Biochemical Testing for Neuroendocrine Tumors

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Abstract: In this review, we focus on the use of biochemical markers for the diagnosis of neuroendocrine tumors and exclusion of conditions that masquerade as neuroendocrine tumors. In addition, we outline the use of biochemical markers for follow-up, response to intervention, and determination of prognosis. Previous publications have focused only on markers specific to certain tumor types, but the uniqueness of this chapter is that it presents a new approach ranging from biochemical markers that relate to symptoms to the use of markers that facilitate decision making with regard to optimizing the choices of therapy from the complex arrays of intervention, The sequence of presentation in this chapter is first to provide the usual view, that is, biochemical markers of each tumor type and thereafter the diagnosis of the underlying condition or exclusion thereof and finally the algorithm for their use from the clinical presentation to the suspected diagnosis and the biochemical markers to monitor progression and therapeutic choice. There is also a specific description of the properties of the most important biochemical markers and 2 complications, bone metastasis and carcinoid heart disease, from the biochemical point of view.

Key Words: biochemical tests, chromogranin A, pancreastatin, neurokinin, Ki-67

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Neuroendocrine tumors (NETs) are rare, usually slow-growing, neoplasms characterized by the ability to store and secrete different peptides and neuroamines.[1] Some of these substances cause a specific clinical syndrome.[2] It is important to be able to recognize from the clinical presentation the most useful markers to reduce time and costs and that way facilitate the diagnosis and make wise use of resources. Unfortunately, there is no "ideal neuroendocrine tumor marker,[3]" but according to the presentation, the sensitivity and specificity of each marker vary and it is generally possible to choose those of greatest value for each patient. In addition, it is important to recognize the contribution of each marker to diagnosis, follow-up of treatment response, or prognosis.

The biochemical markers are those hormones or amines secreted by the enterochromaffin (EC) cells from which these tumors are derived. Some of these are nonspecific to any tumor but, in contrast, are produced and secreted by most NETs; other biochemical markers are more specific to the type of tumor.

The incidence of NETs has risen to 40 to 50 cases per million, perhaps largely caused by better diagnosis than a change in the real incidence of the disease, but we still need more accurate and precise biochemical methods for trying to diagnose the presence of a NET as accounting for a symptom complex.

The natural history of this disease is invariably attended by a long history of vague abdominal symptoms, a series of visits to a primary care practitioner, and referral to a gastroenterologist, often with a misdiagnosis of irritable bowel syndrome (Fig. 1). These symptoms persist with a median latency to correct diagnosis of 9.2 years, by which time the tumor has metastasized, causing symptoms like flushing and diarrhea and progressing on its slow but relentless course until the patient dies. Clearly, a greater index of suspicion and a carcinoid tumor profile screen are warranted for all patients presenting with *traditional irritable bowel syndrome symptoms*. The diagnosis of metastases to the liver is generally more obvious but often still takes place only after a delay of many years. Even then, an incorrect diagnosis is not uncommon. Unless biopsy material is examined for the secretory peptides chromogranin (Cg), synaptophysin, or neuron-specific enolase (NSE), tumors may be labeled erroneously as adenocarcinoma, with a negative impact on physicians' attitudes regarding management and underestimation of prospects for survival.[5]

SPECIFIC BIOCHEMICAL MARKERS FOR EACH TUMOR TYPE

Each tumor, depending on the site of origin will be more prone to produce one or another hormone or peptide. In rare cases when the tumor is localized before the symptoms occur, then these biochemical markers (Table 1) will be useful to confirm the diagnosis, follow the progression or treatment response, and may even have prognostic value.

The historic classification of NETs into *foregut*, *midgut*, and *hindgut* carcinoid tumors (see later) still has some virtue even in the era of the recent World Health Organization classification of endocrine tumors.[7] Less than 10% of NETs are *functional*, that is, hormone secreting with an associated syndrome. Most NETs are *nonfunctional*, that is, no associated hormonal secretion.

In general, poorly differentiated NETs are nonfunctional and act as would an aggressive adenocarcinoma often presenting with advanced disease.

Foregut Carcinoid Tumors

Foregut carcinoid tumors occur in the thymus, bronchus, stomach, first portion of the duodenum, pancreas, and ovaries. These tumors produce less serotonin when compared with carcinoid tumors in the midgut and secrete the serotonin precursor 5-hydroxytryptophan (5-HTP). This is caused by a deficiency in dopa-decarboxylase, an enzyme that catalyzes the conversion of 5-HTP to serotonin (Fig. 2). However, after secretion, a small amount of 5-HTP is converted to 5-hydroxyindoleacetic acid (5-HIAA) and serotonin, so modest elevation of these metabolites can be found with foregut tumors. When all these metabolites are measured together, the sensitivity increases to 84%. Other products of foregut carcinoids are histamine, substance P (SP), neuropeptide K, pancreatic polypeptide (PP), and CgA[5]

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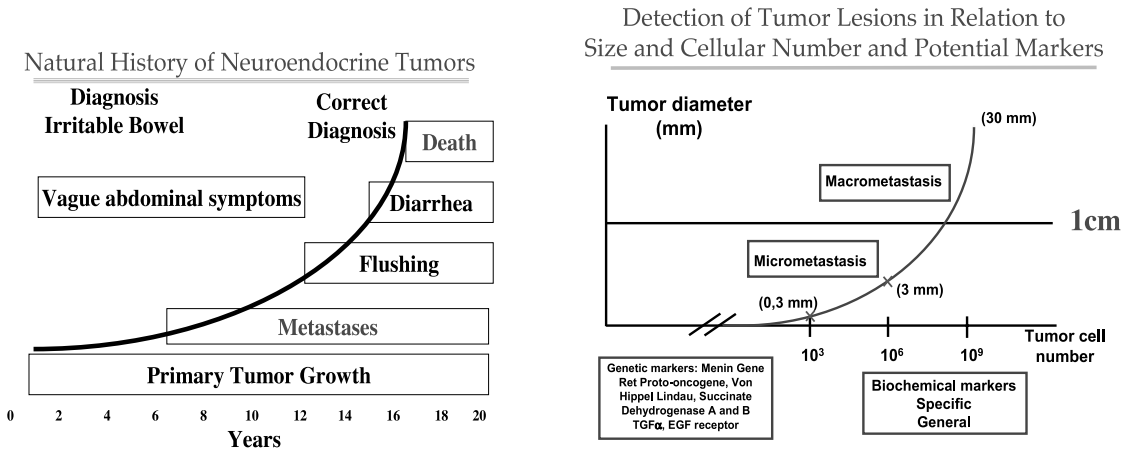

FIGURE 1. The natural history of NETs. Vague symptoms such as abdominal pain precede the diagnosis by a median of 9.2 years, and flushing and diarrhea, the major manifestations of carcinoid NETs, occur after the tumor has metastasized. On the right, the figure shows the relationship between tumor size and when the biochemical markers are positive when measured in blood, usually, after the tumor reaches a diameter of approximately 3 mm and contains about 1 million cells.[4]

(Fig. 3). A further point of interest is that a sex variation is present when a NET coexists with multiple endocrine neoplasia type 1 (MEN-I) syndrome; 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.[5]

Midgut Carcinoid

These tumors occur in the second portion of the duodenum, jejunum, ileum, and ascending colon. They are argentaffin positive on cytochemical staining. They produce huge amounts of serotonin, but the serotonin precursor 5-HTP is rarely produced. These tumors also secrete other vasoactive compounds such as kinins, prostaglandins, and SP.[5] Both functional and nonfunctional midgut NETs produce CgA.

Hindgut Carcinoid

Hindgut carcinoid tumors include those tumors of the transverse colon, descending colon, and rectum. They are argentaffin negative, rarely contain serotonin, rarely secrete 5-HTP or other peptides, and usually are silent in their presentation. Plasma CgA is usually elevated as may acid phosphatase.

