies or FNAC of liver metastases from NETs originating in extra-GEP anatomical sites, such as the lung, where terminology (typical and atypical carcinoid instead of NETs) and defining criteria (grading based on morphology rather than Ki-67 LI) are quite different [39]. In these cases, clinical correlation is always fundamental and the relevant terminology should be adapted and commented to avoid an improper generalization of classification criteria.

9.4 Surgical Specimens

Surgical specimens represent the ideal and irreplaceable material of investigational studies, where most features of the GEP-NETs can be elucidated, although representative only of a fraction of lesions, predominantly NETs, amenable to surgical resection [40].

They are the basis for the deepest knowledge on the pathobiology of these neoplasms, inasmuch as many biological, diagnostic, prognostic and predictive characteristics can be here developed [1]. Therefore, surgical specimens should be optimally fixed, processed and stored to avoid technical artifacts and then accurately evaluated on gross and microscopic pathology [1]. Furthermore, storing frozen material and blood samples from paired patients is a powerful tool for many research activities on genomics, metabolomics or proteomics of NETs. In general, gross features do not help differentiate GEP-NETs from each other and from the more common non-neuroendocrine tumors. However, the distribution and number of lesions may provide helpful hints to recognize varying subtypes of NETs.

Most gastrinomas (74%) and somatostatinomas (84%) arise on the right of the superior mesenteric artery in the pancreatic head, whereas most insulinomas (74%) and glucagonomas (77%) are positioned on the left of this artery in the body and tail of the pancreas [1]. Inside the pancreas VIPomas are more prevalent in the distal pancreas (77%), while non-functioning NETs have been reported to be either uniformly distributed within the whole organ or preferentially restricted

to the cephalic region. Gastrinomas and somatostatinomas may frequently arise in extrapancreatic locations, such as duodenum or proximal jejunum. Ninety per cent of gastrinomas occur within the so-called "gastrinoma triangle", a virtual anatomic area of triangular shape bounded by the junction of the cystic and common bile ducts, the junction of the second and the third portions of the duodenum, and the junction of the head and the body of the pancreas. The most common site of sporadic gastrinomas is the head of the pancreas, followed by the first and second portions of the duodenum and the periduodenopancreatic lymph nodes as metastatic deposits of unrecognized duodenal tumors. In turn, the localization in bile ducts or stomach or in other anatomical sites including the jejunum [1], liver, kidney, ovary, and parathyroid is very rare, in opposition to the normal distribution of gastrin-producing neuroendocrine cells in the gastric antrum and the duodenal wall [4].

In MEN1 patients, gastrinomas most commonly originate in the duodenum, and, less frequently, in the pancreas. Duodenal somatostatinomas are more frequent than those arising in the pancreas, and are generally located in the second portion of the duodenum, often in the ampullary-periampullary region. Unlike gastrinomas and somatostatinomas of the pancreas, which are nearly always functionally active, the same duodenal neuroendocrine tumors are associated with hormonal symptoms in only 40–45% of the patients. GEP-NETs may present as either sporadic or familial tumors. Sporadic lesions are generally single, although multifocal tumors, either synchronous or metachronous, have been described in 2–13% of pancreatic insulinomas, 40% of duodenal gastrinomas, 30% of duodenal somatostatinomas and gastric NETs associated with chronic atrophic gastritis [1]. Multifocal pancreatic, duodenal or gastric NETs are instead the rule in MEN1 patients.

9.4.1 Gastric NETs

There are three main categories of gastric NETs, most of which are well-differentiated tumors (G1 and G2 NETs) composed of enterochromaffin-like (ECL) cells arising in the fundal-type mucosa or in the body-antrum border, while neuroendocrine carcinoma (NECs), tautologically G3, accounts for about 10% of them [1, 41]. NETs in turn are split into type I (≈74%, F>M, VI–VII decade) when associated with autoimmune atrophic gastritis, type II (≈6%, F=M, V decade) when related to MEN1 or Zollinger-Ellison syndrome and type III (≈10%, F<M, VI decade) when sporadic and without endocrinological syndrome. Type I and II ECL NETs are small (<1–1.5 cm), non-functioning and multicentric, either synchronous or metachronous, and responsible for low to intermediate incidence of lymph node (5% in type I; up to 30% in type II) or liver (2% and 10%, respectively) metastases. In contrast, type III is solitary, larger (most often, >2 cm) and sometimes functioning, with lymph node and/or liver metastases in 70% of cases [1, 41].

NECs present with non-specific symptoms similar to conventional gastric cancer, and is mostly found to be extensively metastatic [1]. There is also mixed adenoneuroendocrine carcinoma (MANEC) where the neuroendocrine component accounts for at least 30%, and which is usually G3 and, rarely, G1-G2 [1, 42], while other pure neuroendocrine neoplasms composed of enterochromaffin (EC) cells (with no preferential distribution) or G cells in the antrum are exceedingly uncommon [1].

9.4.2 Pancreatic NETs

Pancreatic NETs are usually well-differentiated tumors (G1 and G2 NETs) composed of cells closely resembling their normal counterparts and characterized by mild to moderate cell atypia and solid, gyriform, trabecular or glandular growth pattern [1, 40]. NECs are quite rare, sometimes associated with hormone secretion syndromes and prognostically unfavorable.

Although there is usually no relationship between the staining intensity for hormonal products and the presence or severity of the clinical symptoms, individual functioning tumors may be immunohistochemically negative for the high rate of release of the hormones into the bloodstream. Furthermore, the immunoreactivity for a given hormone does not have clinical implications, because nonfunctioning tumors may also exhibit immunostaining for a variety of hormones which are not released in the bloodstream [1, 40].

Progression of NETs to high-grade NECs is a rare but well-documented phenomenon in the pancreas and elsewhere in the GEP tract [36] or the thymus [37], thus realizing a hybrid category with intermediate tumor behavior according to an innovative concept of secondary high-grade neuroendocrine neoplasm [43].

9.4.3 Small Bowel NETs

Most NETs developing in the small intestine arise from the distal jejunum and ileum, and are mainly composed of EC cells and, rarely, L cells (G1 and G2 NETs) [1]. NECs are virtually undescribed. The typical presentation is in the form of white-yellowish nodules, multiple in about one-third of instances [44], with intact or eroded mucosa, which deeply infiltrate the muscular layers of the intestinal wall reaching the subserosal adipose tissue or the peritoneum, where emboli in lymph vessel and vascular channels are common. Tumor cells usually show a nesting pattern of growth with peripheral palisading, which may be of some value to recognize these tumors in metastatic sites. Extensive fibrosis of the intestinal wall and mesentery and fibrous changes of the intestinal artery walls frequently cause visceral adhesions, volvulus and infarct by vascular occlusion [1].

9.4.4 Appendix NETs

Appendix NETs affect young to middle-aged patients, are slightly prevalent in females, and are usually non-functioning. Most neoplasms arise at the tip of the appendix, but also the middle part or the base may be involved and fulfill criteria for G1 NETs and G2 NETs, whilst NECs are exceedingly rare in the appendix [1].

These tumors show a trabecular to solid histologic appearance and are composed of EC cells (producing serotonin and substance P) or L cells (producing glucagon-like peptides, glicentin and PP/PYY) [45]. Other NETs of the appendix feature gland-like tubular structures resembling metastatic adenocarcinoma, which are positive for neuroendocrine markers, glucagon and serotonin [45] or show a combination of classical NETs and goblet cell carcinoid as separate components [46]. EC cell NETs of the appendix are related to subepithelial neuroendocrine and Schwann cell aggregates present in the lamina propria and submucosa (so-called Masson's neuroendocrine complexes), whereas this association with nerves is lacking in the corresponding small bowel NETs.

Most NETs of the appendix measuring less than 2 cm in diameter are simply cured by local excision, while the risk of lymph node and distant metastases increases in angioinvasive neoplasms, which extend deeply to the mesoappendiceal/subserosal adipose tissue (Fig. 9.3a,b), regardless of the WHO 2010 tumor grading [47]. Carcinoid syndrome is a very rare presentation [45].

9.4.5 Large Bowel NETs

Large bowel NETs affect elderly patients in the VI–VII decade, with a slight prevalence of males in the distal portion of large bowel and females in colonic NETs, while NECs do not show gender predilection in elderly patients in keeping with conventional large bowel adenocarcinoma [48, 49]. High-grade NECs can arise in either the right colon or the rectosigmoid, with small cell carcinoma appearing in the anal canal and small cell, large cell (Fig. 9.3c,d) or mixed histology developing in the colon and rectum [49, 50].

NETs presenting with a number of mitoses and a Ki-67 LI higher than 20% and a well-differentiated morphology have also been identified in the large bowel, and named well-differentiated grade 3 neuroendocrine neoplasms, with an intermediate prognosis and non-platinum agent therapy [51]. Most NETs developing in the right colon present with large growing masses composed of EC cells frequently positive for CDX-2, whereas distal colon and rectal NETs feature solitary, submucosal polypoid nodules, uncommonly larger than 2 cm, composed of L cells lacking CDX-2 and reactive for prostate acid phosphatase [52], Islet 1 and PAX-8 [53]. They correspond to NETs G1 or NETs G2 on the basis of mitotic count and Ki-67 labeling index [1], while NECs can be associated with an overlying adenocarcinoma or adenoma until realizing mixed neuroendocrine/

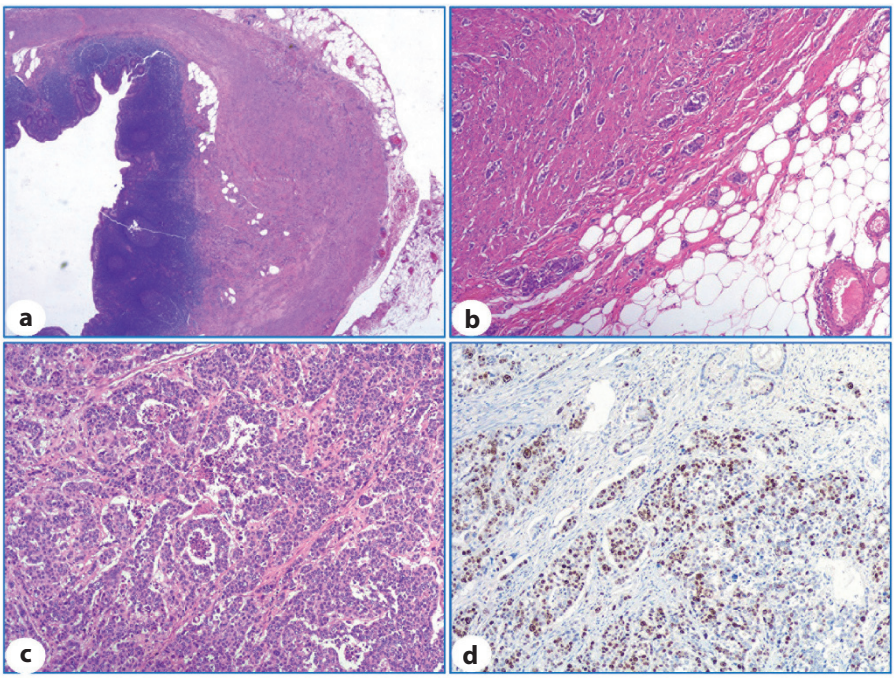

Fig. 9.3 Features of neuroendocrine neoplasms of the appendix and colon as seen in surgical specimens. A well-differentiated NET invades the entire wall of the vermiform appendix (**a**) reaching the mesoappendiceal fat (**b**). A poorly differentiated neuroendocrine carcinoma with necrosis and large cell appearance (**c**) shows a high Ki-67 labeling index (**d**)

non-neuroendocrine neoplasms (MiNENs) [54], formerly known as MANEC [1], when either component accounts for at least 30% of the neoplastic mass [42].

9.5 Conclusive Remarks

An accurate and thorough examination of cytology, small biopsy and resection specimens is the backbone for the best clinical handling of patients with GEP-NETs. In every patient the pathology diagnosis must include information about classification, grading and staging to allow personalized treatments according to updated knowledge of internationally shared and evidence-based guidelines.

The clinical and pathology approach to GEP-NETs must also take into account the anatomical site and the context where these tumors develop, inasmuch as both these factors can influence the tumor biology. The term carcinoid, in the absence of further information about an associated functioning endocrine syndrome, should not be generalized to include all NETs, as it fails to stratify patients for behavior and clinical handling.

Furthermore, an expert pathologist's opinion and multidisciplinary teaming are essential so as to successfully render challenging diagnoses and identify treatment options at the level of individual patients.

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Part III

Management

Management of Gastric Neuroendocrine Tumors

10

Massimo Carlini, Daniela Apa, Domenico Spoletini, Michele Grieco, Marialuisa Appetecchia, Francesca Rota, Salvatore Palazzo, and Salvatore Turano

Gastric neuroendocrine tumors (G-NETs) differ from the rest of the gastrointestinal tract neuroendocrine tumors and are classified into four types. These types are very dissimilar from one another in many respects and require different approaches, with surgery, endoscopy, chemotherapy and hormones being the pillars of treatment.

G-NETs are usually benign and limited to the mucosa or submucosa, without angioinvasion. Only tumors of large size (>1 cm) exhibit a low rate of lymph node invasion (3–8%) or distant metastases (2%) at diagnosis [1]. The main characteristics of G-NETs are reported in Chapter 6 (Table 6.1).

10.1 Type 1 G-NETs

10.1.1 Endoscopy

The majority of type 1 G-NETs are managed by endoscopy. It has been well recognized that conservative treatment (endoscopic excision and follow-up) is better than surgery [2, 3].

The National Comprehensive Cancer Network (NCCN) guidelines recommend only surveillance or endoscopic resection (ER) for smaller tumors without invasion of the muscularis propria and in the absence of metastasis, regardless of the number of lesions [4].

Lesions <1 cm should undergo annual endoscopic surveillance as they have a low risk of invasion or metastases

M. Carlini (✉)
Department of Surgery, Division of General Surgery S. Eugenio Hospital
Rome, Italy
e-mail: maxcarlini@tiscali.it

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Lesions >1 cm should be resected as there is a small risk of lymph node metastases. ER is indicated if endoscopic ultrasound (EUS) shows that the lesion is localized to the mucosa or submucosa [5]. Similarly, the ENETS guidelines propose ER for the treatment of type 1 G-NETs [6].

In larger lesions >2 cm, both single or multiple, endoscopic mucosal resection (EMR), endoscopic submucosal dissection (ESD) or surgical resection are indicated [7].

EMR or ESD must be carried out by centers with long-standing experience, due to the high risk of complications such as perforation and to maximize complete resection rates [8, 9]. However, these techniques are not widely available in Europe and the majority of the ESD evidence for NETs comes from the literature on rectal carcinoids [10].

Endoscopic resection of non-metastatic localized tumors, <2 cm in diameter and with less than 6 lesions if multiple, has been demonstrated to be as effective as surgical resection [5].

Other aspects of the endoscopic treatment of type 1 G-NETs are discussed in Chapter 6.

10.1.2 Surgery

EMR and ESD are effective on small lesions and are minimally invasive, but they do not have any effect on hypergastrinemia and therefore fail to remove the root cause of gastric carcinoids, which have a high rate of recurrence. Furthermore, in chronic atrophic gastritis all the gastric mucosa is subjected to the direct stimulation of hypergastrinemia and it is chronically impaired. ER does not have any effect on this alteration and cannot be applied to very small lesions, which are not visible endoscopically. Moreover, it is difficult to perform when the lesions are multiple and/or recurrent.

