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Pregledni članak

NEUROENDOCRINE TUMORS – LABORATORY DIAGNOSIS

NEUROENDOKRINI TUMORI – LABORATORIJSKA DIJAGNOZA

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Summary: Neuroendocrine tumors (NETs) are a heterogeneous group of neoplasms originating from endocrine cells, which are characterized by the presence of secretory granules as well as the ability to produce biogenic amines and polypeptide hormones. These tumors originate from endocrine glands such as the adrenal medulla, the pituitary, and the parathyroids, as well as endocrine islets within the thyroid or the pancreas, and dispersed endocrine cells in the respiratory and gastrointestinal tract. The clinical behavior of NETs is extremely variable; they may be functioning or not functioning, ranging from very slow-growing tumors (well-differentiated NETs), which are the majority, to highly aggressive and very malignant tumors (poorly differentiated NETs). Classically, NETs of the gastrointestinal tract are classified into 2 main groups: (1) carcinoids and (2) endocrine pancreatic tumors (EPTs). Most neuroendocrine tumors produce and secrete a multitude of peptide hormones and amines. Some of these substances cause a specific clinical syndrome: carcinoid, Zollinger-Ellison, hyperglycemic, glucagonoma and WDHA syndrome. Specific markers for these syndromes are basal and/or stimulated levels of urinary 5-HIAA, serum or plasma gastrin, insulin, glucagon and vasoactive intestinal polypeptide, respectively. Some carcinoid tumors and about one third of endocrine pancreatic tumors do not present any clinical symptoms and are called 'nonfunctioning' tumors. Therefore, general tumor markers such as chromogranin A, pancreatic polypeptide, serum neuron-specific enolase and subunits of glycoprotein hormones have been used for screening purposes in patients without distinct clinical hormone-related symptoms. Among these general tumor markers chromogranin A, although its precise function is not yet established, has been shown to be a very sensitive and specific serum marker for various types of neuroendocrine tumors. This is because it may also be elevated in many cases of less well-differentiated tumors of neuroendocrine origin that do not secrete known hormones.

Kratak sadržaj: Neuroendokrini tumori (NETs) jesu heterogena grupa neoplazmi poreklom iz endokrinih ćelija, koje odlikuju prisustvo sekretornih granula i sposobnost produkcije biogenih amina i polipeptidnih hormona. Ovi tumori potiču od endokrinih žlezda kao što su adrenalna medula, hipofiza i paratiroide, kao i endokrinih insula u okviru tiroide ili pankreasa i raspršenih endokrinih ćelija u respiratornom ili gastrointestinalnom traktu. Kliničko ponašanje NETs varira u znatnoj meri. Oni mogu biti funkcionalni ili nefunkcionalni, kao i spororastući (uspešno diferentovani NETs), koji čine većinu, ili veoma agresivni i vrlo zloćudni tumori (nedovoljno diferentovani NETs). NETs gastrointestinalnog trakta obično se dele na dve glavne grupe: 1) karcinoide, i 2) endokrine tumore pankreasa (EPTs). Većina neuroendokrinih tumora proizvodi i sekretuje peptidne hormone i amine. Neke od tih supstanci izazivaju specifične kliničke sindrome: karcinoidni, Zolinger-Elisonov, hiperglikemijski, glukagonom i Verner-Morisonov. Specifični markeri za te sindrome su bazalni i/ili stimulisani nivoi 5-HIAA u urinu, gastrina, insulina, glukagona i vazoaktivnog intestinalnog polipeptida u serumu ili plazmi. Neki karcinoidni tumori kao i otprilike trećina endokrinih tumora pankreasa ne daju nikakve kliničke simptome i nazivaju se »nefunkcionalnim« tumorima. Stoga se uobičajeni tumorski markeri, poput hromogranina A, pankreasnog polipeptida, neuron-specifične enolaze u serumu i podjedinica glikoproteinskih hormona, koriste za skrining kod pacijenata bez jasnih kliničkih simptoma vezanih za hormone. Me u uobičajenim tumorskim markerima kao veoma osetljiv i specifičan serumski marker za različite tipove neuroendokrinih tumora pokazao se pankreasni polipeptid hromogranin A, iako njegova funkcija još nije ustanovljena. Razlog tome je što može biti povišen i u mnogim slučajevima nedovoljno diferentovanih tumora neuroendokrinog porekla koji ne luče poznate hormone. Hromogranin A se u ovom trenutku smatra najboljim neuroendokrinim markerom u serumu ili plazmi koji je dostupan za dijagnozu i terapeutsku evaluaciju.

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At the moment, chromogranin A is considered the best general neuroendocrine serum or plasma marker available both for diagnosis and therapeutic evaluation, and is increased in 50–100% of patients with various neuroendocrine tumors. Chromogranin A serum or plasma levels reflect tumor load, and it may be an independent marker of prognosis in patients with midgut carcinoids.

Keywords: neuroendocrine tumors, somatostatin, chromogranin A, serotonin

Povišen je kod 50–100% pacijenata s različitim neuroendokrinim tumorima i njegovi nivoi u serumu ili plazmi odražavaju stanje tumora. Hromogranin A može se smatrati nezavisnim prognostičkim markerom kod pacijenata s karcinoidima srednjeg probavnog trakta.

