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Biochemical Testing in Neuroendocrine Tumors

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Neuroendocrine cells are widely distributed throughout the body. They are characterized by the ability to produce, store and secrete peptides and biogenic amines, in response to neural, chemical and other stimuli. The majority of tumors arising from these cells are found in the intestine (particularly jejunum-ileum), pancreas, and lung. As with tumors arising in other endocrine organs, the neuroendocrine tumors (NET) may be functional (secreting one or more products associated with a clinical syndrome) or non-functional. Non-functional tumors either fail to secrete any known product, or may secrete a product with no known associated clinical outcome. Diagnosis of NET involves analysis of the patients' clinical features, imaging (including somatostatin receptor-based imaging), biomarkers, and biopsy. Blood or urine concentrations of amines and peptides secreted by NET have proved to be useful biomarkers for the diagnosis and monitoring of these tumors. Although biomarkers may include cellular, biochemical, or molecular alterations that are measurable in biological media such as human tissues, cells or fluid, we will focus here on currently available biochemical testing of blood or urine for gastro-entero-pancreatic (GEP) and lung NET.

Timely diagnosis of NET can be challenging for multiple reasons:

1. The incidence of these tumors is low, although it does appear to be increasing. Incidence of NET from all sites was 5/100,000 in 2004, increased from 1/100,000 in 1973 [1].

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2. The primary tumor may be very small and is often metastatic at the time of diagnosis. Data from US and European cancer registries suggest that 30–75% of patients have distant metastases at the time of diagnosis [1–3].
3. Clinical presentation may vary depending on the site of origin of the primary tumor. Traditionally, NET are described as arising from the foregut (bronchopulmonary, thymus, gastric, proximal duodenum, pancreas), midgut (distal duodenum, jejunum, ileum, ascending colon), or hindgut (distal colon, rectum). Primary tumors arising from these different anatomical zones differ in their tumor secretions (Table 1). For example, tumors originating in the midgut secrete serotonin and are more likely than tumors of other origins to present with carcinoid syndrome. Carcinoid syndrome, when present, may include the following symptoms: flushing (94%), diarrhea (78%), abdominal cramping (50%), valvular heart disease (50%), telangiectasia (25%), wheezing (15%), or edema (19%) [4–6]. Pulmonary tumors are less likely to secrete serotonin (although they may secrete the serotonin precursor, 5-hydroxytryptophan) and more likely to secrete histamine. While they can present with flushing and wheezing, the full carcinoid syndrome with diarrhea is unusual. Hindgut tumors rarely secrete serotonin or cause carcinoid syndrome.
4. Clinical presentation may vary between tumors of the same originating site, based on the tumor grade and stage. Some biochemical tests have been suggested as markers of tumor grade or differentiation, e.g., neuron-specific enolase (NSE). While only about 10% of NET present with features of carcinoid syndrome, this number increases with higher tumor bulk (later stage), especially when liver metastatic disease increases.

Because of the low incidence, early diagnosis requires highly sensitive and specific biomarkers. It should be noted, however, that although incidence of NET is low, the prevalence is relatively high because of slow tumor progression. The estimated prevalence is 3-fold that of pancreatic cancer and is greater than that of esophageal and gastric cancers combined [1]. Because of relatively slower progression and prolonged follow up period, markers of tumor growth, response to therapy, prognosis, and differentiation state are important.

Over 20 different secretory products of NET have been described (Tables 2 and 3) [7]. Many of these are not assayed in commercial laboratories. Thus, while certain tumor secretions, e.g., chromogranin A (CgA), are widely expressed by NET of different tissues, there are cases of clearly functional tumors with negative biochemical testing for the common tumoral secretions. Thus, due to the heterogeneity of the originating cells and their secretory capacities for amines and peptides, a single biomarker may not allow accurate diagnosis or follow up.

Most of the available biochemical tests do not predict behavior of the tumor or prognosticate survival well. Furthermore, all of the specific biochemical testing used for NET diagnosis and monitoring have specific causes of inaccuracy (Table 3). For different assays, these issues include the effect of diet, fasting state, technique of phlebotomy, and handling of

sample. Standardization of the assays in different laboratories is frequently a concern. This is particularly the case with CgA in the US, where there are multiple assays in commercial use. In Europe, this assay has been standardized across countries and is more clinically useful in follow up.

Biomarkers in Neuroendocrine Tumors - Some patients present with well-defined symptoms associated with overproduction of circulating biologically active hormones, peptides and amines (Table 2). This is similar to other functional endocrine tumors, e.g., prolactinoma, where specific symptoms are associated with specific hormonal secretion. In these patients with functional NET, the specific clinical presentation should guide the initial choice of biochemical testing. Besides NET associated with carcinoid syndrome (most frequently midgut in origin), most of the functional NET are pancreatic endocrine tumors, and assessment of the specific hormone or related fragments allows diagnosis. Other NET may not have an identifiable clinical syndrome and may be asymptomatic or cause local symptoms like obstruction or bleeding. For these tumors, screening tests might include CgA, urine or plasma 5-hydroxyindoleacetic acid (5HIAA), or blood or serum serotonin.