In the case of multiple and relapsing type 1 G-NETs, surgical antrectomy could be considered [11]. This procedure is invasive and cannot always be complete, but it has a protective pathogenetic effect because it reduces the amount of gastrin-secreting antral tissue, thus removing G cell-mediated hypergastrinemia, and leads to regression of the G-NET in over 90% of cases. Gastric antrectomy for type 1 G-NETs is therefore recognized as a therapeutic option in patients with multifocal (>6 lesions, 3–4 lesions >1 cm, or 1 lesion >2 cm), invasive or recurrent disease [2, 12, 13].

Although it may not be effective in preventing recurrence or metastasis, antrectomy seems to be associated, in recent studies [12, 13], with a lower risk of recurrence. Patients who have undergone resection require less endoscopic follow-up than those receiving ER or endoscopic surveillance alone and they avoid the discomfort of repeated endoscopies and the anxiety over a lingering condition.

Laparoscopic antrectomy represents a viable minimally invasive surgical option for treatment of type 1 G-NETs [12, 13] with lower recurrence risk and less postintervention monitoring than polypectomy.

For more advanced lesions – >1 cm with EUS-assessed involvement of the muscularis propria and/or local lymph nodes – a wider surgical resection is always needed. Surgical oncological resection is indicated if there are >6 lesions, 3 or 4 of these >1 cm, or if there is a single lesion >2 cm [12, 13].

However, in the event of persistent or recurrent G-NETs after local or endoscopic resection, antrectomy or partial/total gastrectomy with nodal dissection is mandatory, even though it must be kept in mind that surgical therapy is more invasive and associated with a higher risk of complications.

10.1.3 Medical Treatments

In type 1 G-NETs, somatostatin analogs (SSAs) are of limited use but they represent a potential treatment because of their antisecretory and antiproliferative effects. A morphometric study of gastric endocrine cells in patients with atrophic gastritis and Zollinger-Ellison syndrome (ZES) showed that octreotide has a hypotrophic effect on oxyntic argyrophilic cells, mainly corresponding to enterochromaffin-like (ECL) cells [14].

Twenty years ago, Ferraro et al. showed that the administration of octreotide at a dose of 500 μg once daily for six months in patients with chronic atrophic gastritis resulted in a reduction of chromogranin A (CgA) cells (mostly ECL) and serum gastrin levels [15]. In a small series of nine patients with >5 type 1 G-NETs, each <1 cm in size, without invasion of the muscularis propria and a Ki-67 <3%, disappearance of the lesions was observed after one year of SSAs therapy (octreotide LAR at a dose of 30 mg i.m. every 28 days for 12 months), in addition to a reduction of the levels of circulating gastrin and CgA [16]. In other cases, partial results or early relapse after discontinuation of SSAs were reported [17, 18].

A recent retrospective multicenter Italian study evaluated endoscopic surveillance, ER and therapy with SSAs as viable alternatives in the management of patients with type 1 G-NETs. The study included 97 patients with type 1 G-NETs stage 0–2A, treated from 1998 to 2013 with surgery (3.1%), radical ER (46.4%), and SSAs therapy (37.1%), or followed up with simple endoscopic controls (13.4%). Patients treated with SSAs had a complete response in 76% of cases and stable disease in 24% of cases. Long-term therapy, the use of full doses of SSAs and high levels of gastrin at diagnosis were related to a complete response to therapy [19].

Nevertheless, the most recent studies [6, 7, 19] do not recommend the routine use of the SSAs in type 1 G-NETs and large prospective randomized controlled studies are needed to demonstrate their effectiveness in these cases. Currently SSAs may be restricted to patients with multiple small lesions, to localized G1

G-NETs with low Ki-67 and cases of multiple lesions and frequent recurrences [7], because in these cases an endoscopic eradication is difficult to perform. Finally, according to the ENETS guidelines, SSAs can be an option in metastatic G-NETs with low Ki-67 and proven SSTR2 expression [6, 7].

10.2 Type 2 G-NETs

10.2.1 Surgery and Endoscopy

Type 2 G-NETs are due to chronic gastrin hypersecretion by a gastrinoma, in most cases in patients with ZES and MEN1 syndrome. Gastrin stimulates ECL cells and contributes to the development of the NET. Type 2 G-NETs have an intermediate behavior between type 1 and type 3 NETs, with lymph node metastases in 30% and distant metastases in 10% of patients at the time of diagnosis.

All lesions should be resected, as there is a greater risk of lymph node involvement and metastases. ER is indicated for all localized lesions and surgery for those with invasive or metastatic disease. Multiple lesions can be managed with both endoscopy and surgery.

According to the NCCN guidelines, the management of type 2 is similar to that of type 1 [4]. By contrast, the ENETS guidelines advocate gastric resection as the treatment of choice for type 2 G-NETs, in view of the high rate of metastases [6].

For type 2 G-NETs, treatment is usually dictated by the possible presence of duodenal or pancreatic NETs as part of MEN1, and local or limited excision can be recommended, but this should be patient-tailored at multidisciplinary NET centers of excellence. Although ECL cell hyperplasia in peritumoral gastric mucosa is comparable among patients with MEN1-associated gastrinoma and sporadic gastrinoma, ECL cell dysplasia and ECL cell NET are found only in patients with MEN1 [20]. Resection of the coexisting gastrinoma elsewhere should always be attempted and extended as much as possible.

There is no role for the antrectomy since the hypergastrinemia does not originate from the gastric antrum. Annual endoscopic surveillance is advocated owing to the high percentage of recurrences, particularly if ZES persists.

Gastrectomy (partial or total) with sentinel-node navigation surgery, developed by Japanese authors [21] and proposed in Italy by Carlini since 2002 [22] to perform a more effective tailored lymphadenectomy, is a useful tool for detecting subclinical lymph node metastases in patients with gastric NETs.

The modern development of minimally invasive techniques allows to perform gastric resections – both typical (distal resections, total gastrectomies) and atypical (wedge) – with minimal trauma for the patient, fully respecting the correct oncologic principles. These techniques are currently also used for the treatment of G-NETs in referral centers.

Recently, a hybrid endoscopic-assisted laparoscopic resection of gastric tumors has been reported to provide acceptable results [23]. Endoscopy provides intraoperative localization of the lesion and allows preservation of the pylorus and cardia during surgery.

Single-incision laparoscopic surgery (SILS) with transumbilical gastric stapling has also been reported as a viable alternative to conventional multiport laparoscopy in patients with gastric submucosal tumors, except in cases arising on the small curvature and close to the cardia/pylorus [24].

10.2.2 Medical Treatments

Also for type 2 G-NETs, the role of medical treatment is limited and experiences with SSAs are reported in small series. An Italian study found a reduction in serum gastrin levels and tumor regression in three patients with ZES and type 2 G-NET as part of MEN1 syndrome, treated for one year with octreotide or lanreotide [25]. The SSAs may act in the pathogenesis of Type 2 G-NETs by reducing levels and directly inhibiting the ECL cell proliferation [7, 25]. However, these therapeutic options do not act on gastrinoma, which is the source of hypergastrinemia and therefore requires simultaneous treatment.

In recent years another drug, netazepide, was investigated for its potential use in type 1 and 2 G-NETs. Netazepide is an orally active antagonist of gastric/cholecystokinin type 2 receptor [26, 27]. A Norwegian [28] and a British [29] pilot study evaluated the safety and efficacy of oral administration of netazepide for 12 weeks in eight patients with chronic atrophic gastritis, hypergastrinemia and type 1 gastric carcinoid in each study. The drug was well tolerated and no significant side effects were recorded. A reduction in serum CgA, lesion number and size of the larger lesions was observed, while serum gastrin levels were unchanged, in agreement with persistent achlorhydria. Following the results obtained, the UK Medicines and Healthcare Products Regulatory Agency and the Norwegian Regulatory Agency authorized therapy with netazepide for 52 more weeks. Netazepide was administered to 13 of the 16 patients who had participated in the previous studies. After netazepide was discontinued for an average period of 14 months, an increase in plasma CgA levels and an increase in the number and size of tumors were observed. Netazepide therapy for 52 weeks normalized circulating CgA in all patients and cleared all lesions in 5/13 patients, while in others it reduced their number and size. There were no changes in the serum gastrin levels. Only one patient reported a serious adverse event [27]. A phase II study with netazepide in patients with type 2 gastric carcinoid associated with ZES, which has, as its primary outcome, the regression of gastric carcinoid and/or hyperplasia of ECL cells, is ongoing at the U.S. National Institutes of Health [30]. Clearly, randomized controlled studies involving a larger number of patients are needed to demonstrate the safety and efficacy of netazepide.

Chemotherapy is not indicated in type 2 G-NETs, as in type 1, owing to the limited malignant potential and low Ki-67 of these tumors [7].

10.3 Type 3 G-NETs

10.3.1 Surgery

Type 3 G-NETs are sporadic, isolated lesions with a mean size of 5 cm, located at the body/fundus and surrounded by normal mucosa [31]. They are very aggressive, with metastases described in 50–100% of patients [32]. The primary treatments of type 3 G-NETs are gastrectomy and chemotherapy. The decision to surgically resect type 3 G-NETs should reflect the principles and guidelines of gastric adenocarcinomas, and partial or total gastrectomy with regional lymph node dissection are mandatory. In patients with metastatic disease at presentation, systemic treatments are often the first-line therapy.

The ENETS guidelines suggest that type 3 G-NETs must be treated as gastric adenocarcinoma and recommend surgical resection with chemotherapy [6]. The NCCN guidelines also propose radical resections with regional dissection of lymph nodes. These guidelines also report that local or ER could be adopted to treat lesions <2 cm [4]. Scherübl [33] and Kwon [34] also proposed an ER conservative treatment for small G1 type 3 G-NETs confined to the submucosal layer and not showing lymphovascular invasion.

Due to the absence of easily observable symptoms, non-functioning NETs are less likely to be detected early, presenting late as large primary tumors and advanced disease. However, non-functioning NETs may release bioactive amines at subclinical levels, causing non-specific symptoms such as increased tumor mass and other under-recognized syndromes [4, 35].

Adequate lymphadenectomy is a fundamental aspect of oncologically sound gastrectomies, as is the volume of gastric resection. Laparoscopic and robot-assisted laparoscopic gastrectomies [36] allow functional imaging to be easily integrated to the surgical field and may aid in intraoperative identification of lymphovascular bundles.

The more recent technique with indocyanine green near-infrared fluorescence imaging represents a useful method to visualize lymph nodes during minimally invasive gastrectomy. Surveillance with computed tomography (CT) and endoscopy following a resection of type 3 G-NET is similar to that of gastric adenocarcinomas.

The treatment of metastatic liver disease is multimodal and does not differ from that of other neuroendocrine tumors. Type 3 tumor-related 5-year mortality is 25–30% for well-differentiated and 75–87% for poorly-differentiated tumors.

10.3.2 Medical Treatments

In type 3 G-NETs there is no chance for SSAs, except in the presence of carcinoid syndrome, which has been found very rarely in type 1 and 2 G-NETs [32]. In some type 3 tumors, an atypical carcinoid syndrome has been described, due to the production of histamine and characterized by cutaneous flushing, profound itching, bronchospasm and lacrimation [32]. If carcinoid syndrome occurs, even in type 3 G-NET it is recommended to start therapy with SSAs. Slow-release SSAs in standard doses are used [37]. As in classic refractory carcinoid syndrome not controlled by the maximum dose of slow-release SSAs (octreotide LAR 30 mg every 4 weeks or lanreotide 120 mg every 4 weeks), shorter intervals, an increased dose, adding short-acting octreotide, or switching to another SSA may be considered [37]. Chemotherapy plays a significant role. However, given that the protocols used for type 3 NETs are not so different from those used for type 4, these will be discussed in Section 10.4.2.

10.4 Type 4 G-NETs

10.4.1 Surgery

Type 4 G-NETs include neuroendocrine carcinoma (NEC) and the very rare mixed adenoneuroendocrine carcinoma (MANEC). Gastric NECs (G-NECs) are rare forms of gastroenteropancreatic NECs, with about one thousand cases reported in the literature, and representing up to 1.5% of gastric/gastric resected cancers (Fig. 10.1). These neoplasms are often solitary, ranging in size between 4 and 8 cm, presenting with regional lymph node or distant metastases at diagnosis, as well as vascular and lymphatic invasion. NECs can occur in almost every site throughout the human body and likewise they can originate in any part of the

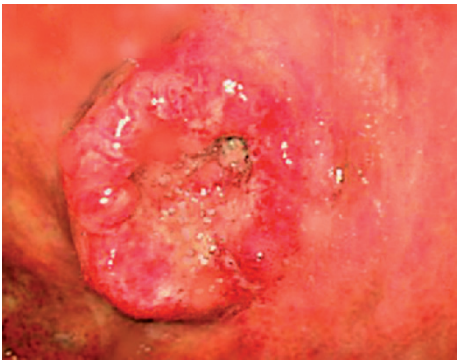

Fig. 10.1 Gastric neuroendocrine carcinoma (NEC)

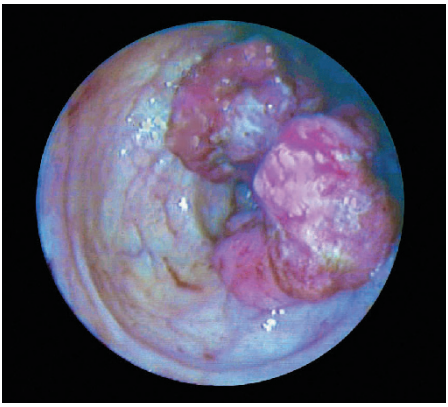

Fig. 10.2 Gastroscopy showing a NEC of a gastric stump. The stomach was resected thirty years earlier for peptic ulcer

stomach. Nevertheless G-NECs usually arise in the upper third of the stomach [38–41]. Fig. 10.2 shows gastroscopy revealing a NEC of a gastric stump, after resection for peptic ulcer.

Since type 4 G-NETs behave similarly to gastric adenocarcinomas, with a high incidence of invasion beyond the submucosa and distant metastasis on presentation (50–100%), radical surgical resection is recommended [31].

A recent retrospective study [42] examined a series of 43 patients with poorly differentiated G-NECs with Ki-67 >60%. Among them were 39 small cell carcinomas and 4 large cell NECs. The authors reported a 5-year survival rate of 35%, without differences between the two subtypes. Other previous studies reported similar results [41, 43–45], and it is noteworthy that in patients with tumors located in the cardiac region, those with less than seven metastatic nodes and no liver metastasis had better survival [46].

In patients with poorly differentiated localized gastric neuroendocrine carcinoma, partial or total gastrectomy plus regional lymph node dissection should be performed and adjuvant chemotherapy should also be provided after surgery.

Currently, if the G-NEC is resectable, surgery should be the initial treatment, to be applied with a basic principle of radical resection [5]. It is generally accepted that surgical resection of both the primary tumor and metastases is the most beneficial treatment, and it is the only possible curative approach [43, 47, 48]. Fig. 10.3 shows the surgical specimen of a total gastrectomy for NEC of a gastric stump (same case as Fig. 10.2).