Ključne reči: neuroendokrini tumori, somatostatin, hromogranin A, serotonin

Introduction

Neuroendocrine tumors constitute a heterogeneous group of neoplasms that have been postulated to originate from a common precursor cell population (1). The system includes endocrine glands, such as the pituitary, the parathyroids, and the [neuroendocrine (NE)] adrenal, as well as endocrine islets within glandular tissue (thyroid or pancreatic) and cells dispersed between exocrine cells, such as endocrine cells of the digestive and respiratory tracts, the diffuse endocrine system (2). NE tumors (NETs) originating from the gastrointestinal (GI) tract, along with similar tumors originating from the lungs and thymus, have traditionally been defined as »carcinoid tumors« (3). Some NETs may occasionally show very aggressive behavior and become highly malignant (poorly differentiated NETs), but the great majority tend to be relatively slow growing (well-differentiated NETs) and retain many multipotent differentiation capacities. Such features include the ability to produce and secrete a variety of metabolically active substances (amines and peptides) and cause distinct clinical syndromes (4). In addition, NETs possess neuroamine uptake mechanisms and/or specific receptors at the cell membrane, such as somatostatin (SS) receptors, which can be of great value in identifying and localizing these tumors, besides being useful in their therapy (5). NETs may occur either sporadically or as part of familial syndromes; some of them are associated with particular genetic defects, a number of which have recently been delineated at the molecular level (6).

Although Theodor Langhans (1839–1915) was the first to describe the histology of a carcinoid tumor in 1867 (7), it is generally Otto Lubarsch (1860–1933) who is credited with the first report of two patients with ileal carcinoid tumors discovered at autopsy, in 1888 (8, 9). In 1907, Siegfried Obendorfer (1876–1944), a German pathologist at the University of Munich, coined the term *karzinoide*, or »carcinoma-like«, to describe the unique feature of behaving like a benign tumor despite resembling a carcinoma microscopically (10). Recognition of the endocrine-related properties of carcinoid tumors did not occur until much later. In 1948, Rapport and colleagues isolated and named serotonin (5-hydroxytryptamine), initially identified as a vasoconstrictor substance in the serum (11); by 1952, it was determined that the origin of this amine was the Kulchitsky cell (12). Just one year later, Lembeck identified serotonin in an ileal carcinoid and confirmed it as the major hormone responsible for carcinoid syndrome (13).

Tumor Biology

Carcinoids are rare neuroendocrine tumors (NETs) thought to arise from the enterochromaffin (Kulchitsky) cells found throughout the crypts of Lieberkuhn of the gut (6). Specifically, the term *enterochromaffin* refers to the ability to stain with chromium or chrome salts, a common feature of serotonin-containing cells. Similarly, the granules of carcinoid tumors have a high affinity for silver stains, justifying the use of the nomenclature *argentaffinoma*, which was used interchangeably with carcinoid tumor years ago. The diagnosis of carcinoid tumor is initially based on histology with confirmation by positive immunohistochemical staining, defined as positive staining for one or more neuroendocrine markers (such as chromogranin A [CgA]or synaptophysin), or electron microscopy in which the cells in most tumors are found to contain membranebound secretory granules with dense-core granules in the cytoplasm (14). Within these granules are a wide variety of biogenic amines and hormones characteristic of NETs. The most common biologically active substance secreted from carcinoid tumors is serotonin, a vasoactive peptide whose biosynthesis is accomplished nearly exclusively by the enterochromaffin cells. Synthesized from the amino acid tryptophan, the release of serotonin into the systemic circulation can cause the classic symptoms of carcinoid syndrome, which include diarrhea, episodic flushing, bronchoconstriction, and eventual right-sided valvular heart disease (15).

Genetic defects. NETs can occur sporadically or in a familial context of autosomal dominant inherited syndromes such as multiple endocrine neoplasia (MEN) (15) (*Table I*). Four major MEN syndromes, MEN I, MEN II, von Hippel-Lindau (VHL) disease, and Carney complex, represent the most common forms of inherited predisposition to NETs with variable but high penetrance in various NE tissue; early screening can be used for presymptomatic diagnosis (6, 16). Less commonly, endocrine tumors of the pancreas,

Table I Characteristics of Multiple Endocrine Neoplasia (MEN) Syndromes (15).

Organ	Neoplasm	Patients Affected (%)
MEN 1		
Parathyroid	Hyperplasia	90
Pituitary	Adenoma	65
Pancreas	Islet cell	75
Adrenal	Cortical adenoma	–
Thyroid	Adenoma	–
Adipocyte	Lipoma	–
Multiple	Carcinoid	–
MEN 2A		
Thyroid	Medullary carcinoma	100
Adrenal	Pheochromocytoma	50
Parathyroid	Hyperplasia	20
MEN 2B		
Thyroid	Medullary carcinoma	75
Adrenal	Pheochromocytoma	50
Parathyroid	Hyperplasia	<1%
Neuroma	Mucosal neuroma, Intestinal ganglioneuroma	100

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parathyroids, and adrenal glands have been observed in phacomatoses, such as neurofibromatosis (NF) type 1 and tuberous sclerosis (16). Most NET-predisposing diseases have been related to inactivation of tumor growth suppressor genes, except in MEN II and the inherited form of MTC, which occur through dominant activation of the RET protooncogene (16, 17). The RET protooncogene encodes a transmembrane tyrosinekinase receptor that causes cellular proliferation, differentiation, and increased cell motility. MEN II comprises three clinical subtypes, MEN IIA, MEN IIB, and familial MTC (FMTC) (17); in MEN IIA, all patients develop MTC, about 50% pheochromocytoma, and about 15% primary hyperparathyroidism (17). Patients with MEN IIB may have a marfanoid habitus and mucosal neuromas but not hyperparathyroidism; in these patients, MTC occurs at a younger age and behaves more aggressively compared with MEN IIA (16). Approximately 95% of MEN II cases are accounted for by germline RET mutations (≈98% of MEN IIA cases, 97% of MEN IIB cases, and 85% of FMTC cases).