Bronchus

Bronchopulmonary NETs comprise 20% of all lung cancers and up to 30% of all NETs.[9] The biochemical findings are

TABLE 1. Specific Biochemical Markers for Each Tumor Type[6]

Site	Tumor Type	Marker	Specificity
All		CgA and CgB	High
		PP, NSE, neurokinin, neurotensin	Intermediate
		HCG-α and HCG-β	Low
Thymus	Foregut carcinoid	ACTH	Intermediate
Bronchus	Foregut carcinoid, small-cell lung carcinoma	ACTH, ADH, serotonin, 5-HIAA, Histamine, GRP, GHRH, VIP, PTHrp	Intermediate Low
Stomach	Foregut carcinoid, gastrinoma, ghrelinoma	Histamine, gastrin Ghrelin	Intermediate Low
Pancreas	Gastrinoma, insulinoma, glucagonoma	Gastrin, insulin, proinsulin, glucagon, somatostatin	High
	Somatostatinoma, PPoma, VIPoma	C-peptide, neurotensin, VIP, PTHrp, calcitonin	Low
Duodenum	Gastrinoma, somatostatinoma	Somatostatin, gastrin	High
Ileum	Midgut carcinoid	Serotonin, 5-HIAA	High
		NKA, neuropeptide K, SP	Intermediate
Colon and rectum	Hindgut carcinoid	Peptide YY, somatostatin	Intermediate
Bone	Metastasis	bAP, N-telopeptide	High (blastic lesions), modest (lytic lesions)
		Vitamin D25, 1:25-OHD	Universal vitamin D deficiency
		PTH, PTHrp	Intermediate
Cardiac involvement	Carcinoid	BNP	Intermediate

Shows the specific biochemical markers used for each tumor and their specificity.
1:25-OHD indicates 25-hydroxyvitamin D; ADH, antidiuretic hormone; GRP, gastrin-releasing peptide; HCG, human chorionic gonadotropin.

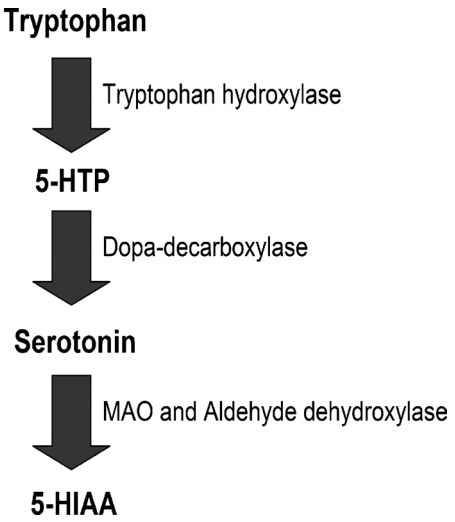

FIGURE 2. Synthesis of 5-HIAA. MAO indicates monoamine oxidase.

dependent on the histological type of bronchial NET. The typical carcinoid may present with increased plasma levels of CgA. When hormone-related symptoms are rarely present, plasma adrenocorticotropic hormone (ACTH), growth hormone–releasing hormone (GHRH), insulin-like growth factor I (IGF-I), urine cortisol, urine 5-HIAA, or histamine metabolites (U-methylimidazole acetic acid) may be elevated.[10]

Thymus

The overall age-adjusted incidence of thymic carcinoids is 0.01 per 100,000 per year. These tumors might be part of the MEN-I syndrome. These tumors may be similar to bronchial carcinoids in their biochemical profile.[10]

Stomach

There are different neuroendocrine cells in the stomach: G cells (antrum), D cells (corpus and antrum), EC-like (ECL) cells (corpus and fundus), D1 cells, EC cells, parietal cells, and X cells, which have different products and are prone to tumor formation. The gastric NETs are most likely to derive from ECL cells. They constitute up to 30% to 40% of the neuroendocrine cells of the stomach and release histamine.[11] Gastric NETs are divided into: type 1 (multiple, small, relatively nonaggressive tumors, associated with achlorhydria often in the presence of pernicious anemia, and high gastrin levels); type 2 (associated with high levels of gastric acid and gastrin [Zollinger-Ellison syndrome {ZES}], they are larger and more prone to metastasize than type 1 tumors); and type 3 tumors (the largest gastric NET with the highest metastasis rate, are sporadic, and usually present with normal gastric acid and gastrin levels).[5] Prolonged increased gastrin levels can produce ECL cell hyperplasia and subsequently the development of a gastric NET.[5]

With the discovery of the orexigenic gastric hormone, ghrelin, it has been known that its circulating levels rise before and decrease after a meal.[12] The physiological functions of this new gut hormone are being elucidated and a role in NETs is anticipated. Despite the frequent occurrence of ghrelin-immunoreactive cells in both the neoplastic parenchyma and the oxyntic mucosa, plasma total ghrelin concentrations do not increase above the reference range and therefore cannot be used as a clinical marker to identify ghrelin-expressing ECL cells, NETs, or ghrelin cell hyperplasia.[13]

Thus, there are 3 different biochemical markers useful for gastric NETs: (1) fasting serum gastrin (FSG) levels, which will be elevated in types 1 and 2 gastric NETs. Of great importance is to stop the use of proton pump inhibitors (PPIs), if possible, 7 days before the test to avoid false-positive results (patients may still need acid suppression with, eg, histamine type 2 receptor antagonists).[5] Histamine, the main secretory product of the ECL cells, and ghrelin have been shown to be of limited value as serum markers. Plasma CgA is often significantly elevated particularly in patients with types II and III gastric carcinoid.

Pancreas

Neuroendocrine tumors of the pancreas are frequently (40%–50%) nonfunctioning or secrete peptides with low biological impact such as PP or neurotensin. The functioning tumors are named according to their secretory product: insulinoma, gastrinoma (nb. most gastrinomas are found in the duodenum), VIPoma, glucagonoma, and somatostatinoma. The first 2 (insulinomas and gastrinomas) are the most frequent functioning pancreatic NETs.[1]

Insulinomas

There are 6 criteria for the diagnosis of insulinomas: documented blood glucose levels less than or equal to 2.2 mmol/L (≤40 mg/dL), concomitant insulin levels greater than or equal to 6 μU/mL (≥36 pmol/L, ≥3 μU/mL by immunochemiluminometric assay), C-peptide levels greater than or equal to 200 pmol/L, proinsulin levels greater than or equal to 5 pmol/L, β-hydroxybutyrate levels less than or equal to 2.7 mmol/L, and absence of sulfonylurea (metabolites) in the plasma and/or urine. Further controlled testing includes the 72-hour fast, which is the criterion standard for establishing the diagnosis of insulinoma.[14] Actually, 98% of patients with insulinomas will develop symptomatic hypoglycemia within 72 hours.[1] When the patient develops symptoms and the blood glucose levels are less than or equal to 2.2 mmol/L (≤40 mg/dL), blood should also be drawn for C-peptide, proinsulin, and insulin. Failure of appropriate insulin suppression in the presence of hypoglycemia substantiates an autonomously secreting insulinoma.[14] It has been proposed that the sensitivity of the 48-hour fasting test is between 94.5% and 95.7% and should be enough for diagnosis of insulinoma instead of the 72-hour fast test.[15,16] In a case of suspected insulinoma, it is important to keep in mind the possible differential diagnoses: nesidioblastosis, noninsulinoma pancreatogenous hypoglycemia syndrome (see discussion later), and multiple adenomas.

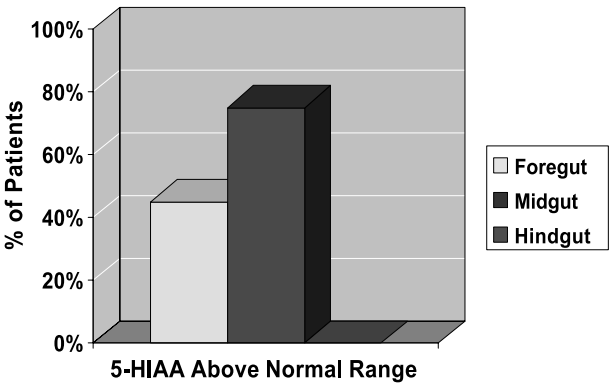

FIGURE 3. Percentage of patients with carcinoid tumors with elevated 5-HIAA.[8]