Because of the difficulties of choosing an optimal biomarker, along with the interpretation issues of each test, the professional societies have not provided strong recommendations related to minimal biochemical testing. In general, guidelines from expert committees (European and American NET societies and National Comprehensive Cancer Network) suggest consideration of CgA for small intestinal, non-functional pancreatic, pulmonary, high grade GEP-NET, and metastases of unknown origin. A 24 hour urine 5HIAA could be considered for small intestinal and pulmonary NET and for all tumors with carcinoid syndrome. Pancreatic polypeptide is recommended for non-functional pancreatic NET, and NSE can be considered for high-grade tumors. Specific markers to assess functionality in patients with syndromes should be guided by the specific symptoms (e.g., urine or plasma 5HIAA for carcinoid syndrome, insulin for hypoglycemia etc). Our own practice is to test a broad range of markers initially and then follow markers that are positive.

Novel NET biomarkers - Despite the development of multiple biomarkers and imaging studies, diagnosis of NET is often delayed by years and the disease is frequently metastasized at the time of diagnosis. This observation suggests that the existing markers are not sufficiently sensitive for diagnosis. There is also an unmet need for markers that offer greater diagnostic and prognostic value, as well as information about response to therapy. An ideal biomarker would have a low false positive and false negative rate, measure aggressiveness of the tumor, identify treatment response to medical or surgical treatment, measure aggressiveness of remnant disease, detect relapse and serve as prognostic indicator.

While new individual biomarkers continue to be assessed, e.g., connective-tissue growth factor for carcinoid heart disease or Paraneoplastic Ma antigen 2 for small intestinal NET [8], there is an increasing interest in multi-analyte assays, which address the deficiencies of the current mono-analyte or single marker assays. These tests utilize multiple simultaneous measurements of different parameters related to the disease, along with algorithms, which improve sensitivity and specificity. This concept is already used for other conditions, e.g., FibroSure® test for hepatitis C [9] or MammaPrint® for metastatic breast cancer [10]. There

have been recent developments in nucleic acid-based technologies, like microRNA profiling, and strategies to collect circulating tumor cells, which may become helpful in future for NET diagnosis and management. At this time, however, the only commercially available multi-analyte NET test, which may be clinically helpful, is the NETest®.

The NETest®, developed by Wren Laboratories, Branford, CT, USA, is a multi-analyte qRT-PCR assay, based on 51 marker genes with an algorithmic analysis, claims a high sensitivity (>95%) and specificity (>95%) in the detection of all GEP-NET tumors. The test offers an assessment of disease status and treatment effectiveness [11, 12]. Early data suggest that the NETest® may be more accurate than single biomarker or mono-analyte tests and is not known to be affected by age, gender, fasting status or use of proton pump inhibitor drugs. The test requires a single laboratory, Wren Laboratories, to offer the specialized analysis. More information is needed on whether the test is positive in non-GEP-NET or cancers with mixed epithelial and NET phenotype, e.g., prostate cancer. Additionally, more prospective verification studies by investigators independent of the manufacturing company are needed. At this time there is no specific recommendation from any professional organization supporting the NETest®.

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KEY POINTS

- Neuroendocrine tumors secrete chemicals which can be used as circulating biomarkers.
- Tumors originating from different sites may differ in the tumor markers secreted.
- All tumor markers have potential for false positive and false negative results.

Table 1

NET sites of origin, potential associated symptoms, and specific biomarker tests.

Primary Tumor Location	Symptom	Test
Bronchopulmonary and thymic	Local symptoms Flushing, wheezing Cushing syndrome	CgA, Serotonin, 5HIAA, 5 hydroxytryptophan ACTH, cortisol
Jejuno-ileal	Local symptoms Carcinoid syndrome	Serotonin CgA Pancreastatin NKA
Colo-rectal	Local symptoms Incidental findings	CgA
Pancreatico-Duodenal		
Non Functional	Local symptoms / Incidental	CgA, (serotonin), PP
Functional (See Table 2)	Specific Syndrome	See Table 2

5HIAA: 5-hydroxyindoleacetic acid, CgA: chromogranin A, NKA: neurokinin A, PP: pancreatic polypeptide.

Table 2

Specific syndromes associated with pancreatico-duodenal NET tumor sites and suggested biochemical testing.

