GEP-NETs UPDATE

Secreting gastro-enteropancreatic neuroendocrine tumours and biomarkers

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Abstract

Neuroendocrine tumours (NETs) are rare tumours with an annual incidence in the population in a range of 2–5 new cases per 100 000 inhabitants. NETs are widely variable in terms of anatomical location, hormone production, clinical behaviour and syndromes they can cause. This article reviews the many localizations and clinical presentations of NETs with a main focus on clinical biomarkers and their use in medical practice.

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Introduction

Neuroendocrine tumours (NETs), which originate from neuroendocrine cells, are found widely distributed throughout the body. The first description of these tumours was over 100 years ago by Lubarsch (1), who found multiple tumours in the distal ileum of two patients at autopsy. These tumours were firstly named carcinoid tumours (Karzinoid) in 1907 by Oberndorfer (2), to describe a small intestinal tumour that was morphologically distinct and less aggressive in behaviour than intestinal adenocarcinoma. Traditionally, these tumours were then described as originating from the foregut, midgut or hindgut (3). In most patients the primary tumour is found in the midgut and less frequently in the lung (3, 4). In 2010 the WHO released a new classification scheme for the digestive system based on histologic grade (5, 6, 7). This classification

divides NETs into well-differentiated NET, including grade 1 and 2 NET and poorly differentiated (grade 3) neuroendocrine carcinoma (NEC). The annual incidence of NET is estimated to be roughly 2–5 cases per 100 000 population (4, 8), though autopsy studies showed they can be found incidentally in up to 1% of necropsies (9, 10). Due to their relative indolent nature, there is often a delay in the diagnosis of NET, also caused by failure to identify symptoms or to establish the biochemical diagnosis. These tumours are a form of cancer that differs from other neoplasia in that they can synthesise and excrete various polypeptide hormones (e.g. Chromogranin A (CgA)) that cause specific clinical syndromes in 20–30% of cases, classified as ‘functioning tumours’. However, most are clinically silent until late stages of the disease (11).

Invited Author's profile

Dr M E T Tesselaar is head of the Antoni Van Leeuwenhoek/UMC Utrecht Centre for Neuroendocrine Tumours, which is an ENETS centre of Excellence, one of the few dedicated centres in the Netherlands. Her fields of interest are head and neck oncology, including thyroid cancer and neuroendocrine tumours.

Over the last two decades, the development of a variety of sensitive and specific plasma and/or serum assays for peptides and amines produced by NET and the development of immunohistochemistry panels has facilitated both blood and tissue diagnosis (12), followed by subsequent anatomical delineation of NET to ensure early surgical and/or pharmacological intervention to improve quality of life and survival. Management strategies for NET include surgery for cure (which is achieved rarely) or cytoreduction, radiological intervention (by radiofrequency ablation and (chemo)embolization), chemotherapy, targeted therapy and somatostatin analogues to control symptoms that result from the release of peptides and neuroamines (11). In this article we aim to give an overview on the current biomarkers secreted by NETs.

Clinical presentation and localization of NET

Neuroendocrine cells occur throughout the length of the entire gut and are the largest group of hormone-producing cells in the body (11, 13). At least 13 gut neuroendocrine cells exist, all of which produce various bioactive peptides or amines, including serotonin, somatostatin, histamine and gastrin. These secretory products are stored in large dense-core vesicles, and the proteins of these vesicles (e.g. CgA) are markers of neuroendocrine cells (11, 14). About two-thirds of NETs are of gastrointestinal or pancreatic origin (GEP-NETs), and among these the most common site of origin is the small intestine.

The clinical presentation of GEP-NET mainly depends on the site of the primary tumour, and whether the peptides secreted by the tumour cause symptoms, also called functioning or non-functioning. In most NETs histology strongly correlates with specific primary sites (Fig. 1): grade 1 NETs mostly originate in the gastrointestinal tract.

Appendiceal NETs are usually found incidentally in relatively young patients and are slow-growing tumours mostly occurring in the distal appendix where they often do not cause any symptoms. Besides location, mesoappendiceal invasion (>3 mm), histological atypia and the size of the tumour (>2 cm) are the best predictors of prognosis (15, 16).