MEN I is an autosomal dominant syndrome characterized mainly by hyperplasia and/or multiple tumors of the parathyroid, endocrine pancreas, anterior pituitary, foregut-derived NE tissues, and adrenocortical glands (18). Somatic mutations of the *MEN I* gene have been reported in sporadic forms of endocrine tumors with a variable incidence of 20–30% in parathyroid, endocrine pancreas (33% gastrinomas, 17% insulinomas), 25% of lung carcinoids, but less than 1% in pituitary and adrenocortical tumors (15). In clinical practice, genetic analysis is useful to assess the syndromic diagnosis of MEN I, but the diagnosis cannot be excluded with certainty when a mutation is not found (6). Some VHL-related lesions include pheochromocytomas, pancreatic islet cell tumors, and papillary cystadenomas of the pancreas, epididymis, the broad ligament, and the lymphatic sac of the middle ear (19). However, the incidence of specific tumors depends on the phenotypic class of VHL, of which four have been described (type 1 and types 2A, 2B, and 2C). The Carney complex is an autosomal dominant disease predisposing to various types of tumors, including cardiac and cutaneous myxomas, spotty pigmentation of the skin, and nonneoplastic hyperfunctioning endocrine states, such as nodular adrenocortical hyperplasia associated with Cushing's syndrome and pituitary and thyroid adenomas (20, 21). Approximately 1% of patients diagnosed with pheochromocytomas may have NF1, a dominantly inherited disorder with complete penetrance but highly variable expressivity. Diagnostic criteria for NF1 include cutaneous or sc neurofibromas, cafe-au-lait spots appearing early in life, optic glioma, benign iris hamartomas (Lisch nodules), and specific dysplastic bone lesions. Digestive tract carcinoid tumors have rarely been described in patients with NF1 and tuberous sclerosis (22). Knowledge of the particular genetic defects in these familial syndromes is essential for the early screening and counseling of other family members.

Growth factors. Malignant progression of NETs may also be triggered by overexpression of growth factors involved in endocrine and endothelial cell proliferation such as TGFα, endothelial growth factor, nerve growth factor, and vascular endothelial growth factor (VEGF)/VEGF-related factors (16). Among various growth factors promoting angiogenesis, VEGF was found to be overexpressed, mainly in midgut carcinoid and some pancreatic tumors, suggesting that it may be involved indirectly in the growth of these tumors (23). The genetic markers so far identified in various sporadic types of NETs are not specific enough to be used for diagnostic purposes, but they provide some clues as to the genetic mechanism of tumor development.

Incidence

Though relatively rare, carcinoid tumors represent the most common gastrointestinal NETs. In a series of 13,715 carcinoid cases reported to the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute, the age-adjusted incidence rates for white men and women over the last decade were 2.47 and 2.58 per 100,000 population per year, respectively, whereas they were somewhat higher for black men and women (4.48 and 3.98 per 100,000 population per year, respectively) (24). In a

separate report from the SEER database of 20,436 carcinoid cases from 1973–2004, carcinoid tumors comprised 0.66% of all malignancies over this time frame and demonstrated a 3–10% increase in tumor incidence depending on the subtype. The majority of carcinoids are found within the gastrointestinal tract (55%) and bronchopulmonary system (30%). According to population-based statistics for the gastrointestinal tract, the small intestine is the most common site of carcinoids (45%), followed by the rectum (20%), appendix (17%), colon (11%), and stomach (7%). Traditionally, the appendix had been identified as the most common site of carcinoid tumors within the gastrointestinal tract; however, recent studies suggest that the incidence of primary appendiceal carcinoid disease is declining, while the incidence of gastric and rectal carcinoid disease is on the rise. This evolution of carcinoid epidemiology presumably stems from improvements in technology and diagnosis over the last several decades (25, 26).

Tumor Classification

Carcinoid tumors have traditionally been classified based on embryologic site of origin, morphologic pattern, and silver affinity. This classification system, originally proposed by Williams and Sandler in 1963 (27), subdivided carcinoids into tumors of the foregut (respiratory tract, thymus, stomach), the midgut (small intestine, appendix, proximal colon), and the hindgut (distal colon, rectum, genitourinary tract). Sporadic primary *foregut* tumors include carcinoids of the bronchus, stomach, first portion of the duodenum, pancreas, and ovaries. *Midgut* carcinoid tumors derive from the second portion of the duodenum, the jejunum, the ileum, and the right colon. *Hindgut* carcinoid tumors include those of the transverse colon, left colon, and rectum. This distinction assists in distinguishing a number of important biochemical and clinical differences among carcinoid tumors because the presentation, histochemistry, and secretory products are quite different.

Foregut carcinoids are argentaffin negative. They have a low content of serotonin (5-hydroxytryptamine [5-HT]). They often secrete the serotonin precursor 5-hydroxytryptophan (5-HTP), histamine, and a multitude of polypeptide hormones. Their functional manifestations include carcinoid syndrome, gastrinoma syndrome, acromegaly, Cushing's disease, and a number of other endocrine disorders. Furthermore, they are unusual in that flushing tends to be of protracted duration, is often purplish or violet instead of the usual pink or red, and frequently results in telangiectasia and hypertrophy of the skin of the face and upper neck. The face may assume a leonine appearance after repeated episodes. It is not unusual for these tumors to metastasize to bone.