Palliative surgery, before or after medical treatment, also plays an important role in the treatment of unresectable metastases by debulking or bypassing the tumor to make medical treatment more effective or to decrease the secretion of bioactive hormones [49]. Other therapies, such as embolization/chemoembolization, radiofrequency ablation and liver transplantation should also be considered in selected patients with disseminated liver metastases.

Because radical surgery alone is rarely curative in G-NECs, even in apparently localized disease, medical treatments are needed [43, 48].

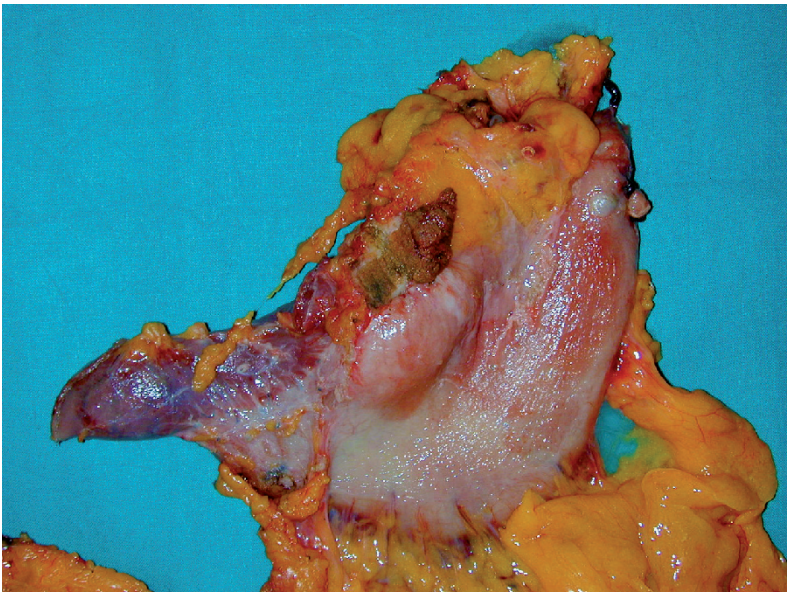

Fig. 10.3 Total gastrectomy for NEC of the gastric stump. Surgical specimen (same case as Fig. 10.2)

Brennan et al. [50], after evaluating 120 mixed-location small-cell extrapulmonary carcinomas, do not recommend surgery, even for limited disease. A later study by Li et al. [51], with a similar patient group, found that surgery and chemotherapy prolonged survival time significantly in small cell NECs. Another study by Brenner et al. [52], specific for small-cell gastrointestinal NECs, supports the conclusions of Li et al., finding it reasonable to treat patients with neoadjuvant and adjuvant chemotherapy and suggests a potential role for surgery in limited small-cell NEC. In conclusion, there is general consensus that surgery should be combined with adjuvant chemotherapy due to the risk of metastatic spread and recurrence. Clearly, surgery is also considered in the prevention or elimination of obstructive NECs.

A recent retrospective study [48] performed on 135 patients with surgically treated G-NETs reported a worse prognosis for males and high-grade G-NETs (NEC G3 and MANEC). The prognosis was better in females, with lesion ≤4 cm, NLR (neutrophil-lymphocyte ratio) ≤2.8, number of positive lymph nodes ≤4, and R0 resection. In other series, type 4 G-NETs have a mortality of 100% in 5 years and a mean survival of 6.5–14 months after the diagnosis [3].

10.4.2 Medical Treatments

Chemotherapy is the cornerstone in the treatment of NECs. As the biological characterization is similar to that of small cell lung cancer (SCLC), the European Neuroendocrine Tumor Society guidelines [53, 54] recommend that metastatic

gastric NECs, as well as all other gastroenteropancreatic NECs, should be treated in a similar way to SCLC, i.e., with etoposide or irinotecan in combination with platinum compounds such as cisplatin or carboplatin, which is the standard therapy. Although objective remission rates are high (40–67%), median progression-free survival is limited to 4–6 months. These results are difficult to interpret due to the heterogeneity of the treatments and the low number of patients enrolled in the study.

The WHO 2010 classification defines NEC as a poorly differentiated neuroendocrine neoplasm when Ki-67 is >20% and the grade is G3, but this neuroendocrine cancer can be further subclassified on the basis of the Ki-67 value less than or greater than 55%, since it shows different response rates to chemotherapy, as reported in the largest cohort of advanced gastrointestinal-NEC patients ever studied.

NEC patients with Ki-67 index >55% vs. 20–55% responded better to platinum-based chemotherapy (42% vs. 15%, respectively; p <0.05) but nevertheless had a median survival of 10 vs. 14 months. Thirty months after chemotherapy was started, 23% of patients with a Ki-67 <55% were alive, compared with only 7% of those with a Ki-67 ≥55% (p <0.001) [55]. Patients with a primary site in the esophagus, stomach and pancreas responded better than those with a primary site in the colon and rectum (complete response/partial response 44%, 50%, 30%, vs. 16%, 23%, respectively) and showed a better overall survival (median 14, 11, 15, and 8, 10 months, respectively). Cox regression on prognostic baseline factors for survival in patients treated with first-line chemotherapy demonstrated that gastric localization had a better odds ratio [56].

Other agents – such as taxanes, gemcitabine, pemetrexed, and topotecan in very small phase II single-agent studies – have not been encouraging, with global response rates less than 10% [57–61].

Increasing the number of chemotherapic agents (triplet versus doublet therapy, particularly with paclitaxel, etoposide plus carboplatin), although feasible, does not lead to clinical benefits.

Amrubicin has demonstrated to be active with a 22% of response rate in patients with platinum-refractory metastatic NEC and MANEC of the gastrointestinal tract [62].

There is no standard of treatment for second-line chemotherapy in NEC patients. With 29% and 31% response rates, the FOLFOX regimen (folinic acid, 5-fluorouracil and oxaliplatin) [63] and FOLFIRI regimen (folinic acid, 5-fluorouracil and irinotecan) [64] could be considered as a second-line option in poorly differentiated NEC patients after cisplatin-based first-line treatment, but they necessitate further confirmation in future larger prospective studies. Welin et al. reported similar results in a series of 25 patients with mainly gastrointestinal NEC, who had progressed on first-line platinum-based chemotherapy and were treated with temozolomide alone or in combination with capecitabine with or without bevacizumab, with a response rate of 33% [65].

Even though platinum-based treatment has historically shown interesting results in terms of response rate in undifferentiated forms, the real impact on

overall survival is minimal, so these results remain controversial and the question of what is the best treatment schedule is still debated. Therefore etoposide plus cisplatin remains a virtual standard of therapy, and its traditional use stems from old studies, with small statistical evidence due to the small number of patients enrolled in clinical trials.

Given the paucity of adequately powered definitive phase III studies incorporating modern response assessments and up-to-date histological subtyping aimed to reducing patient heterogeneity, patients themselves and clinicians should be encouraged to participate in well-designed, prospective clinical trials of chemotherapy or novel targeted therapies [66].

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Management of Pancreatic and Duodenal Neuroendocrine Tumors

11

Luca Landoni, Sara Cingarlini, Salvatore Paiella, Stefano Severi, Marco Miotto, Chiara Nessi, Elisabetta Grego, Maddalena Sansovini, Massimo Carlini, and Claudio Bassi

11.1 Surgical Treatments for Pancreatic Neuroendocrine Tumors

The surgical approach to pancreatic neuroendocrine tumors (P-NETs) is often challenging [1]. Surgery should be considered only after a proper radiological, functional, clinical and pathological investigation. Preoperative work-up for functioning and non-functioning P-NETs should be based on biochemical tests – hormone secretion and chromogranin A (CgA) assessments – and computed tomography (CT) or magnetic resonance imaging (MRI) to stage the disease [2]. Recently, for P-NETs some authors suggested performing positron emission tomography (PET)/CT with ^{68}Ga-labeled somatostatin analogs (SSAs) as the first-line diagnostic method, with the exception of insulinomas where the sensitivity is low (25%) [2, 3].

11.1.1 Surgery for Functioning P-NETs

11.1.1.1 Zollinger-Ellison Syndrome (ZES)

The outcome of surgery for pancreatic gastrinomas is two-fold: to control hormone-related symptoms and to treat the oncological aspects of the disease. Once the presence of sporadic pancreatic gastrinoma is confirmed, a typical pancreatic resection (Whipple procedure) with regional lymphadenectomy should be performed in a high-volume center with specific surgical expertise [2]. The extension of lymphadenectomy is still a matter of debate but recent updated guidelines suggest that the systematic removal of lymph nodes in the

C. Bassi (✉)
General and Pancreatic Surgery Unit, Pancreas Institute, University of Verona Hospital Trust
Verona, Italy
e-mail: claudio.bassi@univr.it

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peritumoral area should be part of any surgical treatment of gastrinomas [2, 4, 5]. In multiple endocrine neoplasia type 1 (MEN1)/Zollinger-Ellison syndrome (ZES) the surgical treatment is still controversial due to the tendency of the disease towards multifocality and due the high risk of recurrence.

Surgical enucleation is commonly recommended only for lesions >2 cm and/or tumors growing and metastasizing during follow-up. Indications for extended surgery should concern specific selected cases (e.g., multiple small duodenal gastrinomas with lymph node metastases) due to the higher complication rates [1, 2].

Gastrinomas ≤2 cm have an excellent long-term prognosis with survival rates around 100% at 5 years, hence a conservative approach is suggested [2, 6].

11.1.1.2 Insulinomas

Surgical treatment results in a high cure rate. In sporadic and MEN1 disease (25%), laparoscopic enucleation is the gold standard [2, 7, 8]. Preoperative work-up should include cross-sectional imaging (CT or MRI), endoscopic ultrasound (EUS) and intraoperative ultrasound (IOUS) to measure the proximity of the tumor to the main pancreatic duct (MPD) to reduce the risk of intraoperative damage to the duct and to establish the best indication for parenchyma-sparing resection [9]. A distance of at least 3 mm between tumor and MPD is considered enough to perform enucleation safely [10]. To avoid major duct damage and minimize the risk of a postoperative high-grade pancreatic fistula, a preoperative endoscopic retrograde cholangiopancreatography (ERCP) with the insertion of a 5-French stent in the MPD has been suggested [11]. When enucleation is not feasible or deemed at high-risk of MPD injury, a typical or a parenchyma-sparing pancreatic resection should be performed [2].

11.1.1.3 Unknown Primary

When primary tumor cannot be assessed (10–20% of cases of gastrinomas and insulinomas) but the presence of a hormonal syndrome has been assessed, the main aim is to identify the lesion. In these cases, an exploratory laparotomy should include a Kocher maneuver, superior and inferior margin dissection and bi-digital manual examination aided by IOUS. If this approach fails to find the lesion, "blind" resections are discouraged and the patient should undergo a strict follow-up and symptoms should be controlled by medical therapy [1].

11.1.2 Surgery for Non-Functioning P-NETs

Surgery for non-functioning P-NETs has been limited to lesions >2 cm in diameter and/or biological-morphological features of localized, aggressive disease (G2 according to WHO 2010 [12], MPD and/or biliary duct dilatation, jaundice, radiological signs of vascular infiltration). The surgical treatment consists of typical and atypical resections, depending on the tumor site [2, 13].

Typical resections Head P-NETs are treated with pancreatoduodenectomy (PD) while lesions of the body/tail with laparoscopic left pancreatectomy (LP) with or without splenectomy. In large series, PD and LP show mortality rates of 3% and 0%, postoperative pancreatic fistula rates of 30% and 19%, perioperative bleeding rates of 20% and 6%, and secondary diabetes rates of 18% and 26%, respectively [14–16]. Lymphadenectomy should always be performed, but the extent of node resection remains controversial [5, 17]. Total pancreatectomy with or without splenectomy should be performed if the whole gland is involved by the neoplasm, for multifocal lesions or if the leftover parenchyma is not enough to guarantee adequate function [13].

Atypical resections These resections have been proposed for small, low- and intermediate-grade P-NETs. No consensus exists on the dimensional cut-off. Although the risk of malignancy cannot be completely ruled out, a 2-cm cut-off should be safe enough. Middle pancreatectomy is usually performed for tumors of the pancreatic body, whereas an enucleation should be considered only when the MPD can be safely preserved. Parenchyma-sparing resections are associated with a lower incidence of endocrine/exocrine pancreatic insufficiency when compared to standard resections. On the other hand, they are associated with a high incidence of pancreatic fistula (50%) [10, 13]. Lymph node sampling is not indicated [5].

For MEN1-associated non-functioning P-NETs <2 cm, surgery is not generally suggested [2]; lesions >2 cm are treated as previously described. Due to the tendency of MEN1-associated P-NETs to be multifocal, an accurate IOUS examination is recommended to guarantee the best indication for a possible total pancreatectomy [13]. In young MEN1 patients, parenchyma-preserving surgery should be considered to reduce the incidence of diabetes.

Von Hippel-Lindau patients with P-NETs, (a) tumor size ≥3 cm, (b) mutation in exon 3, and (c) tumor doubling time ≤500 days, should be referred for surgery [18].

11.1.3 Conservative Treatment for Non-Functioning P-NETs

An observational approach can be safely adopted for small (<2 cm) and indolent (G1) [12] lesions without signs of aggressiveness, especially when a major pancreatic resection would be required [2]. In these patients the risk of nodal involvement is around 8–14% [19]. Morphological characterization should include non-functional and functional imaging [20], while the histological one, when possible, should be based on EUS-guided fine needle aspiration biopsy [21, 22].

The decision to undertake a follow-up versus surgery approach should be collectively shared in a multidisciplinary group and should consider morphological and biological data including patient age, comorbidities, performance status, tumor site, biological impact of a possible surgery, and patient wishes. In the case of a conservative approach, follow-up should be performed with abdominal MRI or EUS every 6–12 months [13].

11.1.4 Surgery in Advanced-Disease Scenarios

11.1.4.1 Locally Advanced P-NETs

Debulking surgery for unresectable, locally advanced P-NETs could be performed in selected cases such as in patients with uncontrolled functioning tumors like carcinoid syndrome, refractory insulinoma, glucagonoma, VIPoma or parathyroid-related peptide-secreting tumors. For locally advanced non-functioning P-NETs, with disease stability in the last 6 months, surgery could alleviate mass-related symptoms by reducing tumor burden [2].

Radical surgery should be achieved by extended lymphadenectomy and, where necessary, multivisceral resection. Criteria for non-resectability are: (a) circumferential invasion of portal vein system with portal cavernoma (tumor thrombus excluded), and (b) circumferential invasion of superior mesenteric artery. The infiltration of the celiac trunk is not a total contraindication to LP [13].

The presence of portal vein thrombi in patients with P-NETs is not rare and does not represent a contraindication to surgery. A recent study described the removal of the thrombus as a safe and feasible procedure for highly selected patients and provided specific precautions are taken: (a) the tumor thrombus must be mobile as an appendage from the primary tumor (without vessel encasement); (b) if needed, multivisceral and/or additional vascular resections should be performed before thrombectomy and (c) a multimodal therapeutic strategy should be considered before performing surgery (e.g., cytoreductive treatment) [23].

11.1.4.2 P-NETs with Liver Metastases

In metastatic liver disease, resection of the primary pancreatic lesion is still under debate but it seems to improve prognosis. In this case, debulking resection is recommended only for G1-G2 P-NETs [24].

