National Academy of Clinical Biochemistry Guidelines for the Use of Tumor Markers in Neoplasms of the Dispersed Neuroendocrine System

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Key words: Melanoma, tumor markers, guidelines, TA90, S100, melanoma-inhibiting activity (MIA)

Abbreviations: CgA, chromogranin A; CgB, chromogranin B; 5-HIAA, 5-hydroxyindoleacetic acid; RIA, radioimmunoassay; EIA, enzyme immunoassay; MEN, multiple endocrine neoplasia.

INTRODUCTION

Tumours of the dispersed endocrine system represent neoplasms ranging from small benign incidental findings to functional hormone-secreting neoplasms to aggressive malignancies. Unlike classical neuroendocrine neoplasms that arise in native endocrine glands, these tumours arise from neuroendocrine cells that are dispersed throughout the gut and lung and more rarely in other sites, including prostate and ovary (1). Hence, this group of neoplasms are collectively referred to as tumors of the dispersed neuroendocrine system. The term “carcinoid” meaning “carcinoma-like”, which was originally introduced by Oberndorfer in 1907, has since, somewhat indiscriminately and with resulting confusion, been used to refer either to all well-differentiated neuroendocrine tumours or to the subset presenting with the classical clinical carcinoid syndrome caused by serotonin excess.

The currently recommended diagnostic approach to these lesions is (i) to base the diagnosis on microscopic features and immunohistochemical identification of hormone, bioactive amines and other markers of neuroendocrine differentiation (ii) to classify tumours according to the differentiated cell type and presumed site of origin and (iii) to stratify tumours by biological behaviour into benign, low-grade malignant and high-grade malignant categories, the latter including poorly differentiated endocrine carcinoma and small cell or oat cell carcinomas. The determination of malignant potential is based primarily on architectural and cytological features, although the location and hormone content of individual lesions is of importance, especially when attempting to predict the likelihood of metastatic behaviour of tumours of histological low-grade malignancy. The wide spectrum of clinical symptoms associated with lesions of the dispersed neuroendocrine system results from their collective ability to secrete an extensive array of peptide hormones and bioactive amines that differ according to the tumour type. Hormone markers of potential value in selected patients include plasma gastrin in gastrinoma, insulin and/or pro-insulin in hypoglycemic syndromes, glucagon in glucagonoma and plasma vasoactive intestinal hormone (VIP) in the Verner-Morrison syndrome.

The National Cancer Institute recently compiled a large database of 13,715 cases of neuroendocrine tumor covering five decades from 1950 to 1999 (2). The most frequently involved anatomic sites were the gastrointestinal tract (67.5%) and the bronchopulmonary system (25.3%). Within the gastrointestinal tract, neuroendocrine tumors were diagnosed in declining frequency in the small bowel (42%), rectum (27%), and stomach (9%). For all body sites, age-adjusted incidence rates were highest in the black male population (~5 per 100,000 per year). Five-year survival rates of 88%, 74% and 71%, respectively, were recorded for patients with the most frequent forms of neuroendocrine cancer, namely those located at the time of diagnosis in the rectum, bronchopulmonary system, and appendix. Of these tumours, 4%, 28%, and 40%, respectively, demonstrated invasive growth or metastatic spread. In nearly 13% of patients with

neuroendocrine tumors, distant metastatic disease was already evident at the time of diagnosis, highlighting the importance of early detection.

The relative infrequency of neuroendocrine tumors has not permitted large-scale systematic prospective studies of the same calibre as those performed for non-endocrine neoplasia. Most studies on the clinical application of tumor markers to neuroendocrine malignancy have relied on much smaller populations. Chromogranin A has emerged as the single most useful general marker of neuroendocrine neoplasia, while urinary 5-hydroxyindoleacetic acid (5-HIAA) and blood serotonin appear to have specific utility as markers of mid-gut and foregut neuroendocrine tumours. Pancreatic polypeptide and other granins such as Chromogranin B and molecular markers of tumorigenesis have either little or no clinical utility at this time.

CURRENTLY AVAILABLE MARKERS FOR NEUROENDOCRINE TUMORS

Table 1 lists the most widely investigated circulating and genetic tumor markers for neuroendocrine tumours. Also listed is the phase of development of each marker as well as the level of evidence (LOE) for its clinical use.