Midgut carcinoids, in contrast, are argentaffin positive, have high serotonin content, rarely secrete 5-HTP, and often produce a number of other vasoactive compounds such as kinins, prostaglandins (PGs), and substance P. The clinical picture that results is the classic carcinoid syndrome of flushing and diarrhea with or without wheezing. These tumors may produce adrenocorticotropic hormone (ACTH) on rare occasions and infrequently metastasize to bone.

Hindgut carcinoids are argentaffin negative, rarely contain serotonin, rarely secrete 5-HTP or other peptides, and usually are silent in their presentation. However, they may metastasize to bone. A further point of interest is that a gender variation is present when a carcinoid tumor coexists with MEN-I; more than two-thirds of the time the tumor is in the thymus in males, whereas in females, more than 75% of the time it is in the lung.

All these tumors arise from similar cells with origins from neural tissue which form part of the endocrine system and produce and release hormones. Hence these are called Neuro Endocrine Tumors (NET) or more accurately Gastro-Entero-Pancreatic-Neuro Endocrine Tumors (GEP-NETs). They have two distinct groups: Pancreatic Endocrine Tumors (PET) and Carcinoid (25, 27).

Laboratory Markers in Neuroendocrine Tumors

Serum and immunohistochemical tumor markers

The various cell types of the NE cell system can secrete specific products, such as peptides and biogenic amines, that are tumor-specific and may serve as markers for the diagnosis and follow-up of treatment; it is also probable that some tumor markers may have prognostic implications (4) (*Table I*). A number of other components specific for all NE cells and associated with secretory granules or cytosolic proteins can also be used as tumor markers; among these, the chromogranin family is the one most commonly used (28).

Specific tumor markers. Peptide hormones are synthesized as precursors, which are cleaved in a sequence- and tissue specific manner to yield the biologically active peptides; however, their fine processing is usually deficient in NET cells (4). Therefore, direct measurement of these peptides, and when necessary of their precursors, establishes the diagnosis and occasionally also provides information regarding the size of the tumor (28). In addition, there are cases in which multiple hormone production is evident, which can also fluctuate throughout the course of the disease (15). The measurement of serum hormone concentrations can also be useful in the diagnosis of clinically nonfunctioning tumors in which the hormonal products may not be associated with clinical syndromes. More recently, the α- and β-subunits of human chorionic gonadotropin (α- and β-

Table II Common tumor markers and distribution of SS receptors in patients with GEP tumors, chromaffin cell tumors, and MTCs (15).

Tumor types	Specific serum tumor markers	Nonspecific serum tumor markers	SS receptors (positive scintigraphy with ^{111}Inoctreotide)
Thymus	SS, serotonin	CgA, NSE	50–80%
C-thyroid cells	Calcitonin, CGRP, ACTH, SS, serotonin	CgA, CEA	70–75%
Lung	GRP, CT, SS, POMC, ACTH, ADH, serotonin, β-hCG	CgA, NSE	80%
GI tract	Gastrin, CCK, GIP, VIP, motilin, glucagon, GRP, PP, GHRH, POMC, ACTH, serotonin	CgA, NSE, hCG	80–90%
Pancreatic islet cells	Insulin, gastrin, VIP, glucagon, SS, serotonin	CgA, NSE, hCG	60–95%
Ovary	Serotonin, hCG, PTHrP, POMC, CGRP	CgA, NSE	
Chromaffin cells	Noradrenaline, adrenaline, dopamine, POMC, calcitonin, neuropeptide Y, neurotensin, SS	CgA, NSE	85–95%
Adenocarcinomas with NE differentiation	POMC, CGRP	CgA, NSE	20–35%

POMC, Proopiomelanocortin; GIP, gastric inhibitory peptide.

hCG) have been shown to be markers of nonfunctioning GEP tumors, as well as MTC and small-cell lung carcinoma (SCLC) (4) (*Table II*).

Nonspecific tumor markers. In addition to specific hormones secreted by NE cells, other proteins that exert regulatory activities on the packaging, processing, and secretion of hormones are increasingly recognized as NET markers (4, 17). Chromogranins belong to a unique family of secretory chromogranin and secretogranin proteins. CgA, CgB, and CgC form a group of acidic monomeric soluble proteins that are localized within secretory granules in which they are co-stored and co-secreted with the locally present peptides. Chromogranin A (CgA) is the granin mostly used in clinical practice, although the other chromogranins are relevant, particularly as CgA-negative, but CgB-positive tumors are increasingly being recognized (29). Several assays for the measurement of intact CgA and the different cleavage products have been developed using either monoclonal or polyclonal antibodies, and thus exhibiting substantial differences in sensitivities and specificities.

Chromogranin A determination for diagnosis and follow-up in patients with gastroenteropancreatic endocrine tumors (GEP-ET) and MEN-I is considered the standard of care in many institutions. Although the absolute value of a single measurement of CgA is not a determinant of tumor bulk nor the presence or absence of metastasis, the trend in serial CgA levels over time has been proven to be a useful predictor of tumor growth. Changes in CgA levels of more than 25% over baseline are considered significant (30).

Serial measurements (every 3 to 6 months) of CgA levels in blood can be used to monitor the progression of a variety of gut-derived NETs. Serum CgA level is also an effective tumor marker in patients with pheochromocytoma. Increased levels strongly correlate with tumor mass. The concordance between CgA level and the results of iodine-131 meta-iodobenzylguanidine (^{131}I-MIBG) scintigraphy is high. A CgA level in the reference range is highly predictive of normal scintigraphy findings.