Gastrinomas

For gastrinomas (ZES), 2 measurements are critical: FSG and basal gastric acid output. Fasting serum gastrin alone is not enough because of its lack of specificity, making it impossible to distinguish hypergastrinemia caused by a gastrinoma from that caused by achlorhydric states. For these measures, a washout period from PPI treatment of 1 to 4 weeks is recommended.[17] If the FSG is greater than or equal to 1000 ng/L (pg/mL) and the gastric pH is less than 2.5, the diagnosis is established[1] if the patient is normocalcemic, free of pyloric obstruction, and has a normal renal function.[18] The 2006 European Neuroendocrine Tumor Society guidelines had cutoff values of greater than 10-fold elevation for FSG and gastric pH less than or equal to 2.[17] In case that the FSG values are not high enough to make a definitive diagnosis, then a provocative test should be done. Following administration of secretin after an overnight fast, serum for estimation of gastrin levels are collected during fasting and 2, 5, 10, 15, and 30 minutes after the secretin bolus. In healthy people, the increase in gastrin is not higher than 50% over the baseline level; in the presence of a gastrinoma, the increase is greater than 100 ng/L above the baseline levels, which will also distinguish patients with hypergastrinemia from achlorhydric states (ie, type 1 gastric NETs, use of PPIs, pernicious anemia, atrophic gastritis), who do not respond to the administration of secretin, unlike patients with a gastrinoma.[5]

Miscellaneous Pancreatic NETs

For VIPomas, glucagonomas, somatostatinomas, and PPomas, the biochemical markers are vasoactive intestinal peptide (VIP), glucagon, somatostatin, and PP, respectively.[1] For every pancreatic NET, always screen for MEN-I syndrome, measuring ionized calcium, serum parathyroid hormone (PTH), and prolactin.[19] Biochemical screening for pancreatic NETs, in the presence of suspected MEN 1 syndrome, should include gastrin, insulin/proinsulin, PP, glucagon, and CgA, which together have a sensitivity of approximately 70% that can be increased if α- and β-hCG subunits, VIP, postprandial gastrin, and PP measurements are added.[18]

Duodenum

Five types of duodenal NETs can currently be distinguished.

Duodenal Gastrinomas

Duodenal gastrinomas are either sporadic or associated with MEN-1 and are combined with a ZES. In both situations, these gastrinomas are usually not bigger than 1 cm and are located predominantly in the upper part of the duodenum. If associated with MEN-1, they are usually multiple, in contrast to sporadic gastrinomas.

Duodenal Somatostatin-Producing Tumors

Duodenal somatostatin-producing tumors account for approximately 15% of all duodenal NETs. Their preferential localization is in the region of the papilla of Vater or periampullary. They are not associated with any hormonal syndrome but often occur in patients with neurofibromatosis type 1. In this situation, a bilateral pheochromocytoma may simultaneously occur.

Nonfunctioning Duodenal NETs

Nonfunctioning duodenal NETs usually consist of serotonin-producing cells. Occasionally, there are also tumors with gastrin- or calcitonin-positive cells. The prognosis of this group of nonfunctioning tumors is much more favorable than ZES-associated gastrinomas or ampullary somatostatin-producing tumors. Metastases are not to be expected until the tumor extends beyond the submucosa.

Duodenal Gangliocytic Paragangliomas

Duodenal gangliocytic paragangliomas occur in the vicinity of the papilla of Vater. Although the tumors are often greater than 2 cm and infiltrate into the muscularis propria, they generally follow a benign course.[20] Thus, the most common products of duodenal NETs are gastrin and somatostatin, and these are the markers considered for diagnosis.

Poorly Differentiated Duodenal Carcinomas

Poorly differentiated duodenal carcinomas occur primarily in the region of the papilla of Vater. They are hormonally inactive. At the time of diagnosis, advanced metastasis into the regional lymph nodes and the liver has usually occurred.

Small Bowel

These are the most frequent gastrointestinal (GI) tract NETs, especially appendiceal tumors. Most are well differentiated and grow slowly. Rarely, they are less differentiated with fast growth and poor prognosis. Symptoms are atypical; diagnosis is often accidental. In 4% to 10% of patients, typical symptoms of carcinoid syndrome are present. The biochemical markers that should be measured in these patients are CgA and urinary excretion of 5-HIAA, which is used for the diagnosis and monitoring of the disease.[21] Some clinicians also measure blood serotonin, which occasionally may be the only elevated marker.

Colon and Rectum

Most of the tumors from the distal colon and rectum are nonfunctioning hindgut carcinoids. It is possible to measure peptide YY (PYY), which is a naturally occurring gut hormone with mostly inhibitory actions on multiple tissue targets, which has been identified in several carcinoid tumors; a decreased expression of PYY may be relevant to the development and progression of colon adenocarcinoma.[22]

Pheochromocytoma

Pheochromocytoma is a rare catecholamine-producing tumor usually localized in the adrenal gland that arises from neuroendocrine chromaffin cells of the adrenal medulla. Guller et al[23] published in 2006 that the tests of choice to establish the diagnosis of pheochromocytomas are urinary normetanephrine and platelet norepinephrine, with sensitivities of 96.9% and 93.8%, respectively. In a study conducted in Switzerland by Giovanella et al[24] in 2006, plasma metanephrines and CgA showed 95% sensitivity with comparable high specificity and diagnostic accuracy (96% and 96% for CgA, 94% and 95% for metanephrine, respectively). If both were used, then sensitivity increases to 100%. The difference found between these 2 markers is that only CgA was correlated with tumor mass.[24] In 2008, Bilek et al[25] also studied the use of CgA for pheochromocytoma and found that it is a great marker for following response to treatment and that the levels of CgA were correlated with the size and the malignancy of the tumor.

Paragangliomas

Paragangliomas are NETs that arise from the paravertebral axis. Sympathetic paragangliomas usually hypersecrete catecholamines and are localized in the thorax, abdomen, or pelvis. Parasympathetic paragangliomas are nonsecretory tumors usually localized in the head and neck area.[26]

Diagnosis of paragangliomas is similar to that of pheochromocytomas because these 2 entities only differ in their places of origin, extraadrenal versus adrenal, respectively. Algeciras-Schimnich et al[27] suggested that when plasma

fractionated metanephrines are measured and values are not 4-fold above upper normal limit, then serum or plasma CgA and urine fractionated metanephrines should be measured to confirm the diagnosis. After surgery, the biochemical follow-up should be done 1 to 2 weeks later with 24-hour urine fractionated catecholamines and metanephrines; if normal, complete resection is claimed, but if it is persistently elevated, a second primary or occult metastasis should be suspected and investigated. Young[28] also proposed an annual biochemical testing follow-up for life, with 24-hour urinary excretion of fractionated catecholamines and metanephrines or plasma fractionated metanephrines; and only in the case of elevated levels, imaging follow-up is then considered.

All patients with paragangliomas should be considered for genetic testing with *VHL*, *RET*, *NF1*, *SDHD*, *SDHB*, and *SDHC* genes.[26] If positive, then genetic testing of first-degree relatives should be suggested and genetic counseling should be offered. First-degree relatives should always undergo biochemical testing with 24-hour urine fractionated metanephrines and catecholamines.[28]

Medullary Carcinoma of the Thyroid

Medullary carcinomas of the thyroid (MCT) originate from the parafollicular cells of the thyroid, which secrete calcitonin. These represent 4% to 10% of all thyroid neoplasms.[29] The MCT can present as 2 different forms, sporadic (75%) or inherited (25%), and the last can be either isolated or part of the MEN-2 syndrome.[30] A germline autosomal-dominant mutation in the RET proto-oncogene, which encodes for a transmembrane tyrosine kinase receptor, predisposes individuals to develop MCT. Screening for RET germline mutations has allowed for early and accurate diagnosis of patients at risk for developing MCT.[31,32]

The most common clinical presentation of MCT is a thyroid nodule, either singly or as a multinodular goiter. Usually, no other manifestations are present unless the tumor is already in stage IV (metastatic disease), when diarrhea and/or flushing can present.[33]