11.1.5 Minimally Invasive Approach

Pancreatic NETs are the ideal entity for laparoscopy because they are often small and with an indolent biological behavior. A laparoscopic approach in both benign and malignant lesions is safe and feasible and associated with a lower complication rate and a shorter hospital stay [8].

11.2 Surgery for Duodenal Neuroendocrine Tumors

Since the biological behavior of duodenal NETs (D-NETs) is largely undefined, the management of D-NETs is not clear. A large population-based study including 1,258 patients suffering from D-NETs showed an overall excellent prognosis,

with a 5-year survival rate of 93.8% and 75.3% when they were treated with resection or no resection, respectively ($p < 0.001$). However, the extent of surgical resection remains controversial.

The surgical treatment of D-NETs ranges from Whipple to local resection. In selected cases the endoscopic removal of D-NETs has become the preferred treatment option, able to guarantee low recurrence rates and an excellent overall survival. As a rule, the treatment of D-NETs should be based on their potential of malignant transformation and on the risk of recurrence, either local or nodal.

Preoperative and staging work-up should include an accurate upper gastrointestinal (UGI) endoscopy to perform biopsies to assess nature and grading [25]. Thereafter, EUS is necessary to assess the depth of invasion and the potential presence of nodal metastases. Finally, cross-sectional imaging should be adopted for disease staging, rather than for detecting regional nodal metastases [26].

NETs of the ampulla of Vater must be distinguished from other D-NETs. They are extremely rare and their prognosis is worse, tending to metastasize more frequently, even when they are small, hence their management is different.

11.2.1 Endoscopic Treatment

Well-differentiated (G1/G2), non-functioning D-NETs ≤1 cm and confined to the submucosa (low-risk D-NETs) can be safely managed with endoscopy [25, 27, 28]. The two most common endoscopic techniques are endoscopic mucosal resection (EMR) and endoscopic submucosal resection (ESD).

It is unclear whether surgery is more appropriate than endoscopy in the case of well-differentiated D-NETs with a diameter ranging from 10 to 20 mm [25, 28]. Since the risk of nodal metastases is modest for tumors limited to the submucosa, some authors proposed to offer local excision (surgical or endoscopic) to this subgroup of patients [29].

For D-NETs ranging from >1 to ≤2 cm, the treatment of choice should be based on the expertise of the endoscopists. To accurately assess resection margins, an *en bloc* resection rather than a piecemeal one should be preferred. EMR is indicated for D-NETs ≤1 cm, with a polypoid structure and with invasion of the mucosal layer. ESD, instead, may be useful in the case of larger D-NETs or those extending to the muscularis propria layer.

Some papers show considerable rates of adverse events (perforation and bleeding rates up to 39% and 18.4%), especially when the lowest margin of the tumor is just before the muscularis propria [30–32], demonstrating that the risks associated with ESD can be even remarkable and close to the classically reported complication rates of duodenopancreatic surgery. When vertical or lateral resection margins are found positive at histology and no further endoscopic approach is feasible, surgery becomes mandatory.

Regarding the follow-up strategy after endoscopic resection, the ENETS guidelines recommend UGI endoscopy, cross-sectional imaging and CgA plasma level assays at 6 and 12 months and then biannually [33].

In consideration of their metastatic potential, functioning D-NETs should not be treated with endoscopy unless surgery is deemed at high risk of morbidity and mortality.

11.2.2 Surgical Treatment

Tumor resection of D-NETs guarantees excellent long-term survival results. In fact, D-NETs are a disease with an indolent nature, hence the benefits of any aggressive surgical treatment should be weighed against its risks.

Current data do not allow us to draw clear conclusions on the surgical treatment of D-NETs. It must be remembered that the overall survival of D-NETs is good, with a 5-year survival ranging from 73.9% to 89.3% [34–36], hence any attempt to reach oncological radicality should be performed.

Several authors reported that the larger the tumor the higher the risk of nodal spread [29, 35, 37], but others did not, at least for the proposed dimensional cut-offs of 1, 1.5 or 2 cm [36, 38]. It is likely that the cut-offs provided did not reflect tumor biology, thus they should be considered as one of the parameters to look at during the therapeutic decision-making, but not the only one.

Regarding the type of surgery, Whipple, transduodenal submucosal excision with or without lymphadenectomy, segmental duodenectomy and antrectomy with D1 duodenectomy have all been adopted as feasible surgical approaches to D-NETs [35–37]. However, none of them has been proved to be more effective than the others.

The ENETS guidelines do not recommend a specific treatment for tumors ranging from 1 to 2 cm [25]. Such cases with a limited vertical invasion (until muscularis propria), without any cross-sectional or EUS suspicion of nodal metastases and with a favorable biology, could be treated with transduodenal local excision without regional lymphadenectomy associated with a strict follow-up or with regional lymphadenectomy, indifferently. A Whipple procedure is indicated for D-NETs >2 cm, for smaller tumors with unfavorable biology, for periampullary and for rare functioning D-NETs (somatostatinomas) [25].

As for functioning D-NETs, the duodenum is the most frequent location of gastrinomas (60% to 75%), whether or not they are MEN1-associated [39]. Nodal metastases from duodenal gastrinomas have been reported in 40–70% of cases [28], but they do not affect the prognosis dramatically or, at least, not as much as pancreatic gastrinomas [40]. Surgery of duodenal gastrinomas can be challenging and depends on the size of the tumor and should always be associated with a regional lymphadenectomy [28, 41]. Intraoperative ultrasound and a wide duodenotomy are required to increase the rate of detection of duodenal gastrinomas [41]. Usually, these D-NETs are excised with a rim of normal

duodenum. In cases of large and/or multiple duodenal gastrinomas, not removable by enucleation, a Whipple procedure is the treatment of choice. When a MEN1-associated Zollinger-Ellison syndrome is diagnosed, surgery is recommended for tumors >2 cm. For smaller tumors, surgery should be considered in selected cases only. In fact, smaller tumors are invariably multiple, very often they have already metastasized at the time of diagnosis, their prognosis is good and positive results have been described with the use of proton pump inhibitors to control the acid hypersecretion [28]. A postresection clinical and laboratory (with fasting gastric/secretin tests) follow-up is generally enough [41].

After surgical treatment of non-functioning D-NETs, the follow-up should include cross-sectional imaging, CgA plasma level assay and somatostatin receptor scintigraphy, and it should be scheduled at 6 months and then annually for at least 3 years [33]. At least a 5-year follow-up would be reasonable.

11.3 Ampullary Neuroendocrine Tumors

Ampullary NETs are extremely rare [42]. They are biologically different from D-NETs, as they tend to metastatize more frequently and their prognosis is worse [34]. Within the family of ampullary NETs, carcinoids and ampullary neuroendocrine carcinoma (NEC) must be considered differently. Some data report notable differences in prognosis for ampullary carcinoids when compared with high-grade ampullary NEC, with a 5-year survival of 82% and 15.7%, respectively. In addition, up to 62% of ampullary NEC have already spread to regional lymph nodes at the time of diagnosis [42]. For these reasons a radical Whipple resection seems more than justified, rather than a transduodenal ampullectomy, which would be oncologically inadequate [43].

Any endoscopic approach to ampullary NETs should be decided multidisciplinarily, individually and providing adequate patient information. Finally, in the case of nodal recurrence during follow-up, surgery with radical resection and lymphadenectomy becomes a valid therapeutic option.

11.4 Medical Treatments

Treatment for advanced G1-G2 NETs consists of various systemic therapies. The choice between different chemotherapeutic regimens, peptide receptor radionuclide therapy (PRRT) or targeted agents is based on the clinical characteristics of the patient, the biology of the disease and the therapeutic goal. When the aim is cytoreduction, both for a symptomatic disease or neoadjuvant intent, the treatments with higher objective response rate are preferred. They include chemotherapy or, in selected cases, PRRT. Among all gastroenteropancreatic NETs

(GEP-NETs), P-NETs have the best chemoresponsivity. Otherwise, when the aim is disease stabilization, somatostatin analogs (SSA) and targeted therapies are reasonable options. For G3 disease chemotherapy is the preferred first-line therapeutic option.

11.4.1 The Role of Chemotherapy

Pancreatic NETs show a moderate/good responsiveness to some antiblastic agents. Single agent chemotherapy in P-NETs have a limited activity. Dacarbazine (DTIC) leads to a response rate (RR) of 26% with a median progression free survival (PFS) of 10 months [44], streptozotocin (STZ) ranges from 21 to 36% with a PFS of 16.5–33 months [45], while chlorzotocin leads to partial response in 30% with a PFS of 17 months [46]. Temozolomide was tested in an heterogenous population of 36 thoraco-abdominal neuroendocrine tumors including only 12 P-NETs. Global RR was 8% and PFS did not exceed 7 months [47].

In one of the first randomized controlled trials comparing mono- and poly-chemotherapy, streptozotocin (STZ) was tested against the combination of STZ and 5-fluorouracil (STZ/5-FU). Both RR (36% vs. 63%) and overall survival (OS) (16.5 months vs. 26 months) significantly favored the combination arm. The same authors compared in a three-arm randomized controlled trial, chlorzotocin alone, and two STZ-based regimens: STZ/5FU and doxorubicin/STZ (ADM/STZ). The latter was significantly superior to STZ/5FU in terms of RR, PFS and OS (45% vs. 69%, 13 vs. 22 months and 17 vs. 26 months respectively) [46]. However, the same combination did not show similar results in two other polychemotherapy single-arm retrospective studies [48, 49].

Oxaliplatin has moderate activity in well-/intermediate-differentiated NETs. It was tested together with capecitabine in a series of 15 patients with well-differentiated P-NETs. A partial response was induced in 27% of patients with a median PFS (mPSF) of 20 months and an OS of 40 months [50]. Once used in combination with gemcitabine, oxaliplatin did not seem to have similar efficacy, probably because of a limited activity of gemcitabine in this histotype [51]. Dacarbazine and cisplatin were the most frequently tested drugs in combination with STZ/5FU or 5FU/ADM backbones. RR for the triplets ranges from 19.5% to 58%; PFS ranged from 9.1 to 21 months, OS from 21 to 38 months [52–56]. The combination of temozolomide and capecitabine (CAPTEM regimen) showed promising results. The efficacy of this regimen was tested in 30 P-NETs as first-line treatment, with a 70% partial remission rate, a mPSF of 18 months and an OS of 92% at 2 years [57]. A small retrospective study explored activity of CAPETM also as a second-line treatment. Among seven treated patients a RR was recorded in 43% and a clinical benefit in 71% of patients [58].

11.4.2 Targeted Therapies

The PI3K-Akt-mTOR pathway is a key regulator pathway in the biology of NETs [59]. Its constitutive activation is described in many malignancies including P-NETs.

Data from clinical trials support the central role of the PI3K-Akt-mTOR pathway in P-NET tumorigenesis. In the first study of the "RADIANT saga" (RADIANT-1) everolimus was given alone or in combination with octreotide LAR if such a treatment was ongoing at baseline. The primary endpoint was RR in the largest stratum of everolimus monotherapy (n = 115 patients). An RR of 9.6% was observed in the everolimus "stratum" vs. 4.4% in the everolimus plus octreotide one [60].

The RADIANT-3 study further explored the role of everolimus in the management of advanced P-NETs. Everolimus significantly increased PFS (adjudicated central review PFS was 11.4 and 5.4 months for the everolimus and placebo arm, respectively) among patients with progressive advanced P-NETs. This resulted in a reduction of the risk of progression for the experimental arm of nearly 65% [61].

Pancreatic NETs are highly vascularized tumors. In P-NETs, a high vascular endothelial growth factor (VEGF) expression has been reported to be a negative prognostic factor. Many different antiangiogenic drugs are available for clinical use. The results of many trials including bevacizumab in combination with both chemotherapy or other targeted agents in patients with advanced GEP-NETs, although heterogeneous, have led to cautious optimism [62].

Sunitinib was also tested as antiangiogenic multitarget agent in NETs. The first experience, in a single-arm phase II trial, showed promising results in 109 patients with advanced NETs (including 66 P-NETs). A partial response was recorded in 16.7% of patients with P-NETs, with a mPSF of 7.7 months [63]. A phase III study then was conducted enrolling advanced P-NET patients only. A total of 171 patients with advanced P-NETs were randomly assigned to receive sunitinib or placebo together with best supportive care. Noteworthy baseline characteristics of the enrolled patients were the following: 22% with tumors having a Ki-67 >10% and 66% chemo-pretreated patients in the experimental arm. Patients could receive SSA in both arms according to investigators' discretion. After assessment of the data on 154 patients the safety monitoring committee recommended discontinuation of the trial because of the great number of deaths and serious adverse events in the placebo group. At that time point, RRs of 9.3% and 0% were recorded in the experimental and placebo arm, respectively. A statistically significant difference in PFS between the two arms was also observed (11.4 vs. 5.5 months). All subgroups of patients benefited from sunitinib but the hazard ratio for progression in the experimental arm compared to placebo seemed to favor patients with a Ki-67 ≤5% [64].

11.4.3 The Role of Peptide Receptor Radionuclide Therapy (PRRT)

Peptide receptor radionuclide therapy (PRRT) is a relatively new treatment option for unresectable or metastatic P-NETs, based on the systemic administration of a radiolabeled SSA with a beta-emitting radionuclide.
The radiolabeled SSA can irradiate neoplastic cells via internalization through the somatostatin receptors (SSTRs) overexpressed on the cell membrane.

Since the 1990s the most investigated radiopharmaceuticals have been ^{111}In-pentreotide, ^{90}Y-DOTA-tyr^{3}-octreotide and ^{177}Lu-DOTATATE, which is the newest and most clinically used radiopharmaceutical. ^{177}Lu is a medium-energy β-emitter with a maximum energy of 0.5 MeV and a maximal tissue penetration of 2 mm. Its half-life is 6.7 days. ^{177}Lu also emits low-energy γ-rays at 208 and 113 keV with 10% and 6% abundance, respectively, which allows scintigraphy and subsequent dosimetry with the same therapeutic compound. The shorter β-range of ^{177}Lu provides better irradiation of small tumors, in contrast to the longer β-range of ^{90}Y which allows more uniform irradiation in large tumors that may show heterogeneous uptake.

Usually PRRT with ^{177}Lu-DOTATATE is administered intravenously in multiple cycles, and the maximum cumulative administrable activity depends on the irradiation of the kidneys, which are the dose-limiting organs. The absorbed dose threshold is conventionally set at 25–27 Gy, or, optimally, at ~40 Gy (for a biological effective dose). The co-infusion of amino acid solution protects the kidneys. Generally, mild acute side effects (amino acid-related) are nausea and vomiting, while those related to PRRT are fatigue, mild hair loss, rare exacerbation of carcinoid syndromes [65].

^{177}Lu-DOTATATE PRRT is generally used after failure of medical therapies in advanced progressive G1-G2 P-NETs.

The efficacy in terms of disease control rate (DCR), PFS, OS and quality of life improvement by the control of hormone-induced symptoms is widely known.