Syndromes	Site of Tumor	Symptoms	Diagnosis	Marker
Insulinoma	Pancreas	Hyperinsulinemic hypoglycemic syndrome	Hypoglycemia with non-suppressed insulin in a 72 hour fast	Insulin Pro-insulin C-peptide
Gastrinoma / ZES	Duodenum Pancreas	Recurrent peptic ulcers Diarrhea	Fasting plasma gastrin >1000 pg/ml; gastric pH <2.5	Gastrin
Glucagonoma	Pancreas	Glucose intolerance Weight loss Erythematous rash Venous thrombosis	Plasma glucagon levels 500-1000 pg/ml	Glucagon
VIPoma	Pancreas Pheochromocytoma	Secretory diarrhea Hypokalemia Dehydration	Plasma VIP >200 pg/ml	VIP
Somatostatinoma	Pancreas Duodenum	Diabetes mellitus Gallstones Diarrhea Weight loss Steatorrhea	Elevated plasma somatostatin with histologically confirmed NET	Somatostatin
Cushing	Pancreas Lung/Thymus	Easy bruising Facial plethora Myopathy Purple striae Hypertension Osteoporosis	Urine 24 hour cortisol, late night salivary cortisol, or 1 mg overnight dexamethasone suppression test	Cortisol ACTH CRH
Acromegaly	Pancreas Lung	Acral enlargement Prognathism	Non suppressible GH after glucose ingestion	GHRH IGF-1

ZES: Zollinger-Ellison Syndrome, VIP: vasoactive intestinal polypeptide, ACTH: adrenocorticotropic hormone, CRH: corticotrophin releasing hormone, GHRH: Growth hormone-releasing hormone, IGF-1: insulin-like growth factor-1.

Table 3

Specific blood and urine markers for NET.

Marker	Sensitivity	Specificity	Advantages	Disadvantages	Comments	Reference
CgA	43-100%	10-96%	Most widely accepted test as it is secreted by almost all NET particularly metastatic midgut carcinoids and pancreatic tumors Levels independent of functional status Correlates with tumor load and tends to be highest in metastatic cancer	Expressed in healthy tissue Elevated in other neoplasia like pancreatic, small bowel, prostate cancer Elevated in inflammatory conditions, cardiac disorders, renal failure, PPI use Not elevated in 15-40% patients Not standardized between laboratories	Granin family of peptides are water soluble acidic glycoproteins expressed in excretory vessels in neurons and endocrine cells Often used to confirm diagnosis, establish baseline, provide insight into tumor burden	[13-17]
PP	50-80%	No data	May be useful for early detection of NET of pancreatic origin in context of MEN 1	Increased PP release after meals, exercise and hypoglycemia; decreased by IV glucose and somatostatin Falsely elevated levels – laxative abuse, age, inflammatory processes of gut, chronic renal disease	Produced by NET of pancreas and colon and thought to play a role in auto-regulation of pancreatic and GI secretion and hepatic glycogen levels	[18, 19]
Pancreastatin	64%	58-100%	Not affected by PPI use More stable measurement of tumor activity Possibly greater sensitivity and specificity compared to CgA	Levels elevated in diabetics and hyperparathyroidism	Derivative of CgA	[20-23]
NSE	33%	Up to 100%	Used in diagnosis of small cell lung cancer or neuroblastoma Elevated in poorly differentiated NET where CgA might be normal Possible use as early response marker with mTOR inhibitor therapy	Cannot differentiate between different subtypes of NET No particular benefit over CgA Poor sensitivity – 33% Erythrocytes have large amount of NSE and can cause falsely elevated levels	NSE is an enolase present in neurons and NE cells and can indicate tumors derived from these cell types Located in cell cytoplasm and enters circulation after tumor lysis	[17]
NKA	88%	No data	Predominantly secreted by midgut NET Correlates with poor outcomes in midgut tumors			[24]

Marker	Sensitivity	Specificity	Advantages	Disadvantages	Comments	Reference
			Signals response to somatostatin analogues			
24-hour urine 5HIAA	35%	Up to 100%	Specific marker for carcinoid syndrome and midgut NET	<p>Many substances affect urinary 5-HIAA levels including:</p> <ul style="list-style-type: none"> -Foods rich in tryptophan – avocado, pineapples, banana etc. -Drugs - acetaminophen, guaifenesin, caffeine, mesalamine Falsely low values can occur with concomitant use of ethanol, levodopa, MAO inhibitors, heparin, methylidopa 		[25, 26]
Whole blood or serum Serotonin	75-80%	Up to 100%	<p>Specific marker for carcinoid syndrome and midgut NET</p> <p>Less cumbersome to obtain than a 24 hour urine 5-HIAA</p>	<p>Levels are somewhat affected by diet and stress. (see 5-HIAA above)</p> <p>Not all NET secrete serotonin</p> <p>Single level can vary during the day compared to 24 hour urine 5-HIAA</p>	Most circulating serotonin is stored in the platelets. The preferred specimens for serotonin analysis are either whole blood (containing all of the platelets) or serum from clotted specimens (since the clotting process releases almost all platelet serotonin).	[25, 27–29]
Plasma 5HIAA	89-95%	75-85%	<p>Specific marker for carcinoid syndrome and midgut NET</p> <p>Less cumbersome to obtain than a 24 hour urine 5-HIAA</p>	<p>Assay not standardized across laboratories</p> <p>Does not offer a significant advantage over blood serotonin levels</p>		[25, 27]

CgA: chromogranin A, PPI: proton pump inhibitors, PP: pancreatic polypeptide, 5HIAA: 5-Hydroxyindoleacetic acid, NSE: neuron-specific enolase; NKA: neurokinin A.