The calcitonin-secreting nature of these tumors and the fact that calcitonin is almost exclusively secreted by C cells explain why this hormone is the preferred biochemical marker for the diagnosis and follow-up of this disease. Besides, it has been shown that calcitonin measurement is more sensitive than fine-needle aspiration for the diagnosis of MCT.[33] A 10-year survival of only 50% for MCT patients is reported in several series. The only possible means to improve the cure and survival rate of these patients consists of early diagnosis and early surgical treatment while the MCT is still intrathyroid.[33] Costante et al[29] reported in 2007 that the positive predictive value of basal calcitonin levels greater than 100 pg/mL is 100% for MCT, and if pentagastrin (PG) stimulation test is used, calcitonin levels greater than 100 pg/mL had a positive predictive value of 40%, but below this cutoff value, the false-positive results increase until the positive predictive value of basal calcitonin levels greater than 20 pg/mL is less than 25%. Cohen et al[30] found that calcitonin levels are not only useful as a diagnostic marker, but they are also correlated with tumor size and metastasis, which gives some prognostic value to this hormone. When levels are less than 50 pg/mL preoperatively, the normalization of calcitonin levels postoperatively is found in 97.8% of the patients.[30] Scheuba et al[34] recently published that values of basal calcitonin greater than 64 pg/mL or stimulated calcitonin levels greater than 560 pg/mL had a sensitivity of 100% for MCT. Increased calcitonin levels can be observed also in parafollicular C-cell hyperplasia (CCH) and other extrathyroidal conditions. The PG test is used to distinguish MCT from CCH because it is thought that the response to this stimulus is typical of pathological thyroid C cells. The cutoff value of calcitonin response between patients with MCT and CCH remains to be established.[35] Pentagastrin stimulation test is no longer available in the United States, but it consists of the intravenous injection of 0.5 μg/kg body weight of PG and measurements of calcitonin at 0, 1, 2, 5, and 10 minutes after the injection; healthy people do not experience an increase in calcitonin greater than 200 pg/mL after the administration of PG.[5] Instead, a stimulation test can also be done with intravenous calcium infusion.

C-Cell Hyperplasia

This entity has been proposed to be a precancerous lesion that eventually transforms into MCT. Schley, Shin, Perry, and Vinik submitted a study where 3 cases are reported in which patients presented with flushing, abdominal pain, diarrhea, and facial telangiectasia, resembling carcinoid syndrome, but the only biochemical abnormalities were elevated calcitonin levels and positive PG and calcium infusion tests. Venous sampling was performed, and it localized the overproduction of calcitonin to the thyroid and histology showed CCH. After thyroidectomy, symptoms resolved and calcitonin levels returned to normal. They proposed that the condition might be a gene mutation, but so far, the site has not been identified, considering that RET proto-oncogene was negative in the 3 patients. These findings (Schley et al, unpublished data, 2009) suggest that every case of flushing and diarrhea should have a calcitonin measurement, considering CCH or MCT in the differential diagnosis.

MEN Syndromes

This entity is classified as either MEN-1 or MEN-2. They are both inherited in an autosomal-dominant pattern. Mutations on the MEN-1 tumor suppressor gene (inactivated) or the RET proto-oncogene (activated) are found in MEN-1 and MEN-2, respectively.[37]

Multiple Endocrine Neoplasia Type 1

Multiple endocrine neoplasia type 1 is characterized by hyperplasia and/or neoplasm of the parathyroid glands, enteropancreatic NETs, and pituitary adenomas. Some patients do not present with all these tumors, so it has been agreed upon that diagnosis is made when a patient presents with 2 of these concomitantly. To diagnose familial MEN-1 syndrome, a first-degree relative has to present at least one of the tumors previously mentioned.[38] Hyperparathyroidism occurs in about 90% of patients; endocrine pancreatic tumors in 60% of patients, usually they are small and nonfunctional, and the most common hormonally active ones are insulinomas or gastrinomas. Pituitary adenomas are present in 40% of patients, and in 60% of the patients, skin manifestations can also be present.[38,39] Genetic studies are available for MEN-1 syndrome; MEN-1 germline mutations are found in these patients, but their presence does not prompt any immediate intervention.[40] Piecha et al[38] proposed a recommendation for carriers of MEN-1 mutation to be screened biochemically every 1 to 3 years for hyperparathyroidism, prolactinoma, gastrinoma, insulinoma, and other enteropancreatic tumors.

Multiple Endocrine Neoplasia Type 2

This syndrome is subclassified into type 2A, 2B, and familial MCT, all sharing the presence of MCT; and they are all characterized by an activating germline mutation in the RET proto-oncogene, specific for each type and which can be identified in almost 100% of the patients with genetic testing. Once the genetic test demonstrates the mutation, a total thyroidectomy is mandatory either prophylactically in carriers or as

TABLE 2. The Clinical Presentations, Syndromes, Tumor Types, Sites, and Hormones[5]

Clinical Presentation	Syndrome	Tumor Type	Sites	Hormones
Flushing	Carcinoid	Carcinoid	Midgut/foregut	Serotonin, NKA, TCT, PP
			Adrenal medulla	GCRP, VIP
			Gastric	SP
Diarrhea	Carcinoid, WDHHA, ZES, PP, MCT	Carcinoid, VIPoma, gastrinoma, PPoma, MCT	As above, pancreas, mast cells, thyroid	As above, VIP, gastrin, PP, calcitonin
Diarrhea/steatorrhea	Somatostatin Bleeding GI tract	Somatostatinoma, neurofibromatosis	Pancreas Duodenum	Somatostatin
Wheezing	Carcinoid	Carcinoid	Gut/pancreas/lung	SP, CGRP, serotonin
Ulcer/dyspepsia	ZES	Gastrinoma	Pancreas/duodenum	Gastrin
Hypoglycemia	Whipple triad	Insulinoma, sarcoma, hepatoma	Pancreas, retroperitoneal	Insulin, IGF-1, IGF-11
			Liver	
Dermatitis	Sweet syndrome	Glucagonoma	Pancreas	Glucagon
	Pellagra	Carcinoid	Midgut	Serotonin
Dementia	Sweet syndrome	Glucagonoma	Pancreas	Glucagon
Diabetes	Glucagonoma	Glucagonoma	Pancreas	Glucagon
	Somatostatin	Somatostatinoma	Pancreas	Somatostatin
DVT, steatorrhea, cholelithiasis	Somatostatin	Somatostatinoma	Pancreas	Somatostatin
Neurofibromatosis			Duodenum	
Silent, liver mets	Silent	PPoma	Pancreas	PP
Acromegaly	Acromegaly, gigantism	NET	Pancreas	GHRH
Cushing	Cushing	NET	Pancreas	CRH, ACTH
Pigmentation	Pigmentation	NET	Pancreas	MSH
Anorexia, nausea, vomiting, abdominal pain	Hypercalcemia	NET	Pancreas	PTHrp

Summarizes our approach based upon the clinical presentation, the tumor type, their sites of origin and the possible means of diagnosis, and the biochemical markers that should be measured.

CRH indicates corticotropin-releasing hormone; MSH, melanocyte-stimulating hormone; WDHHA, watery diarrhea, hypokalemia, hyperchlorhydria, and acidosis.

treatment in patients who already present with manifestations of the syndrome.[41] Multiple endocrine neoplasia type 2A presents with MCT, bilateral pheochromocytomas, and primary hyperparathyroidism; lately, it has been published that Hirschsprung disease could also be a manifestation of this syndrome, and genetic screening for RET proto-oncogene mutation is recommended in this patients[42]; MEN type 2B is an association of MCT, pheochromocytomas, and mucosal neuromas[43]; these patients usually present with a marfanoid phenotype.

The biochemical studies recommended for these syndromes are the same as previously proposed for each tumor type, depending on the clinical syndrome; and in the case when MEN syndrome is suspected, genetic testing should also be performed in the patient, and if positive, first-degree relatives should also be tested.

SPECIFIC BIOCHEMICAL MARKERS FOR EACH CLINICAL SYNDROME

Flushing

Foregut

The flushing in foregut carcinoid tumors is dry, long lasting, intense, and purplish or violet in contrast to the common red/pink seen in other neuroendocrine-related flushing. It is related to telangiectasia and skin hypertrophy mostly in the face and upper neck but can also involve the limbs, and it can lead to a leonine appearance after repeated episodes (Table 2; Fig. 4).