TUMOR MARKERS IN NEUROENDOCRINE TUMORS: NACB RECOMMENDATIONS

Table 2 summarizes the National Academy of Clinical Biochemistry (NACB) recommendations for the clinical use of blood and urine markers in the diagnosis and management of tumours of the dispersed neuroendocrine system. Below we briefly present a more detailed discussion on the markers listed in these tables.

Chromogranin A and the granins

Granins are ubiquitously distributed in neuroendocrine cells from where they are co-secreted with resident peptide hormones and biogenic amines. As such, they serve as useful markers of normal and neoplastic neuroendocrine cell activity and burden. The distribution of granins in neoplasms generally correlates with their expression in the corresponding normal tissue (3). In addition to neuroendocrine tissue, granins are widely distributed in endocrine cells and throughout the central and peripheral nervous systems.

The granins comprise a family of acidic soluble proteins that are intracellularly concentrated within secretory granules (4, 5). The group includes Chromogranin A (CgA), Chromogranin B (CgB) and Secretoneurin II along with a number of more recently identified members (6-8). Cg A has proven to be the most useful clinically. CgA plays a crucial role in secretory granule formation and hormone sequestration in neuroendocrine cells. Impairing CgA expression with antisense RNA

has been shown to deplete secretory granules, to inhibit regulated secretion of pro-hormone, and to reduce the content of secretory granule protein in neuroendocrine cells (9-11).

CgA contains a number of paired basic amino acids that are cleaved by endogenous proteases to generate biologically active fragments with autocrine, paracrine, and endocrine activities (12-16). Specific immunoassays are available to measure intact CgA while others detect peptide fragments derived from the parent molecule (17-20). To date, measurement of intact CgA in plasma has yielded diagnostic sensitivity for neuroendocrine tumours superior to that obtained by measurement of fragments (20-22). Enzyme immunoassay (EIA) and radioimmunoassay (RIA) CgA methods correlate well (3, 22-27). The detection limit of CgA EIA is frequently lower than that of RIA, but RIA methods tend to have a wider dynamic assay range (23). Reference laboratory granin assays exist for clinical samples, and three RIA kits that detect intact CgA are offered commercially (18, 22-24). When selecting a kit it is essential to compare the clinical sensitivities and specificities of candidate methods.

Elevation of circulating CgA is not specific to neuroendocrine malignancy as elevated levels are found in a number of other conditions including impaired renal function (28) and liver and heart failure (29). CgA levels in end-stage renal disease can be as high as those in neuroendocrine neoplasia, suggesting renal elimination of CgA (28). Plasma CgA rises as a function of neuroendocrine activation in congestive heart failure. CgA levels directly correlate with disease severity; patients with New York Heart Association (NYHA) Class IV heart failure exhibit CgA elevations ~7.6-fold greater than normal (29). CgA is also elevated in individuals with chronic gastritis type A and in patients undergoing treatment with proton pump inhibitors (28). Elevated CgA has been documented in primary parathyroid hyperplasia, thyroid C-cell hyperplasia, and gastric enterochromaffin-like cell hyperplasia (21, 30). Finally, potentially confounding the interpretation of blood levels in the management of established neuroendocrine malignancy, treatment with somatostatin analogues may reduce levels of circulating CgA independently of any effect on tumor burden (17).

CgA is a standard immunohistochemical probe and serum diagnostic test for neuroendocrine tumors. CgA blood levels can be used to follow the progression or regression of neuroendocrine tumors during treatment (30). Measurement of plasma CgA in children provides a sensitive and specific approach to the diagnosis of neuroblastoma (31), and the test can be used to monitor response to treatment and predict survival. In most neuroendocrine tumors, CgA is more abundant than CgB, and thus CgA is usually a better circulating tumor marker than CgB. Rarely, increased circulating CgB is seen when CgA levels are normal, as for example in insulin producing pancreatic neuroendocrine carcinomas (21).