CgA levels may also be elevated in several other endocrine and nonendocrine diseases. It is well known that drugs that suppress gastric acid secretion can increase gastrin levels. Proton-pump inhibitors (PPI) are extensively used to treat patients with ZE syndrome, gastroesophageal reflux disease (GERD) or acid-peptic disease, but their long-term use can cause significant increases in gastrin levels and cause hypertrophy of the EC cells of the stomach. Enterochromaffin-like (ECL) cell hyperplasia secondary inhibitors of acid secretion, atrophic gastritis, and infection with *Helicobacter pylori* are common conditions leading to hypergastrinemia. These ECL cells are the precursor cells for the development of gastric carcinoids. Renal insufficiency and severe hypertension have been associated with increases in CgA levels. Although antihypertensive drugs do not commonly interfere with the analysis of CgA levels, some false-positive results occur in the presence of renal impairment, hypergastrinemia, corticosteroid therapy, and the use of PPI. CgA has a circadian rhythm unrelated to plasma catecholamines; thus, collection of blood for serial measurement of CgA levels should be done at approximately the same time of day (29).

The »value« of CgA for diagnosis and follow-up of NETs has a sensitivity of 62.9% with specificity of 98.4%; levels are higher in secreting versus nonsecreting tumors (7% vs. 45%) and are related to the extent of metastases. In nonsecreting tumors, the positive predictive value for the presence of metastases is 100%, but the negative predictive value is only 50%. In MEN-I, a high value predicts the presence of a pancreatic tumor with 100% specificity, but

the sensitivity is only 59%. During follow-up, the concordance of tumor growth and CgA is 80%, better than that with serotonin (81% vs 54%). Thus, owing to its high specificity, CgA determination may help to discriminate the endocrine character of an NET and to establish a pancreatic tumor in MEN-I syndrome. Serial measurements are also useful for evaluating response to treatment.

In contrast to CgA alone, PP sensitivity for NETs was approximately 50%, but combining the two markers increased sensitivity for all tumors to greater than 95%. Nonetheless, all evidence points to the combined measurement of the following markers: CgA, PP, Gastrin, and Gastric pH. These measurements are a very effective means of discovering a NET, identifying its probable site of origin, and monitoring response to intervention. In carcinoid tumors, neurotensin is elevated in 43% of patients, substance P in 32%, motilin in 14%, somatostatin in 5%, and vasoactive intestinal peptide (VIP) rarely (31).

Synaptophysin and NSE are present diffusely in the cytoplasm of NETs, so they are consistently positive in most NETs. NSE is only present in neurons and NE cells and can also serve as a circulating marker for NETs. NSE is most frequently elevated in patients with small-cell lung carcinoma (SCLC) (74%) but has also been found to be elevated in 30–50% of patients with carcinoids, MTC, islet cell tumors, and pheochromocytomas (32). Elevated levels of NSE are also roughly correlated with tumor size, although the specificity is lower than that of CgA; however, the combination of both CgA and NSE has a higher sensitivity than either parameter separately. Some oncogenic proteins are not specific for NETs but are frequently synthesized in these tumors, i.e., carcinoembryonic antigen (CEA) in MTC (33, 34).

Tumor markers and stimulation tests. When patients present with a high clinical suspicion of a functional syndrome but with normal basal measurements of specific tumor markers, a dynamic test can be used to increase sensitivity. Although the rationale of employing such tests has recently been questioned, several dynamic tests have traditionally been used (35).

Amine and peptide receptor expression and visualization

The demonstration of the presence of amine uptake mechanisms and a high density of peptide receptors on several NETs, as well as their metastases, has been used for both diagnosis and monitoring of these tumors.

Metaiodobenzylguanidine (MIBG) is a guanidine derivative that exploits the specific type 1 amine uptake mechanism at the cell membrane and the subsequent uptake from the cytoplasm and storage within the intracellular storage vesicles. It shows little binding to postsynaptic receptors and has minimal or no intrinsic pharmacological effect. MIBG localizes to adrenomedullary tumors, hyperplastic adrenal medulla and, to a lesser degree, in the healthy adrenal medulla. In addition, several other NETs including carcinoids and MTC exhibit this specific uptake mechanism and can thus accumulate MIBG (36).

Somatostatin (SS) is a 14-amino acid peptide that is widely expressed throughout the central nervous system as well as in peripheral tissues including the endocrine pancreas, gut, thyroid, adrenals, and kidneys (15). SS acts mostly as an inhibitory factor on neurotransmission, intestinal mobility, absorption of nutrients and ions, vascular contractility, and cell proliferation (36). Owing to its short half-life (1–2 min), many SS long-acting analogs have been synthesized, among which octreotide and lanreotide are the ones most commonly used in clinical practice. These analogs are cyclic octapeptides that have a more prolonged half-life (1.5–2 h), and thus, biological activity. The biological effects of SS are mediated by five specific SS receptors (1–5) that all bind the native peptide but show major differences in their affinities for SS analogs; the currently used analogs exhibit a very low affinity for SS receptors 1 and 4, but bind with high affinity to SS receptors 2 (predominantly) and 5, and with moderate affinity to SS receptor 3 (37, 38). Each receptor subtype is coupled to multiple intracellular transduction pathways, but all five are functionally coupled to inhibition of adenylate cyclase and decreased calcium influx, and thus generally inhibit hormonal secretion and intestinal mobility. SS also inhibits the proliferation of both normal and tumoral cells as a result of hypophosphorylation of the retinoblastoma gene product and G1 cell cycle arrest (39). The antiproliferative effects of SS can also result from apoptosis through SS receptor 3 induced by p53 and Bax. There is a close correlation between the presence of SS receptor 2 mRNA, tracer uptake using SS receptor autoradiography, and the therapeutic response to SS analog treatment (15). In addition, other small peptidic receptors that are expressed in cell membranes of NE tissues include vasointestinal peptide (VIP), bombesin, cholecystokinin (CCK), gastrin and/or substance P. Labeled analogs/peptides can also be used as markers for putative receptors for *in vivo* tumor visualization (40).