Midgut

A midgut tumor is faint pink to red and involves the face and upper trunk as far as the nipple line. The flush is initially provoked by exercise, alcohol, and food containing tyramines (eg, blue

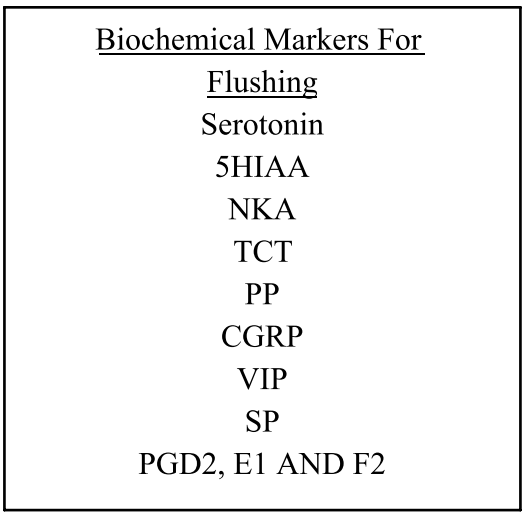

FIGURE 4. Biochemical markers for flushing. TCT indicates thyrocalcitonin.

cheese, chocolate, red sausage, and red wine). With time, the flush may occur spontaneously and without provocation. It usually is ephemeral, lasting only a few minutes, and may occur many times per day. However, over many years, patients may develop a persistent flush with a purpuric malar and nasal hue.

Differential diagnosis of flushing includes the postmenopausal state, simultaneous ingestion of chlorpropamide and alcohol, panic attacks, MCT, autonomic epilepsy, autonomic neuropathy, and mastocytosis.[44] A pseudocarcinoid syndrome with flushing and increased 5-HIAA has been described in men with hypogonadism, which responds to testosterone treatment.[45] To differentiate all those causes from a carcinoid tumor, besides knowing the differences in the characteristics of the flushing, it is also necessary to know what is producing the flushing (Table 3).

Flushing in carcinoid syndrome has been ascribed to prostaglandins, kinins, and serotonin (5-HT). With the advent of sophisticated radioimmunoassay methods and region-specific antisera, a number of neurohumors now are thought to be secreted by carcinoid tumors, including serotonin, dopamine, histamine, and 5-HIAA, kallikrein, SP, neurotensin, motilin, somatotropin release–inhibiting factor, VIP, prostaglandins, neuropeptide K, and gastrin-releasing peptide.

Feldman and O'Dorisio[46] have previously reported the incidence of elevated levels of plasma neuropeptide concentrations. Despite the elevated basal concentrations of SP and neurotensin, these authors were able to document further increases in these neuropeptides during ethanol-induced facial flushing. We support this contention and hasten to add that neuropeptide abnormalities frequently occur in patients with other forms of flushing and may be of pathogenetic significance.

Several provocative tests have been developed to identify the cause of flushing in carcinoid syndrome. These tests are based upon the need to distinguish the flushing from that found in a host of other conditions, particularly in panic syndrome, in which the associated anxiety and phobias usually establish the cause but frequently the physician and patient need reassurance that there is no underlying malignancy.

Ahlman et al[47] reported the results of PG provocation in 16 patients with midgut carcinoid tumors and hepatic metastases. All patients tested had elevated urinary 5-HIAA levels, and 12 had profuse diarrhea requiring medication. Pentagastrin uniformly induced facial flushing and GI symptoms in patients with liver metastases, but it had no effect in healthy control patients. All patients with PG-induced GI symptoms demonstrated elevated serotonin levels in peripheral blood. Administration of a serotonin receptor antagonist had no effect on serotonin release but completely aborted the GI symptoms. The authors emphasized the improved reliability of PG compared with calcium infusion, another provocative test popularized by Kaplan et al,[48] and pointed out that PG provocation occasionally can be falsely negative in patients with subclinical disease. Our own experience is that PG uniformly induced flushing in patients with gastric carcinoid tumors that was associated with a rise in circulating levels of SP in 80%. Thus, SP is one neurohumor that may be involved in the flushing of carcinoid syndrome.

Substance P has been found in tumor extracts and plasma from patients with carcinoid tumors and, in 1 reported case, was useful for tumor localization. Neurokinin A (NKA), its amino-terminally extended form, neuropeptide K, and SP are a group of peptides (ie, tachykinins) with common biological properties. Norheim et al[49] measured peptide responses to PG or ingestion of food or alcohol in 16 patients with metastatic carcinoid tumors and demonstrated 2-fold or greater increases in NKA and neuropeptide K in 75% of patients, as well as variable increases in SP in approximately 20% of patients.

Conlon[50] used region-specific antisera to SP and NKA to measure circulating tachykinins during a meal-induced flush in 10 patients with metastatic carcinoid tumors. Five patients had undetectable levels of NKA and SP after stimulation, thus suggesting that elevated tachykinin concentrations are not a constant feature of such patients. The authors also studied the effect of a somatostatin-analogue administration on meal-induced tachykinin responses in 3 patients with carcinoid tumors. Flushing was aborted in 2 patients, but tachykinin levels were only partially suppressed, indicating that these peptides cannot be solely responsible for the carcinoid flush. When the diagnosis of the underlying cause of flushing has been established, pathogenesis-oriented treatment can be very helpful.[44]

Cunningham et al[51] also performed a study in which they used patients with metastasizing ileocecal serotonin-producing carcinoid tumors and looked for the relationship of flushing totachykinin production. They concluded that metastasizing ileocecal serotonin-producing carcinoid tumors produce many biologically active substances with partially overlapping biological functions. The biological processes underlying the specific symptoms of the carcinoid syndrome are probably multifactorial. They

TABLE 3. Differential Diagnosis of Flushing and Recommended Tests

Clinical Condition	Tests
Carcinoid	Serotonin, 5-HIAA, NKA, TCT, PP, CGRP, VIP, SP, PGD2, PGE1, PGF2
MCT	Calcitonin, Ca2+ infusion, RET proto-oncogene
Pheochromocytoma	CgA, plasma free metanephrines, urine metanephrines, VMA, Epi, Norepi, glucagon stimulation, MIBG
Diabetic autonomic neuropathy	HRV, 2 hs PP glucose
Menopause	FSH
Epilepsy	EEG
Panic attack	PG, ACTH
Mastocytosis	Plasma histamine, urine tryptase
Hypomastia and mitral valve prolapse	Cardiac echo
Male hypogonadism	Testosterone

EEG indicates electroencephalography; Epi, epinephrine; FSH, follicle-stimulating hormone; HRV, heart rate variability; MIBG, metaiodobenzylguanidine; Norepi, norepinephrine; PGD2, prostaglandin D2; PGE1, prostaglandin E1; PGF2, prostaglandin F2; TCT, thyrocalcitonin; VMA, vanillylmandelic acid.

confirmed results from earlier studies showing that tachykinins and 5-HIAA levels are elevated in patients with daily episodes of flushing. The hormone effects were not mutually independent. It is possible that the development of flushing is the result of multihormonal stimulation. Other biologically active substances, such as kallikrein, and prostaglandins, may also contribute.[51]

Diarrhea

Secretory diarrhea is characteristic of NETs (Fig. 5), causing large volume stools, persists with fasting, and there is no osmotic gap between serum and stool. There are several causes of secretory diarrhea that need to be taken into consideration in the differential diagnosis: watery diarrhea, hypokalemia, hyperchlorhydria, and acidosis syndrome, the ZES, carcinoid tumors, MCT, secreting villous adenoma of the rectum, surreptitious laxative abuse, and idiopathic.

Neuroendocrine tumors can produce diarrhea by different mechanisms depending on their secretory products. Gastrin can increase the acid secretion by the stomach, which in turn inactivates lipase, amylase, and trypsin, and damages the mucosa of the small bowel, leading to decreased absorption and impaired digestion in the small bowel, exceeding the absorptive capacity of the colon, what gives an increased fecal volume and malabsorptive syndromes and sometimes steatorrhea. On the other hand, carcinoid or other NETs can produce other substances such as VIP, PP, SP, calcitonin gene–related peptide (CGRP), and/or thyrocalcitonin (TCT), all of which will act on the small bowel increasing the secretion of fluids and ions, which in turn will also exceed the colonic absorptive capacity producing an increased fecal volume as well as great losses of potassium and bicarbonate.

A disturbing cause that may be very difficult to differentiate is laxative abuse, and in all circumstances, a KOH stool preparation to detect laxatives is mandatory. Measurement of intestinal secretion by passing a multilumen tube and quantifying electrolytes and water transport, in addition to the measurement of stool electrolytes, which should account for the total osmolarity, will help to exclude laxative abuse, but is rarely performed.