Levels of serum CgA are elevated in about 80% of patients with gastrointestinal neuroendocrine tumors and appear to correlate with tumor-load and can therefore be used to predict prognosis, particularly in patients with mid-gut type of endocrine tumors (32). As elevation of CgA levels can precede radiographic evidence of recurrence in foregut carcinoid this may prove a useful marker in monitoring the course of disease. CgA may be elevated in 93% of patients with metastatic pulmonary endocrine carcinomas (32b). The sensitivity and specificity are approximately 92% and 96% respectively, which is significantly better than the discrimination that has been obtained with neuron specific enolase (NSE) and the alpha subunit of human chorionic gonadotropin (hCGα), two other markers that have been examined in the diagnosis of neuroendocrine tumors.

In glucagonoma, somatostatinoma, and the VIP pancreatic neuroendocrine malignancy VIPoma, serum CgA levels are usually elevated and readily serve as tumor markers for monitoring such patients. In gastrinoma (Zollinger-Ellison syndrome), the role of CgA is not well defined. Elevated levels of CgA may reflect the associated gastrin-mediated enterochromaffin-like cell hyperplasia, rather than actual tumor (gastrinoma) size, and excision of the stomach alone substantially reduces plasma CgA, even without excision of the gastrinoma (33).

In small cell lung cancers with neuroendocrine differentiation, plasma CgA may be used to monitor treatment response and screen for disease recurrence. While CgA indicates the likely degree of neuroendocrine differentiation within lung cancers, it lacks the sensitivity to reliably diagnose such neoplasms (34).

In prostate cancer, CgA may aid diagnosis and determine prognosis in malignancy arising from cells of neuroendocrine lineage (35). Circulating CgA may be increased in the absence of elevated prostate specific antigen (PSA). Detection of CgA may predict a lack of tumor response to hormone therapy. Elevated levels appear to augur a worse prognosis (36). CgA has also occasionally been detected in some of the tumour cells comprising colorectal and breast malignancy (36, 37). The prognostic significance of this is not yet clear.

CgA can aid in the diagnosis of "clinically silent" or "non-functioning" neuroendocrine tumors (36). Cases of medullary thyroid carcinoma, anterior pituitary adenoma, small cell lung cancer, and pancreatic endocrine carcinoma that are hormone-negative but CgA-positive have been reported (36). In multiple endocrine neoplasia type 1 (MEN1), tumor mass and circulating levels of CgA correlate (32).

Serotonin and Urinary 5-Hydroxyindoleacetic Acid

Serotonin is synthesized from tryptophan by hydroxylation and decarboxylation, and stored in secretory granules prior to its release locally and into the circulation upon appropriate stimulation. Most of the secreted serotonin is taken up by platelets, while free serotonin is rapidly degraded by monoamine oxidase in the liver and lung. The major product of degradation is 5-hydroxyindoleacetic acid (5-HIAA), which is eliminated in the urine in free and conjugated form.

Urinary 5-HIAA is the most important marker for mid-gut tumors, often heralded by the carcinoid syndrome. Measurement of urinary excreted 5-HIAA by HPLC is most accurate. Two 24-hour collections of urine are recommended, avoiding foods and medications that interfere in the assay or physiologically modulate the urinary output. Some centres recommend instead measurement of serotonin in plasma as this has been reported to be more sensitive than measurement of urinary 5-HIAA. A problem with monitoring plasma serotonin is its wide fluctuation in concentration with time, which makes it an unreliable marker for long-term follow up. Elevated 24 hours urinary 5-HIAA levels have about 73% sensitivity and 100% specificity in detecting a “carcinoid” tumour (21, 38, 39).

Pancreatic Polypeptide

Pancreatic polypeptide (PP) is a 36 amino acid polypeptide that is localized in cells in the pancreatic islets and throughout the exocrine parenchyma. Groups of PP rich lobules have been found in the pancreatic head. Elevated levels of pancreatic polypeptide in the circulation are found in most endocrine pancreatic tumours. Unfortunately, pancreatic polypeptide is also elevated in renal failure, laxative abuse and diarrhoeas of uncertain origin. Elderly people have higher circulating concentrations of pancreatic polypeptide than younger people (40).