Recently, Sansovini et al. published the results of a phase II prospective pathology-oriented trial in metastatic G1-G2 P-NETs treated with ^{177}Lu-DOTATATE [66]. In this trial, 60 consecutive patients were scheduled to receive 5 cycles of therapy at intervals of 6 to 8 weeks, with planned activity per cycle of 3.7 or 5.5 GBq of ^{177}Lu-DOTATATE based on the presence or absence of risk factors for kidneys and bone marrow. Overall response was complete response in 4 cases (6.6%), partial response in 14 cases (23.3%) and stable disease in 31 cases (51.7%), with a DCR of 81.7%. The mPFS was 28.7 months (95% CI 20.2–53.8) and the median OS had not been reached. In this study, the prognostic role of ^{18}FDG-PET in G1-G2 advanced P-NETs, already documented also by Severi et al. [67], was confirmed. Among 55 patients who performed an ^{18}FDG-PET scan, 32 (58%) presented an increased glucose metabolism and 23 (42%) were classified as negative, regardless of the Ki-67 grading score. ^{18}FDG-PET-negative patients showed a significantly better outcome after PRRT with ^{177}Lu-DOTATATE as opposed to those with positive scans. In particular, mPFS in the

^{18}FDG-PET positive patients was 21.2 months (95% CI 18.1–28.7), while mPFS in the ^{18}FDG-PET-negative group was 68.7 months (95% CI 53.4-nr, p <0.0002), regardless of total administered activity. The median OS of ^{18}FDG-PET-positive patients was 63.8 (95% CI 28.2-nr), whereas it was not reached in the negative group (p = 0.006).

Currently, several studies are evaluating the potential role of ^{177}Lu-DOTATATE as neoadjuvant treatment in P-NETs, when surgery is not possible. In 2015, a Dutch study reported encouraging results in terms of surgical resection rate and mPFS when surgery was performed after PRRT with ^{177}Lu-DOTATATE. The mPFS was 69 months for patients with successful surgery and 49 months for patients without surgery [68]. In addition, another Italian study on 20 P-NETs documented that patients who underwent neoadjuvant PRRT for a metastatic and/or locally advanced P-NET had a lower risk of pancreatic fistula (25% vs. 65%, p = 0.011) and the 2-year PFS rate was 67% [69].

Another potential therapeutic application of ^{177}Lu-DOTATATE is the treatment of high grade P-NETs which overexpress SSTRs [70]. Nicolini et al. evaluated 26 patients affected by GEP-NETs with a Ki-67 >15% who received a personalized treatment with ^{177}Lu-DOTATATE PRRT. Sixteen patients had Ki-67 between 15% and 35% and 10 patients had Ki-67 >35%. The first group of patients showed a DCR of 80% with a mPFS of 20.9 months (95% CI 10.8–28 months), whereas the second group had a DCR of 30% and a mPFS of 6.8 months (95% CI 2.1–27 months) (p = 0.050) [71]. Although this subgroup of patients benefit from PRRT less than those with well-differentiated P-NETs, the use of ^{177}Lu-DOTATATE in high-grade Ga-PET positive P-NETs should be proposed in cases of tumor progression after the first line of medical therapy.

Finally, considering their biological heterogeneity [72], the use of chemotherapy and PRRT might have a synergistic combination, as already reported by several trials [73–75].

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Management of Ileal, Appendiceal and Colorectal Neuroendocrine Tumors

12

Massimo Carlini, Domenico Spoletini, Michele Grieco, Daniela Apa, Marialuisa Appetecchia, Rosa Lauretta, Salvatore Palazzo, Stefano Minardi, Stefano Severi, and Annarita Ianniello

12.1 Ileal Neuroendocrine Tumors

12.1.1 Surgery

Ileal NETs (I-NETs) show an increasing incidence and prevalence, reflecting the low aggressiveness, the slow growth and the prolonged disease course, together with the good results obtained over the years with current treatments [1–3].

Surgery is the only definitive curative treatment of I-NETs, but it is also used for palliative control of symptoms via cytoreductive procedures. For a proper therapeutic strategy, patients are to be evaluated in a multidisciplinary setting including an experienced visceral surgeon [4]. In fact, all patients with I-NETs are potential candidates for curative surgery of the primary tumor and regional lymph node metastases [5]. Surgery associated with medical treatments is the paramount therapy for stage I-II-III ileal NETs, which have an excellent prognosis.

Resection of the primary tumor, associated with extended lymphadenectomy, achieves a 5-year survival rate of 100% in stage I and II and more than 95% in stage III [5]. Surgery is also needed for small and asymptomatic tumors, because tumor size cannot be directly related with biological behavior, and liver and node metastases can be present even with small lesions [6].

Precise localization of the tumor is mandatory to plan a minimally invasive resection. This can be achieved by radiological examinations and by double-balloon enteroscopy, to avoid conversions to open surgery for missing lesions.

M. Carlini (✉)
Department of Surgery, Division of General Surgery S. Eugenio Hospital
Rome, Italy
e-mail: maxcarlini@tiscali.it

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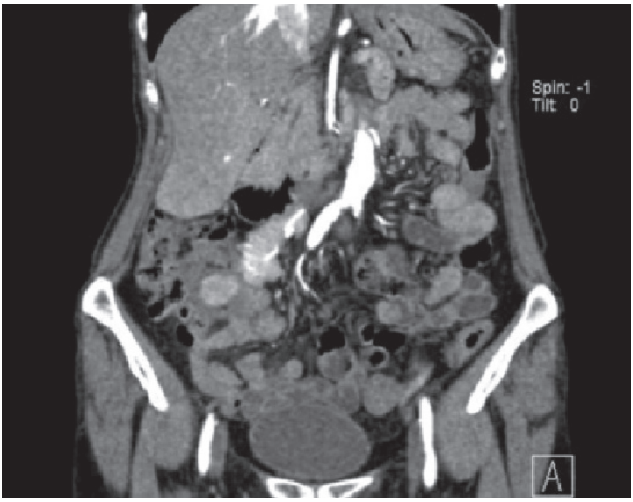

Fig. 12.1 CT scan of patient with ileal NET and bulky lymph node metastases

Considering the main features of ileal NETs, surgery has some important key points. The I-NETs may seem unresectable, with entrapped loops and mesenteric encasement leading the surgeon to believe he is facing an inoperable adenocarcinoma, but these tumors can be patiently and carefully removed by dissection from the superior mesenteric vessels, with preservation of important collaterals, also under laparoscopy. Removal of the tumor and involved bowel loops is recommended before initiation of medical therapy.

Lymph node dissection is the most important and demanding part of the ileal resection for NETs. Lymph node recruitment is directly related to the oncological outcome of the procedure, because these tumors very often produce a severe lymphadenopathy (Fig. 12.1) and a mesenteric fibrosis located proximally to the origin of the superior mesenteric vessels. Nodal metastases are often so bulky that they may be mistaken for the primary tumor. Radical lymphadenectomy can be difficult but is feasible with the laparoscopic approach. In Fig. 12.2 the same ileal NET as shown in Fig. 12.1, with bulky metastases along the ileocolic vessels, was radically resected laparoscopically, with a right hemicolectomy and lymphadenectomy of the superior mesenteric vein. To obtain a wide lymphadenectomy of this region, a high ligature of the vessels and a long bowel resection are often necessary, and for this reason special attention must be paid to avoid resections leading to short bowel syndrome. Intestinal by-passes should be avoided as far as possible, since ischemia may develop and the procedure tends to complicate repeat surgery, which often becomes necessary.

Twenty percent of ileal NETs are multicentric and even though this data does not change the surgical strategy, the resection should not be too close to the tumor. This is not a problem when a high lymphadenectomy is performed, because in this case an extended resection is mandatory.

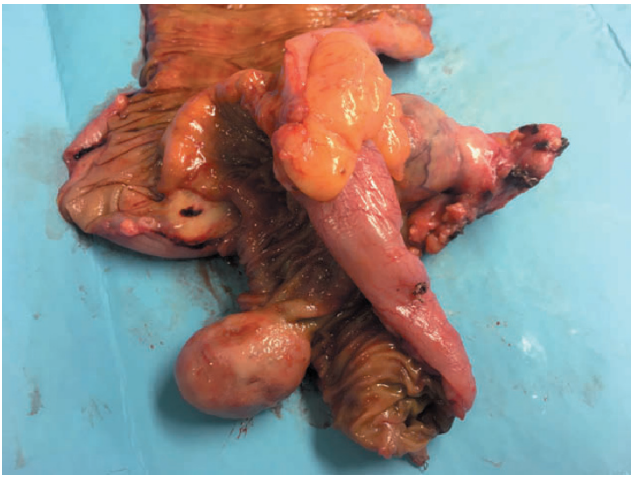

Fig. 12.2 Surgical specimen of a laparoscopic right colectomy (same case as Fig. 12.1)

Some lesions can have important peritumoral fibrosis involving the mesentery root and the retroperitoneum, leading to occlusion, hydronephrosis, and chronic pain. In these patients, surgery must be performed in highly experienced centers.

In referral centers, minimally invasive resection is the procedure of choice. The laparoscopic technique provides the same oncologic outcome as open surgery, with all the advantages of a minimally invasive approach. During the surgical procedure, in candidates for therapy with somatostatin analogs, a simultaneous cholecystectomy is recommended to prevent cholelithiasis.

Curative surgery allows patients with stage I and II 5- and 10-year survival rates of 100% and more than 95% and 80% for patients with stage III disease.

In stage IV patients, medical therapy is the mainstay, but surgery still has an important role. Since abdominal complications remain one of the major causes of death, debulking surgery should always be considered. How surgery, in metastatic patients, can influence oncological outcomes is still debated [7, 8].

Even if a complete surgical resection of the I-NET is performed, recurrence can occur in about 35% of cases [9]. For liver recurrence, surgical resection is the best option, but this is feasible in fewer than 20% of cases. For unresectable liver metastases, local treatment (chemoembolization, thermoablation) can be used, together with systemic therapy like somatostatin analogs or chemotherapy.

12.1.2 Medical Treatments

Somatostatin analogs (SSAs) in I-NETs have their main indications in (a) symptomatic and (b) non-functioning advanced forms [10].

a) In the case of symptomatic disease, like the carcinoid syndrome [10, 11], it is recommended to start immediately the treatment with SSAs [12–14]. The

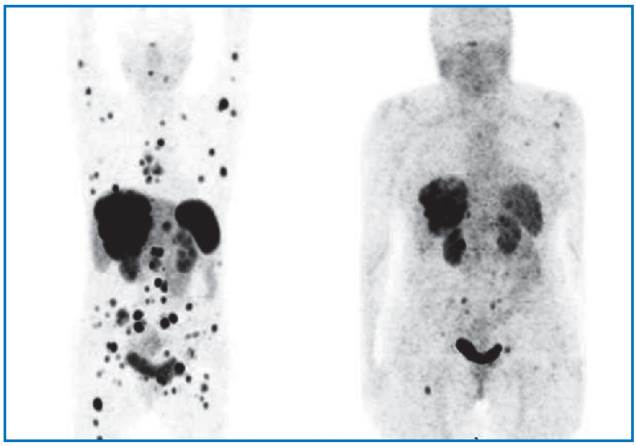

Fig. 12.3 I-NET G2 in a 65-year-old woman. ^{68}Ga PET images pre and post ^{177}Lu-DOTATATE peptide receptor radionuclide therapy (PRRT)

first formulation providing symptom control is short-acting octreotide subcutaneously (s.c.). Since the 1990s, long-acting formulations have replaced the quick-release formulations in the long-term control of carcinoid syndrome. About 40% of patients with carcinoid syndrome under treatment with the maximum dose of long-acting SSAs are not fully controlled. In these cases, the clinician may consider an increase in dose, a reduction of the dosage intervals or the addition of s.c. octreotide (rescue) [15].

In a phase II trial, pasireotide, a multireceptor-targeting SSA with high affinity to all somatostatin receptors (SSTRs) except SSTR type 4, was administered to patients with carcinoid syndrome refractory to SSAs. The trial demonstrated a benefit in controlling the symptoms of carcinoid, following failure with a standard dose of octreotide LAR [16]. However, this drug is not currently approved by the Italian Medicines Agency (AIFA).

Interferon-alpha (IFN-alpha) controls the symptoms of carcinoid syndrome in 40–70% of patients, but it is a second-line therapy because its use is associated with substantial adverse effects including fever, fatigue, anorexia and weight loss, autoimmune diseases and myelosuppression [17].

Telotristat etiprate, an oral serotonin synthesis inhibitor is a potential novel option that significantly reduces diarrhea in patients with refractory carcinoid syndrome [18].

b) In non-symptomatic I-NETs, SSAs can be considered in progressive disease due to their anti-neoplastic activity [19, 20]. The efficacy of SSA therapy (octreotide and lanreotide) in non-functioning I-NETs with progressive disease has been demonstrated in two placebo-controlled trials. Fig. 12.3 summarizes the treatment of advanced locoregional disease or distant metastases from I-NETs.

Data from a study with ultrahigh-dose octreotide pamoate at 160 mg i.m. every 2 weeks for 2 months followed by the same dose once monthly seemed to show some promise [21]. A high-dose formula of octreotide has been

reported to stabilize hormone production and tumor growth in 75% of patients with advanced midgut carcinoid tumors and stabilize progressive disease for 6–24 months [22].

Among the new targeted therapies, everolimus, an oral inhibitor of the mammalian target of rapamycin (mTOR), can be recommended in advanced I-NETs in the case of disease progression as a second- or third-line therapy, after failure of SSAs and/or IFN-alpha or peptide receptor radionucleotide therapy (PRRT) [23]. Everolimus was the first targeted agent showing a robust antitumor activity across a broad range of neuroendocrine tumors, with acceptable tolerability. On June 2016, the European Medicines Agency (EMA) approved everolimus for the treatment of unresectable metastatic gastrointestinal NETs.

There are not enough data to support the use of other targeted drugs including bevacizumab, sorafenib, pazopanib or axitinib in either pancreatic or non-pancreatic NETs.

In metastatic disease, whether or not symptomatic, concomitant locoregional/ablative therapy, where feasible, is recommended. In the absence of any large comparative trials of the different locoregional or ablative therapies (bland embolization, chemoembolization, radioembolization, radiofrequency ablation or microwave destruction), the choice of treatment is based on individual patient features (e.g., size, distribution, number of liver lesions, vascularization, proliferative index) and local physicians' expertise. Fig. 12.4 summarizes the medical treatment options for advanced/metastatic I-NETs.

12.1.3 Chemotherapy

Like other neuroendocrine carcinomas (NECs), poorly differentiated I-NETs are both biologically and morphologically identical to small and large cell NECs of the lungs. Therefore, patients are usually advised to go for a palliative chemotherapy that corresponds to the medical treatment of small cell carcinoma of the lungs. The objective response rate is ≥50% and classic chemotherapy using cisplatin/etoposide (Moertel regimen) [24] is recommended. Alternative regimens substituting irinotecan for etoposide [25] or carboplatin for cisplatin [26] are thought to be acceptable options.

The optimal duration of chemotherapy has not been clearly defined and there is no established second-line therapy. Encouraging results using either 5-FU i.v. or capecitabine orally combined with oxaliplatin or irinotecan are reported, considering this schedule of treatment as an option [27].