It is important to mention that Cunningham et al[51] found in their study of tachykinins and NETs that there is an association between the elevation of tachykinins and the severity of the diarrhea. They concluded that all biochemical marker concentrations were elevated in patients with daily episodes of diarrhea, although the association between increased plasma tachykinins and the severity of diarrhea was independent of both CgA and 5-HIAA concentrations.[51]

Bronchoconstriction

Wheezing caused by bronchospasm occurs in up to one third of patients with carcinoid syndrome. Lung function tests show a prolonged forced expiratory volume in the first second. Differential diagnoses are asthma and chronic obstructive pulmonary disease. In the carcinoid syndrome, the cause of bronchoconstriction is usually SP, histamine, or serotonin that should be measured in patients who present with this symptom.[5]

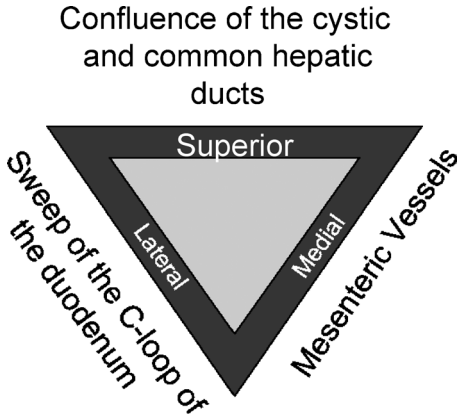

FIGURE 6. Gastrinoma triangle.

Dyspepsia or Peptic Ulcer

The ZES is characterized by peptic ulcers and diarrhea that respond to therapy with proton pump inhibitors (PPIs) in the setting of hypergastrinemia and low gastric pH. Gastrinomas are localized 90% of the time in the "gastrinoma triangle" (Fig. 6). As discussed in the previous section, the measurements that should be drawn for these tumors are FSG and gastric acid output.

Hypoglycemia

The Whipple triad (symptoms of hypoglycemia, blood glucose levels less than 40 mg/dL, and relief of symptoms with glucose) is the clinical presentation of insulinomas, but other causes should be ruled out.

Patients with noninsulinoma pancreatogenous hypoglycemia present with postprandial neuroglycopenia symptoms (within 4 hours of meal ingestion); have negative 72-hour fasting test; negative tumor localization studies; and on histological diagnosis, hypertrophy or nesidioblastosis rather than an insulinoma is found.[18,52] Other possible causes that should be thought of are fasting, autoimmune (insulin antibodies), counterregulatory hormone deficiency, drug-induced, and factitious hypoglycemia. To exclude all the other causes, clinical suspicion together with measurement of hormones or peptides should be used (Fig. 7).

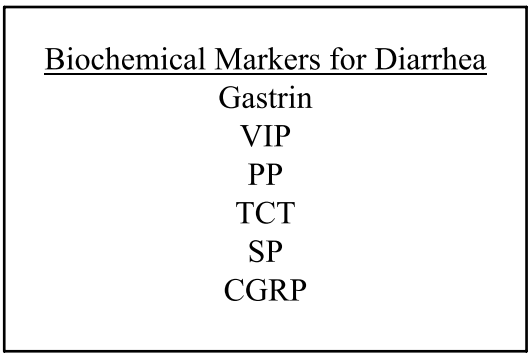

FIGURE 5. Biochemical markers for diarrhea.

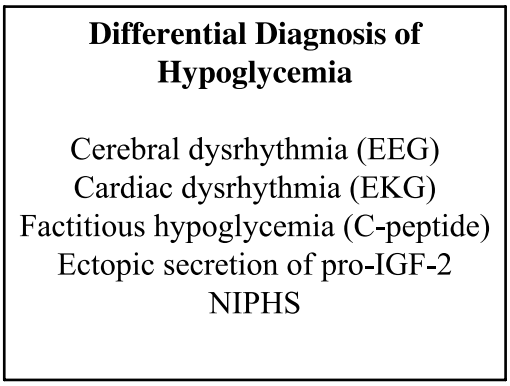

FIGURE 7. Differential diagnosis of hypoglycemia.

In case of hypoglycemia, the recommended biochemical markers are insulin, IGF-2, C-peptide, glucagon-like peptide type 1 (GLP-1), glucose-dependent insulinotropic peptide, sulfonylurea, ACTH, GH, insulin antibodies, and liver enzymes.[5]

Dumping Syndrome

This manifestation occurs after surgery when the pylorus has been resected or inactivated. It can be early, when symptoms resemble shock, or late, which presents as hypoglycemia. For the diagnosis of this syndrome, a provocative test is done, giving the patient a high-calorie carbohydrate-rich breakfast with 750 kcal (21 g protein, 30 g fat, and 99 g carbohydrate) that should be ingested in 10 minutes to produce the maximum response. After completion of the meal, blood sample is collected at 10, 15, 30, 45, 60, 120, and 180 minutes to measure glucose, insulin, C-peptide, motilin, PP, and GLP-1 levels.[5] An exaggerated insulin and GLP-1 response to the meal is found in gastric bypass patients with the syndrome, although the case and relationship between the hormonal overproduction and the clinical syndrome remain controversial.

Pellagra

Pellagra is caused by niacin deficiency caused by the detour of the tryptophan pathway toward the production of increased amounts of serotonin. Biochemical evidence of subclinical pellagra was found in one third of the patients with newly diagnosed untreated carcinoid syndrome in a study by Shah et al.[53]

Glucagonoma or the ''Sweet'' Syndrome

Diabetes accompanied by the 4D syndrome (dermatosis [necrolytic migratory erythema], depression, deep venous thrombosis, and diarrhea) is the clinical presentation of glucagonomas.

Glucose intolerance in the glucagonoma syndrome may relate to tumor size. Fasting plasma glucagon levels tend to be higher in patients with large hepatic metastases than in those without hepatic metastases,[54] and all patients with large hepatic metastases have glucose intolerance. Massive hepatic metastases may decrease the ability of the liver to metabolize splanchnic glucagon, thus increasing peripheral plasma glucagon levels. Glucagon may not directly induce hyperglycemia, however, unless metabolism of glucose by the liver is directly compromised. Another factor may be variation in the molecular species of glucagon that is present in each case and its biological potency.[50]

In previously reported cases of glucagonoma in which plasma glucagon concentrations were measured by radioimmunoassay, fasting plasma glucagon concentrations were 2100 ± 334 pg/mL. These levels are markedly higher than those reported in normal fasting subjects (ie, 150 pg/mL) or in those with other disorders causing hyperglucagonemia, including diabetes mellitus, burn injury, acute trauma, bacteremia, cirrhosis, renal failure, or Cushing syndrome, where fasting plasma glucagon concentrations often are elevated but less than 500 pg/mL.

As with other islet cell neoplasms, glucagonomas may overproduce multiple hormones such as insulin, ACTH, PP, PTH or substances with parathyroid hormone-like activity, gastrin, serotonin, VIP, and melanocyte-stimulating hormone in that order of frequency.[55]

Acromegaly or Gigantism

Acromegaly or gigantism can present when any NET secretes GH or GHRH. Basal levels of GH and IGF-1 are usually enough to make a diagnosis; but in 15% to 20% of the patients, further investigation is needed to show nonsuppressibility of GH to an oral glucose tolerance test, a somatostatin inhibition test, or a bromocriptine suppression test. The oral glucose tolerance test also measures lipids and insulin, which should also be suppressed. Other pituitary and hypothalamic hormones should also be measured, such as prolactin, the α and β subunits of gonadotropins, and thyroid-stimulating hormone.[5]

Cushing Syndrome

A pituitary tumor, small cell carcinoma of the lung (known to produce ACTH), or an ACTH-secreting NET will present clinically as the Cushing syndrome from oversecretion of cortisol, adrenal androgens, and 11-deoxycorticosterone. To reach the diagnosis, several steps should be followed. New guidelines for the diagnosis of Cushing syndrome have been published, although some of the recommendations are based on low-quality evidence. Their proposed approach is as follows.