Small intestinal NETs are most frequently located in the distal ileum and are often multicentric, occasionally appearing as dozens of lesions lining the small bowel (17). Patients with small intestinal NET generally present in the 6th–7th decade of life with abdominal pain or small bowel obstruction, often having years of vague abdominal complaints prior to presentation. These small intestinal

Figure 1

Anatomical distribution of grade 1 and grade 2 NET (adapted from (4)).

tumours can cause extensive mesenteric fibrosis and mesenteric ischemia (15, 16) Liver metastases can lead to carcinoid syndrome in 7–28% of these patients (15, 16), and endocardial fibrosis of the right-sided heart may occur, damaging the tricuspid and pulmonary valves, leading to impairment of cardiac function, also called the carcinoid heart disease. Minority of patients (10–20%) with carcinoid syndrome have heart disease at presentation, sometimes necessitating prosthetic valve replacement, often with a significant increase in survival (18).

Gastric NETs are typically multiple, small and localized tumours associated with hypergastrinaemia,

either secondary to chronic atrophic gastritis (type 1) or as part of Zollinger–Ellison syndrome (ZES) (type 2 in case of MEN-1). They have low malignant potential and metastasize in <3–5% (11). Type 3 large solitary gastric NETs, however, are not associated with hypergastrinaemia, and locoregional and hepatic metastases are very common (15). Most duodenal NETs are gastrin secreting, associated with MEN-1 in 20–30% of cases and causing ZES (11, 15).

NETs of the colon are mostly found in the right-sided colon, patients typically presenting with abdominal pain and weight loss. These tumours are often large, and patients commonly presenting with liver metastases (11, 15). Rectal NETs, however, are typically small, localized and non-functioning asymptomatic tumours, often found incidentally by endoscopy. They rarely metastasize, and local excision is the treatment of choice (15).

Pancreatic NETs are defined as epithelial tumours with a predominance of endocrine differentiation (15). A number of different clinical syndromes are recognized, reflecting the potential of pancreatic, endocrine cells to secrete both peptide and amines (depicted in detail in Table 1) (15). However, about 60–70% are non-functioning tumours and remain clinically silent until symptoms

due to mass effect or metastatic disease occur in an unresectable stage (15).

In general, according to SEER data, the 5-year overall survival rate of NETs is 60–70%, with the best prognosis for rectal tumours (88%) and worst for pancreatic ones (37%).

Biomarkers

General

Chromogranin A ► The neuroendocrine cells in which the NET originates are unique in that each secretes a variety of biomarkers specific to different types of NET. Currently, the most frequently used biomarker for the diagnosis and follow-up of GEP-NETs is CgA (19, 20, 21). CgA is an acidic glycoprotein that is exclusively expressed in the secretory dense core granules of most normal neuroendocrine cells and upon stimulation is co-released with peptide hormones and neuropeptides (12, 22, 23). The CgA molecule can exist in different molecular forms, depending on the type of NE cells and its concerning tumour (24). Therefore, the CgA result, measured in serum or plasma, is dependent of the

Table 1 Anatomical, clinical and biochemical features of hormonally active GEP-, bronchial- and thymic-NETs (adapted from (15, 45, 46)).

Site of the NET	Diagnostic serum biomarkers	Clinical features	Metastases	MEN-1
Foregut Bronchi, thymus, stomach, 1st part of duodenum, pancreas (below)	5-HTP, histamine, ACTH, CRH, CH gastrin	Atypical flush, pulmonary obstruction and hormone syndroms (below)	Liver Lymph node Bone	10%
Midgut Second part duodenum, jejunum, ileum, right colon	5-HT, tachykinins, prostaglandins, bradykinins and others	Bowel obstruction, typical pink/red flush, wheezing, diarrhea (carcinoid syndrome)	Liver (60–80%) Lymph Node	–
Hindgut Transverse colon to rectum	Peptide YY, glicentin, 5-HTP, neurotensin, local production SS, other hormones	Local symptoms Incidental finding	Bone metastases (4–40%)	–
Pancreatic Insulinoma	Insulin, proinsulin	Neuroglucopenia, Whipple's triad	10%	5–10%
Gastrinoma	Gastrin	ZES (peptic ulcer, epigastric pain, diarrhoea)	60–90%	25%
Vipoma	VIP	Watery diarrhea, hypokalaemia, achlorhydria	80%	10%
Glucagonoma	Glucagon	Cachexia, DM, necrolytic migratory erythema	80–90%	5–17%
Other rare functioning tumours	SS, GRF, PTHrp, other hormones	Diverse	60–80%	5–20%