Serotonin. The rate-limiting step in carcinoid tumors for the synthesis of serotonin is the conversion of tryptophan into 5-HTP, catalyzed by the enzyme tryptophan hydroxylase. In midgut tumors, 5-HTP is rapidly converted to serotonin by the enzyme aromatic amino acid decarboxylase (dopa-decarboxylase). Serotonin is either stored in the neurosecretory granules or may be secreted directly into the vascular compartment. Most of the secreted serotonin is taken up by platelets and stored in their secretory granules. The rest remains free in the plasma, and circulating serotonin is then largely converted into the urinary metabolite 5-hydroxyindoleacetic acid (5-HIAA) by the enzymes

monoamine oxidase and aldehyde dehydrogenase. These enzymes are abundant in the kidney, and the urine typically contains large amounts of 5-HIAA.

In patients with foregut tumors, the urine contains relatively little 5-HIAA, but large amounts of 5-HTP. It is presumed that these tumors are deficient in dopadecarboxylase; this deficiency impairs the conversion of 5-HTP into serotonin, leading to 5-HTP secretions into the vascular compartment. Some 5-HTP, however, is converted to serotonin and 5-HIAA, producing modest increases in levels of these metabolites. The normal range for 5-HIAA secretion is 13 to 52 μmol per 24 hours, and the quantitation of serotonin and all of its metabolites usually permits the detection of 84% of patients with carcinoid tumors. No single measurement detects all cases of carcinoid syndrome, although the urine 5-HIAA appears to be the best screening procedure. Other peptides involved include substance P, neuropeptide K, pancreatic polypeptide (PP), and CgA (30).

Carcinoid tumors have been reported in a wide range of organs, but most commonly involve the gastrointestinal tract and bronchopulmonary system.

Bronchopulmonary Carcinoid Tumors

Pulmonary carcinoid tumors account for 1–2% of all lung malignancies in adults and approximately 25–30% of all carcinoid tumors (41). In a recent review of data from the SEER registry, Modlin et al. (42) demonstrated annual incidence rates of bronchial carcinoids of 0.52 and 0.89 per 100,000 population in white males and females, respectively (the corresponding values for black males and females were somewhat higher, at 0.39 and 0.57, respectively). In 2004, the WHO devised a new classification for bronchopulmonary carcinoids based on a spectrum of clinicopathologic entities ranging from hyperplastic neuroendocrine cell lesions (carcinoid tumorlets, diffuse idiopathic pulmonary neuroendocrine cell hyperplasia [DIPNECH]) to high-grade NETs (small cell carcinoma and large cell neuroendocrine carcinoma) (42).

Gastroenteropancreatic Tumors

Endocrine tumors of the gastroenteropancreatic (GEP) axis (involving the gastrointestinal [GI] system, stomach, and pancreas) are comprised of cells capable of amine precursor uptake and decarboxylation, hence the prior name »APUDomas.« In some cases, multiple peptides or hormones are responsible for symptoms, and several organs and/or multiple tumors may be involved in the disease state, confounding the clinical diagnosis. To facilitate the diagnostic process, we can classify GEP syndromes according to their secretory products and the clinical disorder they produce (43).

Carcinoid, gastrinoma, insulinoma, somatostatinoma, glucagonoma, and waterydiarrhea (WDHHA) syndromes are described as individual syndromes according to their secretory hormones and peptides. Distinguishing the signs and symptoms of each syndrome will further aid the diagnosis.

Carcinoid Tumors and the Carcinoid Syndrome

Carcinoid tumors are the most commonly occurring gut endocrine tumors. The prevalence of carcinoids is about 50,000 cases in any 1 year in the United States. The incidence is estimated to be approximately 1.5 cases per 100,000 of the general population (i.e., approximately 2500 new cases per year in the United States). Carcinoids may, however, occur in the bronchus, pancreas, rectum, ovary, lung, and elsewhere. The tumors grow slowly and often are clinically silent for many years before metastasizing. They frequently metastasize to the regional lymph nodes, liver, and, less commonly, to bone. The likelihood of metastases relates to tumor size. The incidence of metastases is less than 15% with a carcinoid tumor smaller than 1 cm but rises to 95% with tumors larger than 2 cm (30, 44) (*Table III*).

The carcinoid syndrome occurs in less than 10% of patients with carcinoid tumors. It is especially common in tumors of the ileum and jejunum (i.e., midgut tumors) but also occurs with bronchial,

Table III Common secretory products of carcinoid tumors (44).

Biogenic amines	Peptides
5-hydroxytryptamine (5-HT)	Atrial natriuretic peptide (ANP)
5-hydroxyindoleacetic acid (5-HIAA)	Chromogranins A/C
5-hydroxytryptophan (5-HTP)	α1-antitrypsin
Dopamine	Neurotensin
Histamine	Vasoactive intestinal polypeptide (VIP)
	Pancreatic polypeptide (PP)
Tachykinins	Motilin
Kallikrein	Neuropeptide K
Other	Human chorionic gonadotropin α/β
Prostaglandins	Somatostatin
	Substance P

ovarian, and other carcinoids. Tumors in the rectum (i.e. hindgut tumors) rarely occur in the carcinoid syndrome, even those that have widely metastasized. The peak incidence occurs in the sixth and seventh decades of life, but carcinoid tumors have also been reported in patients as young as 10 years of age and in those in their ninth decade.