After excluding exogenous glucocorticoid use (iatrogenic Cushing syndrome), patients with unusual features for age such as osteoporosis or hypertension, patients with multiple and progressive features predictive of Cushing syndrome (easy bruising, facial plethora, proximal myopathy or muscle weakness, reddish/purple striae, weight gain in children with decreasing growth velocity), and patients with adrenal incidentaloma compatible with adenoma should undergo testing for Cushing syndrome starting with 1 test with a high diagnostic accuracy: urine free cortisol (at least 2 measurements), late-night salivary cortisol (2 measurements), 1-mg overnight dexamethasone suppression test or longer low-dose dexamethasone suppression test (2 mg/d for 48 hours). If the test is negative and the pretest probability was low, then follow-up in 6 months is recommended if there is progression of symptoms; in case of a negative test but with a high pretest probability, then more than 1 test should be performed. In some cases, a serum midnight cortisol or dexamethasone corticotropin-releasing hormone test should be done.[56]

CLASSIFICATION OF THE BIOCHEMICAL MARKERS ACCORDING TO THEIR USE

Diagnostic

CgA and CgB

Both CgA and CgB are part of the granin family. They are stored and secreted from vesicles present in the neuroendocrine cells, together with other peptides, amines, and neurotransmitters.[57] Chromogranin A is the best studied[58] and most used. But CgA is not perfect. Stridsberg et al[59] reported that there are some common conditions that can increase the levels of this marker and give false-positive measurements including decreased renal function and treatment with PPIs[59] and even essential hypertension[60]; these problems are not seen with CgB, so they proposed the measurement of CgB as a complement to CgA.[59]

The most important characteristic of these markers is that they are not only secreted by the functional tumors but also by those less well-differentiated NETs that do not secrete known hormones.[2]

Chromogranin A has been shown to be increased in 50% to 100% of patients with NETs.[61] Chromogranin A levels may be associated with the primary type (gastrinomas, 100%; pheochromocytomas, 89%; carcinoid tumors, 80%; nonfunctioning tumors of the endocrine pancreas, 69%; and medullary thyroid carcinomas, 50%). In addition, blood levels depend upon tumor mass, burden, or progression, and malignant nature of the tumor.[25,62] Small tumors may be associated with normal CgA levels.

Sensitivity and specificity of CgA depend on many factors. For example, sensitivity varies from 77.8% to 84% and specificity from 71.3% to 85.3%, depending on the assay used, and of great importance is to establish the cutoff value that gives the highest sensitivity without compromising the specificity.[63]

Another use of CgA is to discriminate between patients with and those without metastasis, which also depends on the assay and the cutoff values used, with a sensitivity of 57% to 63.3% and specificity of 55.6% to 71.4%.[63]

Pancreatic Polypeptide

Pancreatic polypeptide is considered another nonspecific biochemical marker (Fig. 8). In a study conducted by Panzuto et al[64] in Rome, Italy, in 2004, PP sensitivity was 54% in functioning tumors, 57% in nonfunctioning tumors, 63% in pancreatic tumors, and 53% in GI tumors. Specificity was 81% compared with disease-free patients and 67% compared with nonendocrine tumor patients. But when combined with CgA, the sensitivity increased compared with either of the markers alone. When used in combination, the sensitivity of these markers is 96% for gastroenteropancreatic NETs, 95% for nonfunctioning tumors, and 94% for pancreatic tumors.[64]

Neuron-Specific Enolase

Neuron-specific enolase is an enzyme that occurs mainly in cells of neuronal and neuroectodermal origin. The NSE has been found in thyroid and prostatic carcinomas, neuroblastomas, small-cell lung carcinoma, carcinoids, gastroenteropancreatic NETs, and pheochromocytomas. Despite its high sensitivity (100%), its use is limited as a blood biochemical marker for NETs because of its very low specificity (32.9%).[65]

Follow-up, Treatment Response, and Prognosis

CgA

Other than the applications of CgA previously discussed, this marker can be used for prognosis and follow-up. Jensen et al[66] found that a reduction in CgA levels greater than or equal to 80% after cytoreductive surgery for carcinoid tumors predicts symptom relief and disease control; it is associated with improved patient outcomes even after incomplete cytoreduction.[66]

Pancreastatin

One of the posttranslational processing products of CgA has been found to be an indicator of poor outcome when its concentration in plasma is elevated before treatment in patients with NETs. A level greater than 500 pmol/L is an independent indicator of poor outcome. This marker is also known to correlate with the number of liver metastasis, so it would be appropriate to use it in the follow-up of NET patients. Furthermore, Stronge et al[67] found that an increase in pancreastatin levels after somatostatin analogue therapy is associated with poor survival. Other studies have shown that pancreastatin should be measured before treatment and monitored during and after it. Plasma levels of this marker greater than 5000 pg/mL pretreatment were associated with increased periprocedural mortality in patients with NETs who underwent hepatic artery chemoembolization.[68]

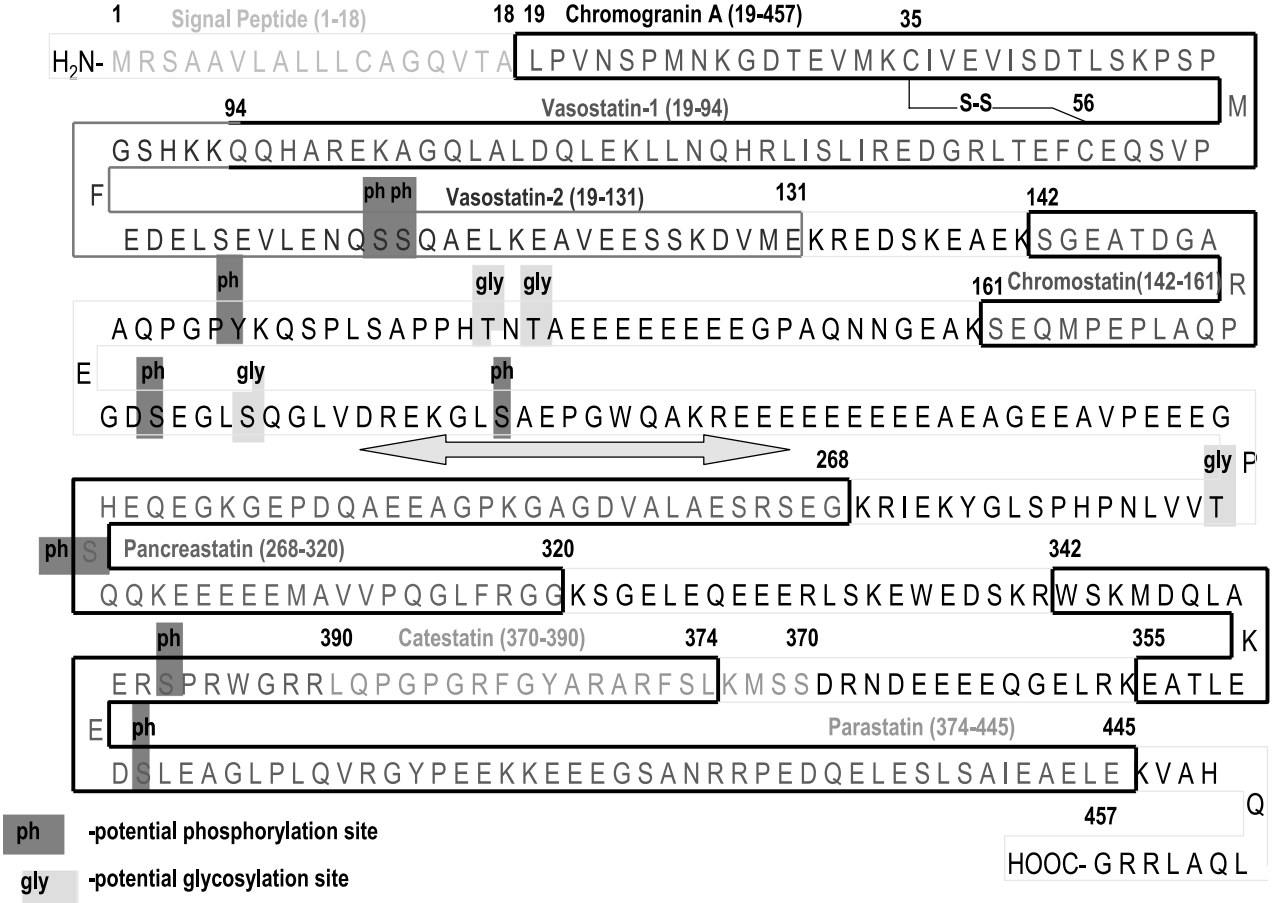

FIGURE 8. Primary structure of the CgA molecule showing several peptides that are derived after the enzymatic cleavage of CgA, such as pancreastatin, catestatin, vasostatin, which have biological activity and may contribute to the clinical syndrome.