12.1.4 Peptide Receptor Radionuclide Therapy

Peptide receptor radionuclide therapy (PRRT) of I-NETs has recently benefited from the publication of a phase-III pivotal study of PRRT with ^{177}Lu-DOTATATE

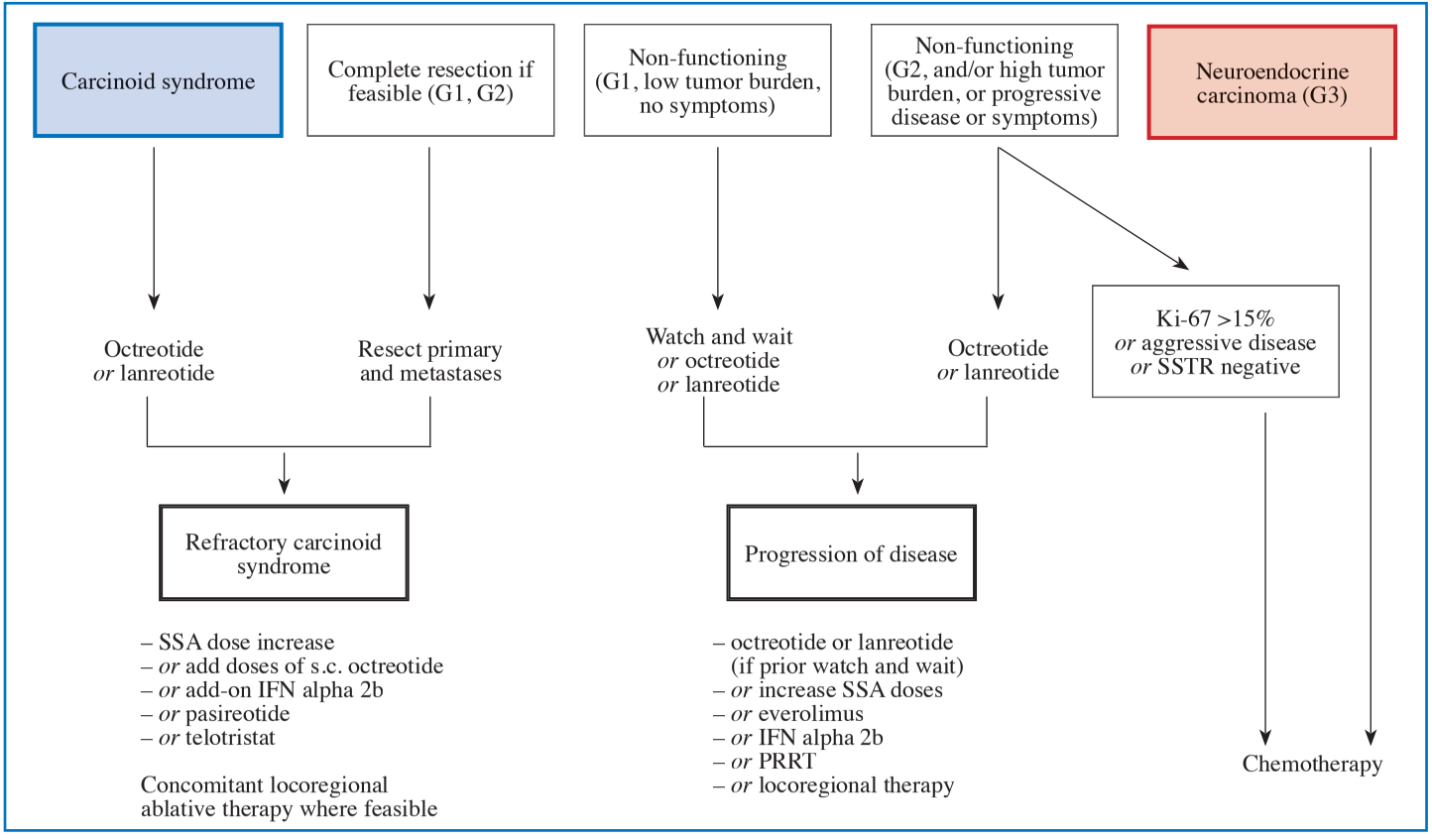

Fig. 12.4 Treatments of advanced locoregional or metastatic I-NETs. *IFN*, interferon; *PRRT*, peptide receptor radionuclide therapy; *SSA*, somatostatin analog; *SSTR*, somatostatin receptor

(Lutathera) for the treatment of midgut NETs [28]. This was the first multicenter randomized protocol to have tested, on 229 patients, the toxicity and efficacy of PRRT with ^{177}Lu-DOTATATE versus a double standard dose of long-acting octreotide therapy. The study showed a 65.2% rate of progression-free survival (PFS) at 20 months (95% confidence interval [CI], 50.0 to 76.8) in the active arm (^{177}Lu-DOTATATE) versus 10.8% (95% CI, 3.5 to 23.0) in the control arm. The response rate was 18% versus 3% (p <0.001). Tolerability was recorded up to a maximum of 9% of transient hematological toxicity, without any renal toxicity. A myelodysplastic syndrome was reported in 2% of the population. These data strongly support the effectiveness of PRRT with ^{177}Lu-DOTATATE in NETs (Fig. 12.3). As the registration of ^{177}Lu-DOTATATE is pending, this therapy can be currently performed only in experimental protocols.

PRRT is indicated both for I-NETs and for other NETs and, since the first applications of PRRT, good tolerability and effective symptomatic response have been recorded for both syndromic symptoms and pain relief. These data, also evident from the use of ^{90}Y-DOTATOC, have been recently confirmed in a study on 90 patients with metastatic carcinoid tumors, 22 of which intestinal. This study showed that symptomatic response had an impact on survival because PFS was significantly longer in those who had improvement of diarrhea [29].

Our group recently published data from a prospective phase-II study on the toxicity and efficacy of PRRT with ^{177}Lu-DOTATATE in 43 patients with I-NETs previously operated or inoperable and in progression after the first line of therapy [30]. Patients were treated with a personalized activity of the radiopharmaceutical in relation to the presence or absence of risk factors for kidney or bone marrow toxicity. The efficacy data showed a disease control rate (DCR) of 84% and a median PFS of 36 months (95% CI, 24–n); the median overall survival (OS) was not reached after a median follow-up of 38 months (range 11–59). In the same study, patients treated with different activities (18.5 vs 26 GBq) showed comparable DCR and PFS. The toxicity was minimal thanks to the extra care given to patients with risk factors.

In another study we evaluated the prognostic value of FDG-PET and found confirmation of a significantly greater reduction in PFS (24.5 months vs. 42 months with $p = 0.025$) in positive patients than in those with normal glucose metabolism, as previously highlighted [31]. In a further study, we enrolled 26 consecutive patients with advanced NET and in progression after a PFS of at least 12 months, following a previous ^{90}Y-DOTATOC treatment in a prospective phase-II study [32]. All patients who had preserved blood chemistry parameters, were treated with ^{177}Lu-DOTATATE administering an activity comprised between 14.8 and 18.5 GBq in 5 cycles performed 6 + 2 weeks apart. With a median total activity of 16.5 GBq in 5 cycles, we achieved a DCR of 84.6% and a median PFS of 22 months (95% CI, 16 months-not reached) compared to 28 months (95% CI, 20–36 months) after ^{90}Y-DOTATOC. The tumor burden and the number of metastases were important prognostic factors. Toxicity was modest for both the bone marrow (1 patient G2 and G3) and the kidneys (1 patient with one with G2 and G3).

^{177}Lu-DOTATATE PRRT offers significant advantages over other therapies, it is effective and should be closely considered in planning treatment for patients with advanced I-NET [33].

12.2 Appendiceal Neuroendocrine Tumors

12.2.1 Surgery

Appendiceal NETs (A-NETs) represent 7% of all NETs and 11% of gastroenteropancreatic NETs (GEP-NETs). They normally arise from the same cells as the ileal NETs, except for the rare goblet cell carcinomas (GCCs) and adenoneuroendocrine carcinomas.

In the majority of cases, the diagnosis is obtained as an incidental finding after appendectomy performed for acute appendicitis. One appendix out of 200/300 appendectomies contains a small A-NET. In these cases, a postoperative evaluation is made to decide if a second surgical step is needed. The parameters to be evaluated are the tumor dimension and signs of lymphatic involvement and are the same that substantiate the indication for right hemicolectomy in the few cases of preoperative diagnosis of appendiceal NET. Serosal, vascular, lymphatic or perineural invasion alone does not constitute an inclusion criterion for right hemicolectomy.

More than 95% of A-NETs are <2 cm [3] and appendectomy itself is the only surgical treatment needed, because the metastatic rate (lymph nodes or liver) is low (<1%). According to some authors, for tumors between 1 and 2 cm, the surgical strategy (appendectomy alone or second-stage right hemicolectomy) has to be evaluated patient by patient on the basis of deep mesoappendiceal invasion (>3mm), positive margin, microscopic lymphatic or venous invasion [34]. Nevertheless, when the size of the primary tumor is >2 cm the metastatization rate is 30–60% and right hemicolectomy is indicated in order to achieve adequate lymphadenectomy.

In females with GCCs, regardless of age, bilateral salpingo-oophorectomy is also advocated. In cases with peritoneal dissemination, cytoreductive surgery and intraperitoneal chemotherapy may offer prolonged survival.

The prognosis of the majority of A-NETs is excellent, with 5-year survival rates close to 100% [35, 36].

12.2.2 Medical Treatment

There is no evidence of the effectiveness of treatment with SSAs in patients with metastatic A-NETs, due to their rarity. In a recent study, patients with A-NETs were pooled together with patients with I-NETs and the appendix cases cannot be extrapolated [37].

In the RADIANT-4 study only one patient with A-NET in the treatment arm with everolimus was reported. Therefore, medical therapy has a limited role in A-NETs and it is confined to the very rare cases of carcinoid syndrome associated with metastatic disease [38].

12.2.3 Chemotherapy

In cases of high tumor grading, GCC or mixed adenoneuroendocrine carcinomas (MANEC) should be suspected, but usually the cases of a "true" neuroendocrine carcinoma (G3 NEC) of the appendix are very rare [39].

NECs of the appendix have very poor outcomes because of frequent lymph node and hepatic metastases, rapid progression and a 1-year survival rate <50% [5]. Because NEC could be misinterpreted as acute appendicitis, it should be considered in the differential diagnosis with immunohistochemical analyses. Appendiceal NEC (grade 3, Ki-67 >20 %) requires a right hemicolectomy and, like adenocarcinoma, adjuvant or intraperitoneal chemotherapy [39, 40]. Because of their clinical and biologic characteristics, de facto similar to those of small cell lung cancer (SCLC), the European Neuroendocrine Tumor Society guidelines [23] recommend that metastatic appendiceal NEC should be treated in a similar way to SCLC (i.e., with etoposide [24] or irinotecan, associated with platinum compounds such as cisplatin or carboplatin [26, 41]). Although objective remission rates are high (40–67%), median PFS is limited within 4–6 months [42].

12.3 Colorectal Neuroendocrine Tumors

12.3.1 Surgery

Colorectal NETs (CR-NETs) are fewer than 1% of all colorectal tumors. Up to 40% of all CR-NETs occur in the cecum and right colon, although this may result from tumors of the appendix extending from the base in the cecum. More than 60% of them are located in the rectum and the diagnosis is mostly incidental during screening endoscopy (1/2500 procedures). Colonic NETs (C-NETs) have a peak incidence in the seventh decade of life, have a male/female ratio of 2:1, and most of them are small, non-functioning tumors.

C-NETs are treated in a similar way to adenocarcinoma of the colon. Well-differentiated C-NETs, <2 cm in diameter, should be treated endoscopically.

Surgery is the only therapy that can cure large colon and rectum NETs (>2 cm) and it is also indicated in cases of incomplete endoscopic resection, deep tumor invasion through the muscularis propria and G3 tumors [43] The surgical options are divided into two types: laparoscopic resections (colectomy, sigmoidectomy and resection of the rectum) and local excision (transanal).

Rectal NETs (R-NETs) are diagnosed at a much younger age than colonic carcinoids, (average age, 48 to 52 years) and about half are asymptomatic; symptoms, when present, are discomfort, mild pain, change in bowel habits, and bleeding. R-NETs are frequently far from the sphincters and can be treated endoscopically or surgically. The treatment of R-NETs is based on the size of the primary tumor. Lesions <1 cm have a low metastatic rate (<3%) and can be completely removed with a transanal excision. Lesions between 1 and 2 cm in diameter have a high metastatic rate, about 10–15% [43–45]. In these cases the endoscopic treatment can be performed if the tumor has a low mitotic index and if there is no ultrasound evidence of deep invasion up to the muscularis propria [46]. Histologic features such as lymphovascular invasion, high mitotic rates, and high Ki-67 should be considered as adverse prognostic factors, but further studies are needed to demonstrate if these factors contribute to recurrence in low risk R-NETs. For tumors >2 cm surgical resection is mandatory, because the incidence of regional metastases is 60–80% [47].

In high-grade lesions, 17% of positive resection margins after standard polypectomy have been reported [48], so that techniques such as submucosal resection with band ligation or endoscopic submucosal dissection are preferred [44].

When surgical resection is indicated, the procedure should follow the rules of standard oncological colorectal resections and preference should be given to the laparoscopic procedure.

C-NET patients have an overall 5-year survival rate of 25–40%. R-NET patients have an overall 5-year survival rate of about 80% if there is only local disease, 50% if regional metastases are present, and 20% if there are distant metastases.

Endoscopic and surgical therapeutic options are summarized in Table 12.1, but at the present time, the best recommendation for CR-NETs is to tailor treatment to the individual case.

Table 12.1 Endoscopic and surgical options in colorectal NETs

	Colon	Rectum
Endoscopy	• Well-differentiated tumors <2 cm	• Well-differentiated tumors <1 cm • Tumors between 1 and 2 cm with low mitotic index and no deep invasion
Surgery	• Tumors >2 cm • Incomplete resection • Deep invasion • G3 tumors	• Tumors between 1 and 2 cm with high mitotic index and/or deep invasion • Tumors >2 cm • Incomplete resection • Deep invasion • High mitotic rate • Lymph node involvement

12.3.2 Medical Treatment

Studies supporting the use of SSAs in CR-NETs are few. In the RADIANT-2 trial, 39 patients with CR-NETs were enrolled. Of those 19/39 (14/19 C-NETs and 5/19 R-NETs) were randomized to receive everolimus plus octreotide LAR and 20/39 (14/20 C-NETs and 6/20 R- NETs) were randomized to receive placebo plus octreotide LAR [49]. Everolimus plus octreotide LAR treatment was linked to a 66% reduction in the estimated risk for disease progression (hazard ratio, 0.34; 95% CI, 0.13–0.89; $p = 0.11$). No complete or partial responses were observed in patients with CR-NETs, but some extent of tumor shrinkage was observed in the everolimus plus octreotide LAR arm (67 vs 37%).

The RADIANT-4 study supports the efficacy of everolimus in patients with advanced, progressive, non-functioning NET of the gastrointestinal tract. The efficacy data on CR-NETs cannot be extrapolated [38], but the study suggests that everolimus could be effective in patients with G1 and G2 CR-NETs.

12.3.3 Chemotherapy

Chemotherapy is an essential part of the multimodality approach for localized colorectal NECs and the mainstay of care in advanced disease. Whereas the neuroendocrine carcinomas have common embryological origin and morphology similar to the small cell carcinoma and Merkel cell carcinoma, the treatment regimens are extrapolated from published data on high-grade SCLC [4]. Therefore, the cisplatin-etoposide doublet is usually the preferred treatment schedule based on the results obtained in metastatic SCLC [24, 26]. It has been observed by Sorbye et al. that the levels of Ki-67 are closely associated with the response to chemo-treatment. Patients with Ki-67 values >55% had greater response rate (42% versus 15%) [26].