Gastric Carcinoid

Originating from the histamine-containing enterochromaffinlike (ECL) cells of the embryologic foregut, gastric carcinoid tumors represent approximately 1.8% of all gastric neoplasms and approximately 7% of all carcinoids (44, 45). Because they are most often discovered incidentally during endoscopy, the incidence of gastric carcinoid tumors has increased in recent years as endoscopic technology continues to improve both technologically and diagnostically.

Carcinoids of the stomach are generally divided into three distinct groups based on their clinical and histological characteristics.

There are three types of gastric carcinoid tumors:

Type 1 gastric carcinoids are associated with achlorhydria, high gastrin levels, and multiple, small, relatively nonaggressive tumors. These tumors are more common in patients with achlorhydria accompanied by pernicious anemia and vitamin B12 deficiency, in which there is loss of gastric acid secretion causing impairment of the normal restraint mechanism suppressing gastrin production. *Type 2* gastric carcinoids are associated with elevated gastric acid, high gastrin levels, and the Zollinger-Ellison (ZE) syndrome. These tumors are larger and have a higher propensity to metastasize than type 1 carcinoids of the stomach. *Type 3* gastric carcinoids are much larger than types 1 and 2 and have a high propensity to metastasize.

The major clinical manifestations of carcinoid tumors include the following: cutaneous flushing (84%), GI hypermotility with diarrhea (70%), heart disease (37%), bronchial constriction/wheezing (17%), myopathy (7%) and abnormal increase in skin pigmentation (5%).

Common amines and peptides produced by carcinoids that cause symptoms are as follows: serotonin, histamine and substance P (31).

Insulinoma

The classic description of insulinoma is of Whipple's triad, which includes symptoms of hypoglycemia with a low blood glucose concentration relieved by the ingestion of glucose. These tumors are most commonly benign (90%) and can be located anywhere within the pancreas. Insulinomas are associated with a memory rule known as »the rule of tens«, which refers to the following characteristics: 10% are malignant; 10% are ectopic; and 10% are related to the MEN-I syndrome. Removal of the tumor, which is invariably in the pancreas, is curative in more than 90% of cases.

The standard diagnostic test remains a 72-hour fast while the patient is closely observed. More than 95% of cases can be diagnosed based on their response to this test. Serial glucose and insulin levels are obtained every 4 hours over the 72-hour period until the patient becomes symptomatic. When symptoms occur, obtain insulin, glucose, and C-peptide levels. A fasting insulin level of greater than 24 μU/mL is found in approximately 50% of patients with insulinoma. Measurements of proinsulin and C-peptide have proven to be valuable in patients suspected of having organic hypoglycemia. Normally, the circulating proinsulin concentration accounts for less than 22% of the insulin immune-reactivity, but a proinsulin level greater than 40% is highly suspicious for a malignant islet cell tumor (31).

Glucagonoma Syndrome

In 1966, McGavran and colleagues (44) called attention to a syndrome that included acquired diabetes and glucagon-producing tumors. Because these tumors usually were accompanied by a very characteristic skin rash, the syndrome is also known as the 4D syndrome, which stands for dermatosis, diarrhea, deep venous thrombosis (DVT), and depression.

In a study of 1366 consecutive adult autopsies, a tumor frequency of 0.8% was found. All tumors were adenomas, and all contained histochemically defined glucagon cells. None of the tumors had been suspected during life. Although these adenomas contained glucagon, it is not known whether they were overproducing or even secreting glucagon. The incidence *in vivo* is probably 1% of all NETs.

In patients with glucagonomas, fasting plasma glucagon concentrations may be as high as 2100 ± 334 pg/mL. These levels are markedly higher than those reported in normal, fasting subjects (i.e., <150 pg/mL) or in those with other disorders causing hyperglucagonemia, including diabetes mellitus, burn injury, acute trauma, bacteremia, cirrhosis, renal failure, or Cushing's syndrome, in which fasting plasma glucagon concentrations often are elevated but remain less than 500 pg/mL (15, 31).

Somatostatinoma

Somatostatin (somatotropin release–inhibiting factor [SRIF]) is a tetradecapeptide that inhibits numerous endocrine and exocrine secretory functions. Almost all gut hormones that have been studied are inhibited by SRIF, including insulin, PP, glucagon, gastrin, secretin, gastric inhibitory polypeptide (GIP), and motilin. In addition to inhibition of the endocrine secretions, SRIF has direct effects on a number of

Table IV Comparison of Pancreatic and Intestinal Somatostatinoma (30).

Pancreatic somatostatinoma	Intestinal somatostatinoma
SLI 50x higher than normal (range, 1–250 m) 75% of patients have diabetes Tumors are large and destroy part of pancreas 59% of patients have gallbladder disease Diarrhea and steatorrhea are common Weight loss in one third of patients Acid secretion inhibited in 87% of patients Café-au-lait spots Neurofibromatosis Paroxysmal hypertension	SLI slightly elevated or normal 11% of patients have diabetes Tumors are relatively small 27% of patients have gallbladder disease Diarrhea and steatorrhea are rare Weight loss in one fifth of patients Acid secretion inhibited in 87% of patients

target organs. For example, it is a potent inhibitor of basal and PG-stimulated gastric acid secretion. It also has marked effects on GI transit time, intestinal motility, and absorption of nutrients from the small intestine. The major effect in the small intestine appears to be a delay in the absorption of fat and reduced absorption of calcium (*Table IV*).