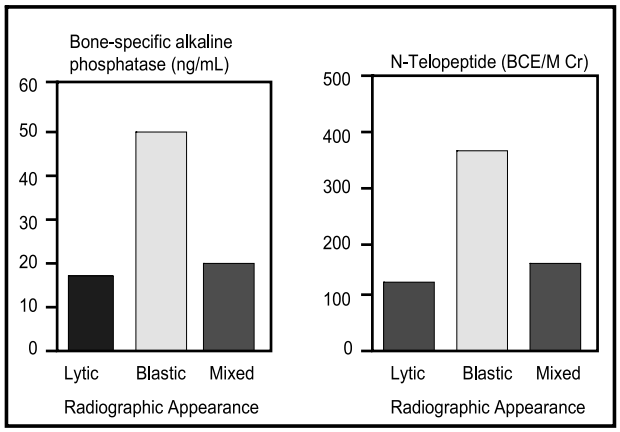

FIGURE 9. Bone markers in patients with lytic and blastic metastases. BCE indicates bone collagen equivalents.[70]

These observations suggest that pancreastatin is potentially a very useful marker not only for diagnosis but more importantly for monitoring treatment response.

Neurokinin A

Neurokinin A has been shown to have a strong prognostic value. Turner et al[69] in 2006 showed that in patients with midgut carcinoid that have raised plasma NKA, a reduction of this biochemical marker after somatostatin analogue (SSA) therapy was associated with an 87% survival at 1 year compared with 40% if it increased. They also concluded that any alteration in NKA predicts improved or worsening survival.[69]

BIOCHEMICAL MARKERS FOR BONE METASTASIS

Metastases from NETs can be either lytic and/or osteoblastic.

There may be an increased osteoclast activity contributing to lytic lesions and/or an increase in osteoblastic activity responsible for blastic metastases. Bone markers in lytic and osteoblastic metastases that may assist in the evaluation of stage as well as response to therapy include bone alkaline phosphatase (bAP), an indicator of osteoblast function, and urinary N-telopeptide, which reflects osteoclast activity or bone resorption. Somewhat paradoxically, only blastic metastases show an increase in both markers as indicated in Figure 9.[70]

Increased osteoclast activity predicts a poor outcome, with relative risks for high N-telopeptide (>100 nmol bone collagen equivalents/mM creatinine) of 3.3 ($P < 0.001$) for skeletal-related events, 2.0 ($P < 0.001$) for disease progression, and 4.6 ($P < 0.001$) for death.[71]

The following markers are recommended.

Bone formation markers: serum PINP, serum bAP, serum osteocalcin, osteoprotegrin.

Bone resorption markers: urine N-telopeptide, serum CTX, serum N-telopeptide, and serum RankL

Markers of malignancy: PTH-related protein (PTHrp) in blood. Perhaps IGF-1. Calcitonin, transforming growth factor-β, and endothelin 1.

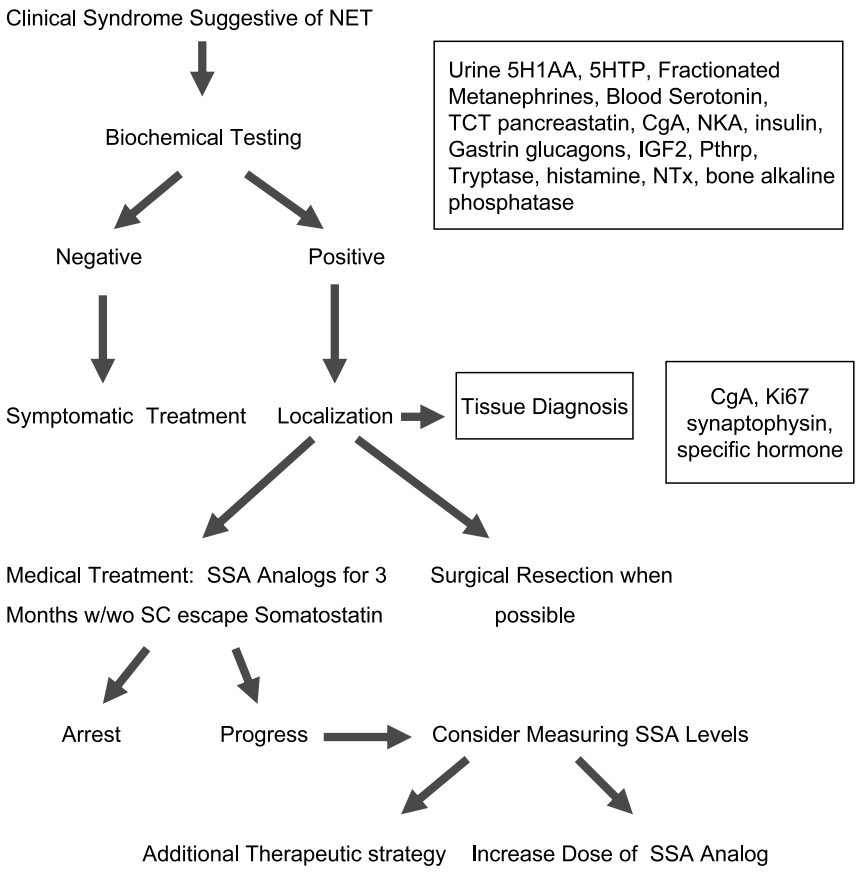

FIGURE 10. Algorithm for diagnosis and follow-up of NETs.[8,76,77] NTx indicates N-telopeptide.

Markers of cytokine excess: serum interleukin 1 and interleukin 6. Vitamin D metabolism: serum 25-hydroxyvitamin D and ionized calcium.

BIOCHEMICAL MARKERS FOR CARDIAC INVOLVEMENT

Carcinoid heart disease is a unique cardiac disease associated with NETs and may be seen in up to 60% of patients with metastatic carcinoid. Valvular disease is the most common pathological feature; tricuspid damage is found in 97%, and pulmonary valve disease is found in 88%, with 88% displaying insufficiency and 49% displaying stenosis. The distinctive carcinoid lesion consists of deposits of fibrous tissue devoid of elastic fibers known as carcinoid plaque. The deposits are found on the endocardial surface on the ventricular aspect of the tricuspid leaflet and on the arterial aspect of the pulmonary valve cusps.[72]

Although the precise cause for the plaque formation is not entirely clear, the direct actions of serotonin and bradykinin have been implicated in animal studies. This finding is corroborated by the observation that the appetite suppressant drug fenfluramine, which releases serotonin, has been noted to cause valvular distortion similar to that seen in carcinoid heart disease.[73] Values of serotonin greater than 1000 ng/mL seem to consort with the development of carcinoid heart disease. Possibly for this reason alone, in treating these patients, all attempts should be made to keep serotonin levels down in addition to relieving symptoms and slowing or abrogating tumor growth.

Pro–brain natriuretic peptide (NT-pro-BNP) can be used as a biomarker for the detection of carcinoid heart disease with a high specificity and sensitivity and used as an adjunct to deciding who requires echocardiography (Bhattacharyya et al[74]).

OCTREOTIDE LEVELS

For those patients "escaping" symptomatic control with somatostatin analogue therapy, one might consider measuring octreotide levels and adjusting the dose of therapy accordingly.[75]

SUMMARY AND CONCLUSIONS

To conclude, this algorithm (Fig. 10) proposes a summary of the steps for diagnosis and management of NETs starting at the presentation of a suggestive clinical scenario.

It is the purpose of this chapter to show the importance of recognizing, as early as possible, the clinical syndromes that suggest a NET as one of the differential diagnosis, and once suspected, look for the appropriate biochemical markers that will confirm the diagnosis or confidently discard it.

Neuroendocrine tumors are small slow-growing neoplasms, usually with episodic expression that makes diagnosis difficult, erroneous, and often late; for these reasons, a high index of suspicion is needed and it is important to understand the pathophysiology of each tumor to decide which biochemical markers are more useful and when they should be used.

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