Alternative regimens substituting carboplatin for cisplatin, or irinotecan for etoposide, have been validated in SCLC and seem equivalent in terms of efficacy in advanced CR-NETs. Evidence for salvage therapy in patients progressing with one first-line platinum-based regimen is very limited [50–52].

Welin et al. reported a 33% response rate with temozolomide, alone or in combination with capecitabine and bevacizumab, in a cohort of 25 patients with poorly differentiated NECs [53].

Retreatment with platinum/etoposide may also be considered in patients who achieved good durable responses upfront and have progressed after a treatment break of at least 3 months, provided no cumulative toxicity (i.e., neurotoxicity, ototoxicity) precludes further treatment with platinum agents.

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Management of Liver Metastases from Gastroenteropancreatic Neuroendocrine Tumors

13

Giuseppe M. Ettorre, Roberto L. Meniconi, Pascal Hammel, Sophie Deguelte, Luca Filippi, and Roberto Cianni

Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) metastasize to the liver in up to 90% of patients and in these cases the main cause of death is liver failure secondary to replacement by metastases [1, 2]. The incidence rate of liver metastases from NETs depends on the primary tumor site, most pancreatic and small bowel NETs being frequently diagnosed with hepatic metastases (70% and 90%, respectively) [3].

Different therapeutic options are available for treating hepatic metastases including surgical resection and liver transplantation, or locoregional and medical treatments such as transarterial chemoembolization, radioembolization, radionuclide therapy, radiofrequency ablation, chemotherapy, and somatostatin analogs. For this reason, a multidisciplinary approach is needed [4].

13.1 Hepatic Resections

13.1.1 Indications, Techniques and Results

In patients with resectable liver metastases from GEP-NETs, liver resection is the main curative treatment for neuroendocrine liver metastases (NELMs) [5]. Liver resection of NELMs has an overall survival rate ranging from 47% to 92%, with resolution of symptoms in more than 90% of the patients and with low operative mortality.

G.M. Ettorre (✉)
General Surgery and Organ Transplantation, Azienda Ospedaliera San Camillo-Forlanini
Rome, Italy
e-mail: gmettorre@scamilloforlanini.rm.it

M. Carlini (Ed), *Abdominal Neuroendocrine Tumors*,
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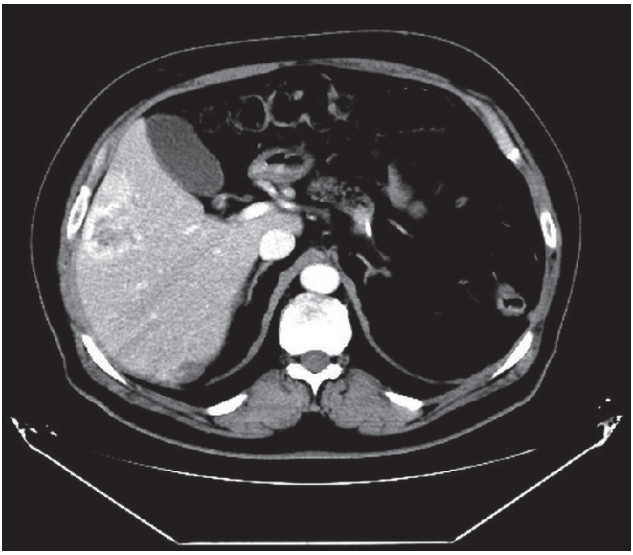

Fig. 13.1 CT scan showing a single liver metastasis (type I) in segment V-VI from pancreatic NET

According to the ENETS guidelines, inclusion criteria for liver resection with curative intent are well-differentiated tumors (G1-G2) without unresectable metastatic lymph nodes, extrahepatic disease or peritoneal carcinomatosis, with a low predictable perioperative risk and the absence of right heart insufficiency (carcinoid syndrome) [3] (Table 13.1). The surgical approach in NELMs includes resection with curative intent (10-20% of cases), liver transplantation or palliative cytoreductive surgery to reduce tumor-related symptoms. The type of liver resection depends on the tumor extension and the future liver remnant.

Frilling et al. classified three types of NELMs: single metastasis (type I), bulky isolated metastasis accompanied by smaller deposits (type II) and disseminated metastases (type III) [6]. For type I patients with a solitary well-differentiated metastasis (Fig. 13.1), curative liver resection is normally feasible and includes wedge hepatic resections or anatomic hepatectomies, achieving clear resection margins (R0) and a sufficient future liver remnant (FLR). Type II and III metastases usually correspond to unresectable disease due to either massive hepatic involvement or small FLR (<30%) (Fig. 13.2). In these cases, curative resection is rarely possible but, when feasible, as in selected type II cases, a staged approach should be considered, including portal vein occlusion (radiological embolization or surgical ligature), two-staged hepatectomy or associating liver partitioning and portal vein ligation (ALPPS) procedure, if necessary coupled with local interstitial treatments in order to decrease the risk of postoperative liver failure [7, 8]. However, surgical resection is not the treatment of choice for type II and III disease, which are well controlled by non-surgical therapies such as transarterial chemoembolisation (TACE), selective internal radiation therapy, radiofrequency ablation or chemotherapy, alone or in combination.

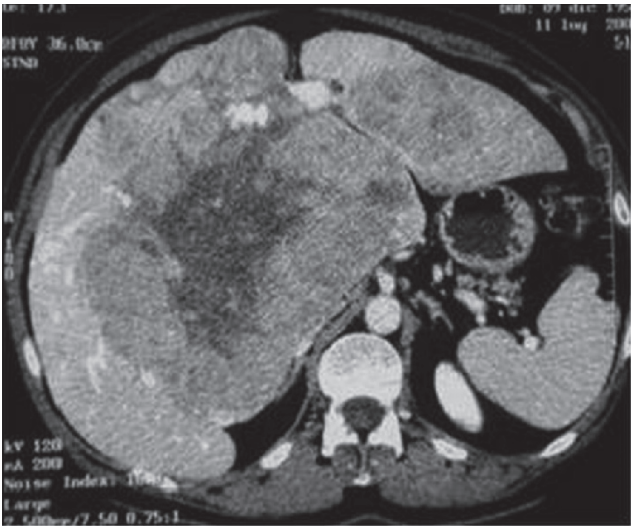

Fig. 13.2 CT scan showing a large tumor bulk in the liver (type III) from a G2 pancreatic NET

Palliative cytoreduction is indicated for unresectable metastases (types II-III) to control either local tumor-related complications (i.e., mass effect) or systemic symptoms secondary to secretion of biologically active peptides. Removal of more than 90% of the tumor bulk achieves a potential survival benefit and relief from symptoms that otherwise may not be feasible by other non-surgical techniques. Sarmiento et al. reported one of the largest experiences of cytoreductive surgery for NELMs (170 patients) achieving symptom control in 96% of patients with functioning unresectable NELMs [9]; however, 59% of these patients experienced symptom recurrence and 84% of patients had evidence of disease recurrence at 5 years after surgery.

In cases of symptomatic NETs, the perioperative use of somatostatin analogs is mandatory for prevention of the "carcinoid crisis" [10].

The overall recurrence or progression rate after liver resection for NELMs is approximately 80% at 5 years. A recent Cochrane review reported 5- and 10-year survival rates of 74% and 51%, respectively, after surgery for NELMs [5]. However, very few patients remained disease-free and the 5-year recurrence rate could be more than 90% [9]. The high incidence of recurrence after resection is related both to the positive margins and to the underestimation of the liver disease. A recent systematic review found a median R0 resection rate of 63% of cases, with a median progression-free survival of only 15 months and a 5-year disease-free survival of 29% [11]. Elias et al. found that preoperative imaging techniques and intraoperative ultrasound underestimated the extent of liver disease in more than 50% of cases, so that would explain the high percentage of incomplete resections and high recurrence rate after resection (75% at 10 years) [12]. However, despite high tumor recurrence, there is a significant increase in

survival time and symptom control, even in incomplete surgical resections (R1 or R2). Other factors related to survival and recurrence of NELMs after surgery have been recently analyzed by an Italian multicenter study which classified patients in three classes of risk based on the number and size of NELMs and the Ki-67 index. The authors found a significant difference between three classes of patients with a 10-year survival rate ranging from 97% (low risk class) to 20% (high risk class) [13].

In cases of intrahepatic recurrence, repeat liver resection may be considered in selected patients, possibly in association with ablative techniques. Glazer et al. reported their experience of repeat hepatectomies for recurrent NELMs and performed up to four re-resections in the same patient. Eighty patients with intrahepatic recurrence were treated by second resection or ablation with a 5-year survival of 62.5%. Among them, 25% (20/80) underwent a third resection/ablation and 60% of them (12/20) underwent a fourth resection with a 50% of survival at a median follow-up of 7.6 years [14]. Patients should be carefully selected based on a number of factors including a thorough assessment of the perioperative risk and following the same criteria as the first liver resection. Alternatively, non-surgical techniques such as TACE, radioembolization or systemic chemotherapy should be considered.

13.1.2 Synchronous Liver Metastases

More than 50% of GEP-NET patients have bilobar and synchronous liver metastases [15]. In these cases, an aggressive surgical approach is widely accepted. The indication for surgical resection of both the primary tumor and liver metastases depends on the site of the primary NET and the type of NELMs (Table 13.1).

Generally, synchronous or staged resections are performed if the primary NET and liver metastases are both amenable to potentially curative resection with acceptable morbidity and mortality rate. Kianmanesh et al. reported a series of 41 patients with synchronous NELMs treated by a two-stage approach [16]: the first step consisted in the resection of the primary NET (small bowel, pancreas tail or rectum), wedge resections of left-sided metastases (segments 1-4) and right portal vein ligation; in the second step a right hepatectomy was performed with a sufficient FLR due to achieved hypertrophy of the left liver. The 5-year overall survival and disease-free survival were 95% and 50%, respectively. These data were also confirmed by Gaujoux et al. who found that concomitant resection of the primary NET and liver metastases can be performed with low mortality (3%) and acceptable morbidity [17].

Another factor to consider for synchronous resection is the location of the primary NET: even though most authors found no significant differences in postoperative complications between pancreatic and digestive tract surgery, perioperative risk should always be taken into account before surgical planning,

Table 13.1 Indication criteria for liver resection of neuroendocrine liver metastases

Well-differentiated tumor (G1-G2) [1]
Resectable extrahepatic disease [2]
Type I or selected type II metastases [3]
Future liver remnant ≥30%
Low perioperative risk
No carcinoid heart disease
Primary NET resected or resectable

[1] Ki-67 index ≤20%; mitotic count ≤20 figures/10 HPF (high-power field)
[2] Including lymph nodes and peritoneal metastases
[3] According to the Frilling at al. classification [6].

especially if the primary NET is located in the pancreatic head and a pancreatoduodenectomy is performed in combination with a major hepatectomy: in this case, patients should be carefully selected based on both operative risk and tumor biological behavior [17].

In cases of unresectable synchronous NELMs, because of the relatively prolonged life expectancy of patients with a well-differentiated slow-growing GEP-NET treated by non-surgical therapies, resection of the primary tumor could be performed to avoid tumor-related complications or hormone secretion symptoms. Some authors suggest resection of the primary NET with unresectable liver metastases even for non-functioning and asymptomatic tumors because it may facilitate liver-directed therapies during active follow-up, improving progression-free survival [18]. These findings have been confirmed in the UKINETS study that found resection of the primary tumor to be one of independent factors of prolonged survival for midgut NETs with unresectable liver metastases [19].

13.2 Neoadjuvant and Adjuvant Treatments

Neoadjuvant and adjuvant treatments have been introduced in single case reports and smaller series. Few authors reported a benefit of neoadjuvant treatment by immunochemotherapy or peptide receptor radionuclide therapy or both, resulting in enhanced tumor resectability. No difference in survival was found between patients treated by adjuvant chemotherapy with streptozocin and fluorouracil versus non-treated patients after liver resection or transplantation [20].

Thus, neoadjuvant and adjuvant chemotherapy are not currently recommended for treating resectable liver metastases from NETs.

13.3 Liver Transplantation

Unresectable NELMs, unlike other metastatic malignancies, have been accepted as an indication for orthotopic liver transplantation (OLT). However clear evidence is lacking regarding the role of OLT in the treatment of unresectable NELMs because of the low incidence of the disease and the wide variety of alternative treatments without adequate available data comparing transplantation for unresectable liver metastases to other treatment modalities. Liver transplantation would ideally allow otherwise unfeasible removal of all metastases of the liver, but it is burdened with higher operative mortality and donor shortage. Moreover, the experience is scarce because liver transplantation for metastatic NETs represents 0.3% and 0.2% of overall liver transplants [21].

OLT for NELMs can offer a significant survival benefit when patients are selected properly. Mazzaferro et al. [22] emphasized the role of patient selection on post-transplant outcomes and proposed the following criteria (Table 13.2): young recipient (less than 55 years), low-grade tumor, Ki-67 index ≤10%, metastatic disease involvement no more than 50% of hepatic volume, primary tumor drained by the portal vein system (midgut and pancreatic NETs) and removed with all extrahepatic deposits (perihilar lymph nodes) before OLT, no metastatic spread to other organs, absence of right heart insufficiency. This approach led to 5-year survival close to 90% and these criteria are currently accepted by most transplantation centers with comparable results. The same Milan group compared two homogeneous groups of patients treated with transplant versus non-transplant techniques and found a significant long-term advantage of liver transplantation over other options (10-year overall survival, 88.8% vs 22.4%; 10-year progression-free survival, 13.1% vs 89%) [23]. These

Table 13.2 Indications and contraindications for orthotopic liver transplantation (OLT) for neuroendocrine liver metastases

Indications	Contraindications
• Low-grade tumor (G1-G2) • Primary tumor and extrahepatic deposits removed before OLT • Primary tumor drained by the portal vein system (pancreas and midgut NETs) • Hepatic involvement ≤50% • Ki-67 index ≤10%* • Stable disease (>6 months of follow-up prior to transplant consideration)** • Age ≤55 years**	• High-grade tumor (G3) • Metastases to other organs • Non GEP-NETs or tumor not drained by the portal system (esophagus, rectum) • Advanced carcinoid heart disease • Other medical or surgical contraindication to OLT

* Cut-off not well defined
** Not universally accepted.