5-HT, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan; VIP, vasoactive intestinal peptide; SS, somatostatin; GRF, growth hormone releasing factor; ZES, Zollinger–Ellison syndrome; DM, diabetes mellitus.

antibodies that are used in the immunoassay. Elevated circulating levels of CgA have been demonstrated in NET patients with both functionally and non-functionally active tumours (25), tend to correlate with tumour burden as well as recurrence and are considered a marker of bad prognosis in both ileal and pancreatic NET (26, 27, 28). The sensitivity of the CgA results is around 60–80% and is dependent of primary site, grade and status of the disease (29). Moreover, significantly higher median CgA levels are found in NET patients with liver metastases (30), the highest levels in patients with functioning ileal NET and carcinoid syndrome (27). Lymph node metastases do not seem to cause a significant increase in CgA levels (27, 30).

CgA can be elevated in several other diseases, such as renal failure, cardiac disease and tumours other than NET, but the main cause of falsely elevated CgA is the use of proton pump inhibitors (PPIs) (12).

In the past, the serotonin-producing NETs were mainly diagnosed and their treatments were evaluated by measuring either serotonin or its breakdown product, 5-hydroxyindoleacetic acid (5-HIAA), in platelet-rich plasma. Stronger correlations of CgA compared to 5-HIAA with physical functioning and well-being, as well as the prognostic value of CgA for survival, make CgA the recommended marker in the management and monitoring response to various treatment strategies of patients with metastatic NETs (28). Unfortunately, however, CgA also has its limitations, as various assays are available and an international standardization is lacking. In addition, elevated CgA levels may be caused by, for example, renal or liver failure and the use of PPIs (31, 32). An overview of all factors increasing CgA levels, possibly contributing to false-positive test results, is shown in Table 2.

Neuron-specific enolase ► Neuron-specific enolase (NSE) is present in neurons and neuroendocrine cells and can be raised in tumours originating from them, especially with a

high tumour burden, poor histological differentiation or a high rate of cell death. NSE is located in the cytoplasm and, unlike CgA, is not secreted. Its diagnostic sensitivity in GEP-NETs is low (32–47%) and is mainly the marker of choice for poorly differentiated NEC (33, 34, 35). Erythrocytes contain a large amount of NSE and can cause falsely elevated NSE levels.

Progastrin-releasing peptide and MonoTotal ► Progastrin-releasing peptide (proGRP) is an established promising tumour marker in small-cell lung cancer (36, 37). In the first cross-sectional marker study in which the possible role of proGRP was evaluated in addition to the established makers CgA and NSE in the diagnosis and prognosis of 573 patients with NET and NEC as well as 282 healthy controls, pro-GRP appeared to be the most sensitive marker for small-cell NEC, especially when located in the lung. MonoTotal, an immunoassay that measured the epitopes on cytokeratin fragments 8, 18 and 19 (CKfr), was also an important prognostic marker for survival in all patients with a NET. Within all histological groups, both the well-differentiated NET as well as the large- and small-cell NEC, a combination of tumour markers is more informative than each marker alone. In well-differentiated NET and large-cell NEC, CgA and CKfr was recommended, whilst in patients with small-cell NEC, proGRP and CKfr were preferred (29).

N-terminal pro-brain natriuretic peptide ► A biomarker which is useful in the early diagnosis of carcinoid heart disease is N-terminal pro-brain natriuretic peptide (NT-proBNP), which is already widely used as a marker for left ventricular dysfunction. The overproduction of serotonin and tachykinines can affect the cardiac valves, especially the tricuspid valve, and subsequently cause the carcinoid heart disease. NET patients with elevated NT-proBNP in addition to elevated CgA levels showed

Table 2 Factors increasing CgA levels, without the presence of a (GEP-)NET (adapted from (12) and Nolting *et al.* (30)).

Renal disease	Renal insufficiency
Gastrointestinal disorders	Chronic atrophic gastritis, pancreatitis, inflammatory bowel disease, irritable bowel syndrome, liver cirrhosis, chronic hepatitis, colon cancer, pancreatic adenocarcinoma, HCC
Non GI-cancers	Small-cell lung cancer, prostate cancer, breast cancer, ovary cancer, neuroblastoma
Cardiovascular disease	Arterial hypertension, acute coronary syndrome, cardiac insufficiency, giant-cell arthritis
Inflammatory disorders	Systemic rheumatoid arthritis, systemic inflammatory response syndrome, chronic bronchitis, airway obstruction in smokers
Drugs	PPI, H2RA
Endocrine disease	Pheochromocytoma, hyperparathyroidism, pituitary tumours, medullary thyroid carcinoma, hyperthyroidism
Others	Food intake and sports shortly before CgA measurement

a worse overall survival than patients with elevated CgA alone (38).