Adrien et al (40) have investigated the diagnostic value of PP as a marker of neuroendocrine pancreatic neoplasia. Sensitivity was quite poor at 45% in the 323 patients with proven pancreatic endocrine tumors who were studied. Only 144 of the group exhibited circulating PP concentrations above the 300 pmol/L positive threshold. One milligram of atropine administered intramuscularly improved specificity by differentially suppressing tumour-associated and non-tumour associated elevations of PP. Elevations due to the former were not suppressed whereas those due to the latter were suppressed more than 50%.

It is unclear whether abnormal stimulation of PP by defined meals is of value in diagnosing early pancreatic involvement in patients with MEN-1. Abnormal meal stimulation tests have been reported in MEN-1 patients up to four years before detection of visible endocrine pancreatic tumour on CT-scan or MRI (21). Other investigators, however, could find no difference among patients with

histologically confirmed endocrine pancreatic tumours, carriers of the MEN-1 mutation and healthy controls. These conflicting findings may be explained by the different composition of the meal used to stimulate pancreatic polypeptide secretion in the various studies. Ideally, the meal should be carbohydrate rich and low in fat and protein as pancreatic polypeptide secretion is particularly stimulated by carbohydrate.

Panzuto et al (41) have reported on the sensitivity of plasma CgA and PP used separately and in combination to diagnose 68 cases of gastrointestinal and pancreatic neuroendocrine tumour. PP was much less sensitive than CgA in detecting both functioning tumours (54% vs 98%) and non-functioning tumours (57% vs 75%). However, combining the two markers boosted the detection of non-functioning tumours to 95%, a significant increase.

Investigational Molecular Markers

Molecular markers specific to neuroendocrine tumorigenesis have yet to be identified, although markers associated with tumorigenesis in general have been described in some preliminary studies of neuroendocrine tumors. None of the latter markers has yet been shown to be widely characteristic of either specific subtypes of neurodocrine tumour or neuroendocrine tumours in general. All such markers are investigational at this time and should be classified as research findings of uncertain clinical utility (Table 1).

Clonality studies (42) have indicated that most neuroendocrine tumours are monoclonal in composition (43, 44). Neuroendocrine tumours are not typically associated with karyotype abnormalities (44), the RAS mutations present in other endocrine tumours (42), or with G-protein mutations, even though one study has documented marked elevation of mRNA from the Gsα gene in insulin-secreting pancreatic endocrine tumours (45). Some studies have found that inhibitors of apoptosis, in particular the oncogenes c-myc, bcl-2, c-erb ß-2, and c-jun, are frequently over-expressed in human gastroenteropancreatic neuroendocrine tumours (46).

Various tumor suppressor genes appear to be mutationally inactivated or lost through gene deletion in neuroendocrine malignancy. One of the most promising markers is the 11q13 menin gene originally identified through its association with MEN-1. Menin gene mutations have been found in a significant number of sporadic tumours of the dispersed endocrine system, primarily gastroenteropancreatic endocrine tumours including 44% of sporadic gastrinomas and 19% of insulinomas, but also in lung endocrine tumours (47-49). Immunohistochemical detection of the menin protein product may prove to be of value in detecting inactivating mutations of this gene, but this remains to be established. Regarding other tumour suppressor genes, a small number of case reports have emerged describing deletions and possible rearrangements of the retinoblastoma (Rb)

gene in insulin-producing neuroendocrine carcinomas (50). Deletions of the putative Wilms' tumor and the transformation suppressor gene k*rev*-1 have also been described in insulin-producing tumors (51). A putative tumour suppressor gene that has been localized to chromosome 1 may predict prognosis in pancreatic neuroendocrine carcinomas (52, 53). Chromosome 10 or 17 monosomy, and loss of the adhesion molecule DCC ("deleted in colonic carcinoma") have also been detected in neuroendocrine tumours as has loss of functioning p53 in a small proportion of neuroendocrine carcinomas including those arising from the appendix (54,55).

CONCLUSIONS

As indicated in Table 2, CgA is the most useful circulating marker for neuroendocrine tumour of the gastrointestinal tract. It not only reflects the metabolic activity of the tumour but also generally correlates with tumour burden. It is also a useful marker for monitoring patients during treatment with the *caveat* that circulating levels may decline due to the suppressive effect of somatostatin analogues without corresponding reduction in tumour mass. Conversely, falsely elevated levels can be seen in patients with impaired renal function, congestive heart disease, liver failure, chronic atrophic gastritis and in those receiving proton pump inhibitors.