Table 13.3 Recurrence and survival rates of liver transplantation for neuroendocrine liver metastases: selection of multicenter and single-center series published since 1998

Authors	Study type	N. of patients	5-year survival	5-year disease-free survival
Mazzaferro et al. 2016 [23]	Prospective Single center	42	97%	NR
Le Treut et al. 2013 [24]	Retrospective Multicenter	213	52%	30%
Nguyen et al. 2011 [25]	Retrospective Multicenter	184	49%	NR
Sher et al.2009 [28]	Retrospective Multicenter	83	49%	32%
Le Treut et al. 2008 [29]	Retrospective Multicenter	85	47%	20%
Mazzaferro et al.2007 [22]	Prospective Single center	24	90%	77%
Olausson et al. 2007 [26]	Prospective Single center	15*	90%	20%
Frilling et al. 2006 [30]	Prospective Single center	15	67%	48%
Rosenau et al. 2002 [31]	Retrospective Single center	19	80%	21%
Lehnert et al. 1998 [32]	Retrospective Multicenter	103	47%	24%

NR, not reported
* 5 multivisceral transplants.

excellent results were not confirmed by other multicenter series (Table 13.3). Le Treut et al. recently reported the largest retrospective multicenter review of 213 patients undergoing OLT for NELMs and found a 5-year survival of 52%, with a 3-month mortality of 10%, and identified three poor prognostic factors affecting postoperative deaths: age more than 45 years, hepatomegaly and concomitant surgery in addition to OLT [24]. The same authors argued that if patients without those risk factors were selected, the 5-year survival rate would reach 80%. These survival rates were also confirmed in another recent review of the UNOS database with a 5-year survival rate of 49.2% in the pre-MELD era that increased to 57.8% after the application of the MELD score (since 2002) [25].

Other authors reported similar results even without applying a strict patient selection according to the aforementioned Milan criteria. Olausson et al. performed 15 transplants (10 OLT and 5 multivisceral) for metastatic NETs with expanded criteria: high proliferation rate (Ki-67 >10%), large tumor burden exceeding 50% of the hepatic volume and older age (>64 years) [26]. However, this experience is too limited for proper evaluation, and other non-surgical treatments should be considered.

Although the short- and long-term outcomes of liver transplantation for NELMs have improved during the last two decades, the recurrence rate after liver transplantation for metastatic NETs still remains high. A recent systematic review of the literature identified the following risk factors for recurrence after OLT: older age (>50 years), symptomatic tumor, primary tumor in the pancreas or a non-gastrointestinal location, non-carcinoid primary tumor, a high Ki-67 index, large liver involvement (>50%) and poor tumor differentiation (G3) [27]. However, in most of the reported series, liver transplantation was offered only after failure of other techniques, whereas it should be considered as a curative option. As for hepatocellular carcinoma, downstaging procedures while on the waiting list should be included in the general protocol of liver transplantation for NELMs [4].

The use of organs from the deceased donor pool for neuroendocrine metastatic disease presents ethical and cost-effectiveness problems. The absence of precise guidelines for liver transplantation for NELMs and the shortage of donors hamper access of these patients to this therapeutic option, as in hepatocellular carcinoma or other rare hepatic tumors. Living donor liver transplantation (LDLT) may be a solution to overcome this obstacle, providing a life-saving organ with acceptable mortality and morbidity. However, large experiences of living donation for NET are not available and current data are limited to very small case series.

13.4 Locoregional Treatments

Surgical resection represents the first-choice approach for the liver metastases from NETs with an overall survival of approximately 114 months as compared to 25 months for the unresectable non-treated lesions [33]. Chemotherapy presents a limited role for NETs with a modest rate of disease control ranging from 0-38% [34]. Preliminary data for novel therapeutic agents demonstrate a promising progression-free survival [35].

13.4.1 Peptide Receptor Radionuclide Therapy (PRRT)

Patients with positive somatostatin receptor (SSTR) tumors can be enrolled for peptide receptor radionuclide therapy (PRRT) with radiolabeled somatostatin analogs [36].

The criterion to enroll patients for PRRT is demonstration of SSTR2 expression by ^{111}In-pentetreotide scintigraphy or by positron emission tomography/computed tomography (PET/CT) with ^{68}Ga-DOTA-peptides. PRRT is administered systemically in multiple cycles up to the maximum administrable activity or to the dose limit of 25 Gy to the kidney, which is the dose-limiting organ. To reduce the irradiation of the kidneys, patients are usually co-infused with cationic amino acids before and after PRRT [37]. Complete or partial response can be

achieved in up to 30-38% of the treated patients, with a significant improvement of the overall survival (i.e., 48 months). Patients treated with ^{177}Lu-DOTATATE showed a significant improvement of quality of life. Recent clinical reports suggest that the combined used of ^{90}Y/^{177}Lu-DOTATATE may be more effective [38].

Among 450 subjects treated with PRRT, complete remission was observed in 5.6% of cases, 22.4% had a partial remission, 47.3% were stable, and 4% were progressive. Overall survival was inferior in the patients treated with ^{90}Y alone compared to any PRRT with ^{177}Lu [39].

Nevertheless, PRRT presents limitations in hepatic lesions from NETs. Around 30% of the administered activity, in fact, is excreted in the urine in the first hours. In patients with liver metastases from NETs, it has been proposed to inject ^{177}Lu-DOTATATE directly in the hepatic artery thus reaching partial response in the 75% of the treated patients, with no nephrotoxicity but with a remarkable myelotoxicity [40].

In NET with exclusive or predominant hepatic involvement, locoregional treatments can be considered, such as radioembolization with ^{90}Y-spheres, transarterial chemoembolization, and radiofrequency ablation.

13.4.2 Radioembolization with ^{90}Y-Spheres

Radioembolization (RE) with ^{90}Y-labeled resin or glass microspheres has emerged as a valuable option for the treatment of primary or secondary hepatic lesions. The principle of ^{90}Y-RE is based on the characteristic dual blood supply of the liver (i.e., from the hepatic artery and the portal vein). The portal vein supplies the normal hepatocytes, while tumoral cells receive blood mainly from the hepatic artery. ^{90}Y-spheres are infused directly into the hepatic arterial circulation with a preferential release of therapeutic material to the tumor.

The indications for ^{90}Y-RE are represented by unresectable and chemotherapy-refractory primary or secondary hepatic tumors. Two kinds of ^{90}Y-microspheres are currently approved for the treatment of unresectable hepatic malignancies in the European market: (a) SIR-Spheres (Sirtex, Australia), biodegradable resin microspheres (20–60 μm in diameter) associated with an embolic effect; (b) TheraSphere (MDS Nordion, Ottawa, Canada), non-biodegradable glass spheres [41].

Several papers suggest that ^{90}Y-RE can be useful in patients affected by hepatic NETs with predominant liver disease. In a cohort of 34 patients with hepatic NETs, King et al. obtained a symptomatic response in 50% of the cases at 6 months with a mean overall survival of 29.4 (±3.4) months [42].

The largest retrospective study on this topic was performed on a cohort of 148 patients with liver metastases from NETs who were subjected to ^{90}Y-RE in a primarily salvage setting: a median survival of 70 months was recorded [43].

One study evaluated the clinical impact of ^{90}Y-RE in 48 patients with unresectable NELMs [44]. The median survival was 35 months (range, 5-63); furthermore, seven patients (15%) had a complete response and 19 patients (40%)

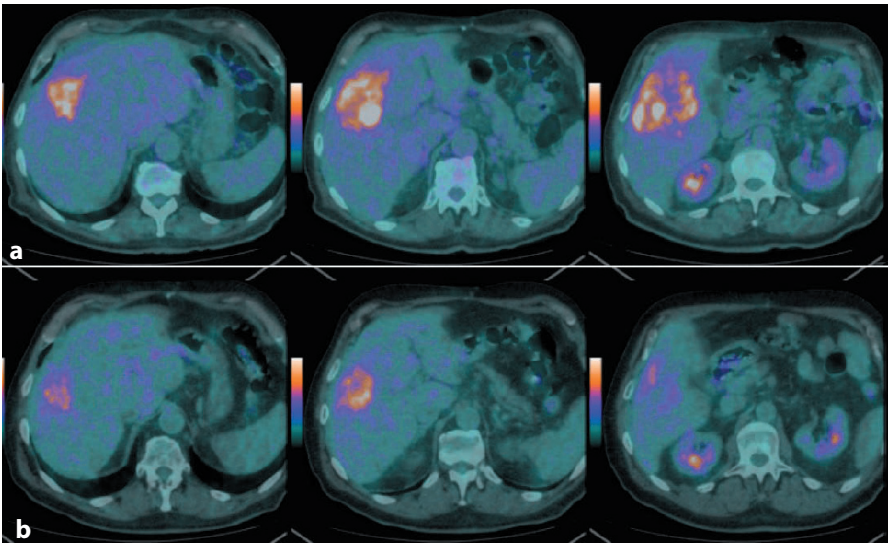

Fig. 13.3 68-year-old male patient who had undergone surgery for ileal NET (G2, Ki-67: 10%). ^{18}FDG PET/CT performed 2 years after surgery revealed a large metastasis in the right lobe of the liver (**a**). After a multidisciplinary meeting, the patient was subjected to ^{90}Y-radioembolization (1.8 GBq of resin spheres) due to the exclusive hepatic involvement. ^{18}FDG PET/CT performed 6 weeks after treatment (**b**) demonstrated significant metabolic response of the liver metastasis (i.e., 67% reduction of total lesion glycolysis). Overall survival since the diagnosis of hepatic metastasis was 41 months

had a partial response to treatment. The study confirmed ^{90}Y-RE to be an effective tool, and also identified important prognostic factors predicting survival: low hepatic tumor burden, well-differentiated tumor, female gender, and no extrahepatic disease.

^{90}Y-RE represents a well-tolerated procedure. Aberrant microsphere deposition may lead to radiation cholecystitis and the risk of liver failure.

To assess the response after ^{90}Y-RE, most clinical studies used contrast-enhanced CT and applied the Response Evaluation Criteria in Solid Tumors (RECIST). Although ^{18}FDG-PET is routinely applied in oncology, several reports suggest that this imaging technique may be of limited usefulness in NETs, since the well-differentiated tumors present a low proliferation rate and poor expression of glucose transporter receptors and only the most aggressive NETs show ^{18}FDG incorporation (Fig. 13.3).

^{68}Ga-DOTA-compounds (i.e., DOTATOC, DOTANOC, DOTATE) characterized by somatostatin receptor-mediated binding have been introduced for PET/CT imaging. In this regard, a recent report from our group demonstrated that an early molecular response assessed by ^{68}Ga-DOTANOC PET/CT may be a useful predictor of clinical outcome in patients affected by hepatic NETs subjected to treatment with ^{90}Y-spheres [45].

13.4.3 Hepatic Artery Embolization and Transarterial Chemoembolization

Hepatic artery embolization (HAE) is performed in NELMs to induce ischemia in the tumoral mass, by using agents such as polyvinyl alcohol, cyanoacrylate, and gel foam particles. HAE may improve symptoms and slow the disease progression.

Transarterial chemoembolization (TACE) is based on the intra-arterial administration of chemotherapeutic agents so as to achieve an intratumoral concentration of the drug much greater than that obtained with systemic administration. The antitumoral effects of the chemotherapy are also enhanced by the ischemic necrosis induced in the malignant mass by the embolization. The drugs currently used for this treatment are: doxorubicin, melphalan, and streptozocin. Relative contraindications for these procedures are represented by coagulopathy, renal failure, portal vein occlusion, and liver failure [46].

In the literature there is little evidence of a significant improvement in the outcome of patients treated with TACE as compared to those subjected to HAE: a 5-year survival between 50%-65% has been reported for TACE and 40-67% for HAE. These results are in agreement with a recent report on a cohort of 30 patients with NELMs: 17 were subjected to HAE and 13 to TACE [47]. Per-lesion reduction was 2.2±1.4 versus 3.3±1.5 cm for HAE (p <0.001) and 2.2±1.5 versus 3.4±1.7 cm for TACE (p <0.001). In the whole population, the median progression-free survival for all patients was 36 months (16.2–55.7 CI), without significant difference between HAE and TACE, but patients treated with HAE showed less toxicity.

Liver abscesses, transient liver failure with or without encephalopathy, carcinoid crisis, pleural effusions, and postembolization syndrome (fever, abdominal pain and transient increases in hepatic enzymes and bilirubin) are possible side-effects associated with the procedures.

13.4.4 Radiofrequency Ablation

The clinical application of this therapeutic technique has gradually expanded. Radiofrequency ablation (RFA) can be performed by using a percutaneous, laparoscopic or open-surgery approach. Current indications consist of limited unresectable liver lesions and non-operable liver tumors. RFA has been widely applied in the treatment of liver lesions (from 1 to 4, ≤5 cm in diameter) from hepatocellular carcinoma and colorectal cancer, but its application in hepatic NETs is still limited.

In a series of 63 patients affected by NELMs, Mazzaglia et al. performed RFA and analyzed perioperative morbidity, symptomatic improvement and long-term survival [48]. Forty-nine patients underwent one ablation session, and 14 (22%) had repeated sessions caused by disease progression. Perioperative morbidity

was 5%, with no 30-day mortality. Fifty-seven percent of patients exhibited symptoms. The median survival was most favorable in patients with a diameter of the dominant liver lesions <3 cm. Another report showed that RFA can be associated with an increased risk of hepatic abscess formation.

13.5 Systemic Therapy

Patients with unresectable NELMs can be treated by systemic therapies, especially in the case of poor-differentiated tumors or symptomatic disease with extrahepatic involvement not suitable for surgical or interventional treatments.

13.5.1 Somatostatin Analogs

Somatostatin analogs (SSAs) – octreotide and lanreotide – have been demonstrated to provide a symptomatic benefit in more than 70% of cases. They also have antiproliferative effects on well-differentiated unresectable NELMs, as demonstrated by the randomized controlled phase III PROMID study, providing significant symptom control and survival benefit especially for limited hepatic involvement and resected primary NET [49].

Thus, in patients with unresectable NELMs from pancreatic or midgut NETs, SSAs can be used both for symptom relief (functioning tumors) and disease stabilization.

13.5.2 Chemotherapy

Systemic chemotherapy with streptozocin, 5-fluorouracil, doxorubicin, capecitabin and temozolomide, as single agents or in combination, are currently used to treat unresectable G1-G2 metastatic NETs from the pancreas, with a survival gain of 74% at 5 years [50]. No effective chemotherapy has been reported for unresectable liver metastases from non-pancreatic NETs, which should be treated by other options (TACE, PRRT, radioembolization, interferon).

For G3 NETs, cisplatin-etoposide-based chemotherapy is currently recommended as first-line therapy, and it should be started immediately. In the case of progression of G3 tumors after first-line treatment, a good response with temozolomide-based chemotherapy (alone or in combination with capecitabine and bevacizumab) was recently reported, with disease stabilization in 71% of patients [51].

13.5.3 Interferon

Interferon-alpha is currently used for symptom and biochemical control of metastatic NETs, but no clear benefits in survival or progression-free survival have been reported. It can be used as an alternative to or in combination with SSAs, with a careful evaluation of secondary effects.

13.5.4 Targeted Therapy

Some of the recent agents such as inhibitors of angiogenesis or mTOR signaling have been proposed as second-line treatments for G1-G2 unresectable neuroendocrine metastases, alone or in combination with other drugs.

The anti-vascular endothelial growth factor (anti-VEGF) monoclonal antibody bevacizumab has been reported to prolong progression-free survival in patients with advanced non-pancreatic NETs, and a recent phase III trial evaluated the efficacy of the tyrosine kinase inhibitor sunitinib in terms of objective response and progression-free survival for advanced G1-G2 pancreatic NETs [52].

Everolimus is an inhibitor of the mTOR signaling pathway and its efficacy has been recently reported for metastatic G1-G2 pancreatic NETs, alone or in combination with octreotide [53].

Targeted therapies are currently indicated as second-line treatments for well-differentiated pancreatic NETs in cases of progressive disease.

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