Somatostatin has been found in many tissues outside the GI tract. Prominent among those are the hypothalamic and extrahypothalamic regions of the brain, the peripheral nervous system (including the sympathetic adrenergic ganglia), and the C cells of the thyroid gland. Not surprisingly, therefore, high concentrations of somatostatin have been found in tumors originating from these tissues. Some patients exhibited the clinical somatostatinoma syndrome. Elevated plasma SS concentrations have also been reported in patients with small cell lung cancer. Pheochromocytomas and catecholamine-producing extra-adrenal paragangliomas are other examples of endocrine tumors that produce and secrete somatostatin in addition to other hormonally active substances. One fourth of 37 patients with pheochromocytomas had elevated SLI levels.

The salient features of the somatostatinoma syndrome are as follows: diabetes, diarrhea/steatorrhea, gallbladder disease (cholelithiasis and dysmotility), hypochlorhydria and weight loss. The mean somatostatin-like immunoreactivity (SLI) concentration in patients with pancreatic somatostatinoma was 50 times higher than normal (range, 1–250 times). Intestinal somatostatinomas, however, present differently and have only slightly elevated or normal SLI concentrations (31, 45).

Ppoma

Pancreatic polypeptide (PP) was discovered in 1972 by Chance and colleagues (46). These authors discovered and purified a single protein peak from a crude insulin preparation. In mammals, 93% of the cells producing PP are located in the pancreas. Meal ingestion, cerebral stimulation, and hormone administration have dramatic effects on circulating levels of PP. The only physiologic effects of PP that are recognized in humans are the inhibition of gallbladder contraction and pancreatic enzyme secretion. The picture is complicated by the fact that mixed tumors, PP-cell hyperplasia in association with other functioning islet cell tumors, ductal hyperplasia of PP cells, nesidioblastosis, and multiple islet tumors producing PP also have been described, either alone or as part of the MEN-I syndrome (30).

VIPoma

VIPomas or vasoactive intestinal peptide-secreting tumors produce the Verner-Morrison syndrome or pancreatic cholera. Patients develop watery (secretory) diarrhea, hypokalemia, and achlorhydria (WDHA). The diarrheal output can be from 700 mL/day up to 8 L/day. Patients can also present with facial flushing, weight loss, dehydration, hyperglycemia, hypokalemia, hypochlorhydria, hypercalcemia, and abdominal pain. Extrapancreatic VIPomas are often located along the autonomic chain and in the adrenals, requiring an exploration of the retroperitoneum if no tumor is found in the pancreas. VIP levels greater than 170 pg/mL are documented with this tumor (30, 47).

Ghrelinoma

Ghrelin is a 28–amino acid acylated peptide related to the oxyntomodulin family of intestinal peptides. This peptide was isolated from the X/A-like neuroendocrine cells of the rat and human stomach. It is predominantly produced by the stomach but is also detectable in many other tissues: bowel, hypothalamus, pituitary, pancreas, co-segregating with pancreatic alpha cells and possibly with pancreatic beta cells.

Ghrelin is the first natural hormone in which a hydroxyl group on one of its serine residues is acylated by n-octanoic acid. This acylation is essential for binding to the GHS-R1a receptor, for the GH-releasing capacity, and also likely for its other actions. Although it has been found to co-segregate with glucagon and insulin by some authors, this is not consistent, and most would agree that its cell of origin in the pancreas constitutes a new cell type. Ghrelin regulates the following: energy balance, increased

appetite and food intake, modulation of insulin secretion negatively, exertion of a tonic inhibitory role on insulin secretion in animals and humans, suppressed by hyperglycemia and insulin, and may, in addition, have a direct role in glycogenolysis. The expression of ghrelin protein and/or mRNA has recently been identified in almost all gastric and intestinal carcinoids as well as pancreatic NETs (30, 47).

Conclusion

When a patient presents symptoms that lead to the suspicion of a gastrointestinal neuroendocrine tumor, determination of serum or plasma levels of peptide markers are employed. Recent data indicate that plasma levels of chromogranins can be used as 'screening markers' for the diagnosis also in patients with a relatively small tumor burden. In the further investigation, however, other tumor markers, including both specific and general markers, should be determined in order to assess what type of tumor and syndrome one is dealing with. As a rule, the whole battery of plausible tumor markers should be analyzed, once the presence of the tumor has been verified, since most of the tumors produce multiple peptides and later in the course a change of hormone profile might occur. Stimulatory tests still have a value because of a higher diagnostic accuracy than the determination of basal peptide levels in many cases. The search for tumor markers that could indicate a malignant potential and possibly also give prognostic information should continue. Clinical suspicion based on the presence of characteristic symptoms and/or syndromes may suggest the presence of an NET, which then requires assessment of specific or general tumor markers that currently offer high sensitivity in establishing the diagnosis and can also have prognostic significance.

Measurement of specific amines or hormones establishes the biochemical confirmation of a GEP tumor, whereas measurement of CgA appears to be a universal marker. Similarly, both urinary catecholamines and plasma metanephrine estimations are highly sensitive and specific for a chromaffin cell tumor, whereas basal or stimulated plasma calcitonin offers the highest diagnostic accuracy for the presence of MTC. Current histopathological and molecular techniques not only provide the diagnosis, but can also, to some extent, predict the biological behavior of the tumor. Molecular screening is gradually replacing other biochemical tests when a familial syndrome is suspected; in such cases, counseling is essential.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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