Measurements of 24 hour urinary 5-HIAA together with plasma CgA should be undertaken in patients with clinical carcinoid syndrome of both foregut and mid-gut type. Two 24-hour urine collections are recommended following appropriate restriction of food and medication.

Additional markers of potential value in selected patients include plasma gastrin in gastrinoma, insulin and/or pro-insulin in hypoglycemic syndromes, glucagon in glucagonoma, and plasma VIP in the Verner-Morrison syndrome of watery diarrhea.

Other general neuroendocrine tumor markers such as NSE, PP and CgB have much lower sensitivity and specificity than CgA, and consequently are not recommended for routine investigation.

Table 1. Currently available serum, urine and tissue markers for neoplasia of the dispersed neuroendocrine system.

Marker	Proposed Use	Phase of Development	Level of Evidence[1]	References
Established serum and urine markers				
Chromogranin A (CgA) (serum)	Diagnosis, recurrence, and monitoring treatment of most types of neuroendocrine tumors	In clinical use (widely available)	I	20,23,30-33
5-Hydroxyindolacetic acid (5-HIAA) (24 hour urine)	Diagnosis, prognosis, recurrence, and monitoring of pulmonary and small bowel neuroendocrine carcinomas	In clinical use (widely available)	I	38,39
Serotonin (serum)	Diagnosis, recurrence, and monitoring of pulmonary and small bowel neuroendocrine carcinomas	In clinical use (limited availability)	IV	38,39
Pancreatic polypeptide (PP) (serum)	Diagnosis and recurrence of pancreatic neuroendocrine carcinomas	In clinical use (widely available)	III	40,41
Insulin, proinsulin (serum)	Diagnosis, recurrence, and monitoring treatment nearly exclusively of pancreatic insulinomas	In clinical use (widely available)	III	21,39
Glucagon (serum)	Diagnosis, recurrence nearly exclusively of pancreatic glucagonoma	In clinical use (widely available)	III	21,39
Gastrin (serum)	Diagnosis, recurrence, monitoring treatment nearly exclusively in gastrinomas	In clinical use (widely available)	III	21,39
Vasoactive intestinal polypeptide (VIP) (serum)	Diagnosis, recurrence, and treatment nearly exclusively in VIP-producing tumors	In clinical use (widely available)	III	21,39
Investigational genetic markers				
Menin	Diagnosis of gastrin-producing carcinomas	Limited availability	III	47,48,49
Krev-1	Diagnosis of insulin-producing pancreatic carcinoma (insulinoma)	Investigational	IV	51
Rb	Diagnosis of insulin-producing pancreatic carcinoma (insulinoma)	Limited availability	IV	50
DCC (deleted in colon cancer)	Diagnosis of bowel neuroendocrine carcinoma	Limited availability	IV	54
p53	Diagnosis of bowel neuroendocrine carcinoma	Widely available	IV	55

[1] According to Hayes et al (56)

Table 2. NACB recommendations for the clinical use of tumour markers in neoplasms of the dispersed neuroendocrine system

Neoplasm	Recommended marker	Clinical Use
Serotonin-producing neuroendocrine carcinomas	24 hour urinary 5-hydroxyindolacetic acid (5-HIAA)	Diagnosis and monitoring of neuroendocrine tumor associated with clinical carcinoid syndrome
Most carcinomas of the dispersed neuroendocrine system	Serum chromogranin-A (CgA)	Diagnosis and monitoring of most neuroendocrine carcinomas including those not associated with clinical endocrine syndrome
Insulinomas	Fasting serum insulin	Diagnosis and monitoring of pancreatic insulinomas
Gastrinoma	Fasting serum gastrin	Diagnosis and monitoring of pancreatic gastrinomas
Vasoactive Intestinal Peptide neuroendocrine bowel carcinoma	Fasting serum Vasoactive Intestinal Peptide (VIP)	Diagnosis and monitoring of VIP-producing tumors

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