NET transcripts ► Modlin *et al.* (39, 40) have developed a PCR-based tool to quantitate (score) the circulating GEP-NET molecular signature ('liquid' biopsy) with high sensitivity and specificity. This test is based on 51 genes and identifies GEP-NETs. Since the blood PCR signature comprises 51 NET-based transcripts that cover a wide biological spectrum, it is also more effective than a single peptide-based ELISA that identifies a secretory peptide unrelated to tumour cell proliferation and not produced by 25% of NETs. Their observations indicate the score is elevated before tumour recurrence is detected by RECIST criteria. This signature can identify all types of GEP-NETs including small, non-metastatic tumours, is significantly reduced after tumour debulking and is absent following surgical 'cure'.

The performance metrics of this test outperformed any other biomarker information currently available for management and assessment of GEP-NETs, in particular CgA; this still, however has to be confirmed by future prospective studies.

Specific biomarkers

Pancreatic polypeptide ► For pancreatic NETs, pancreatic polypeptide may be useful for the early detection of NETs of pancreatic origin in the context of MEN-1, although this is controversial because of its low sensitivity (41). In the absence of a clinical syndrome, serum tests for an extensive set of hormones are not indicated in non-functioning pNETs.

Serotonin ► Serotonin or 5-hydroxytryptamine (5-HT) is enzymatically transformed from the essential amino acid tryptophan following hydroxylation and decarboxylation. Serotonin was discovered and isolated from serum and determined to originate from the enterochromaffin cells found throughout the gastrointestinal and bronchopulmonary system. High concentrations of 5-HT are found in blood platelets and enterochromaffin cells of the gut. With regard to intestinal NETs, endocrine tumours of the jejunum and ileum produce serotonin, but because of the hepatic first-pass effect, this secretion only gives rise to carcinoid syndrome in patients who have developed liver metastases. Quantification of the urinary excretion of the principal metabolite of serotonin, 5-HIAA, is 73% sensitive and 100% specific for detecting the presence of advanced functional NET of the small intestine (42).

Insulin, gastrin, glucagon and others ► Functioning pancreatic tumours can be tested for specific biomarkers such as vasoactive intestinal peptide (VIP), glucagon, somatostatin, growth hormone-releasing hormone (GHRH) or ACTH, as indicated only by the patient's clinical features. For the diagnosis of an insulinoma, insulin levels are used in combination with other criteria, which include: blood glucose levels (<2.2 mmol/l), concomitant insulin levels (>6 μ U/l), C-peptide levels (>200 pmol/l), proinsulin levels (>5 pmol/l), β -hydroxybutyrate levels (<2.7 mmol/l) and absence of sulfonylurea in plasma and urine (43). Further controlled testing includes the 72-h fast, which is the gold standard for establishing the diagnosis of insulinoma (although some studies report a 48-h fast may be adequate) (43). The diagnosis of gastrinoma is based on the existence of hypergastrinaemia (>100 pg/ml) in the presence of fasting hypersecretion of gastric acid. Whenever possible, PPIs should be discontinued for at least a week and H₂-blockers for 3 days before this test is performed. However, discontinuation of PPIs in suspected gastrinoma patients should be done with extreme caution because of the danger of gastro-intestinal bleeding or perforation. If fasting serum gastrin levels are greater than tenfold elevated and gastric pH <2 , the diagnosis of ZES can be made, because the possibility of retained gastric antrum can usually be eliminated by history (44). A gastrin value of over 1000 pg/ml is highly suggestive of gastrinoma. When the fasting serum gastrin levels are between 200 and 1000 pg/ml, the gold standard for the diagnosis of gastrinoma is the secretin test. This hormone, when given intravenously, provokes an increase in serum gastrin and secondarily in gastric acid secretion (44). Pancreatic NET can also produce other even more rarely occurring peptides (some mentioned in Table 2) such as VIP, glucagon, GH, ACTH, PTHrp, calcitonin, renin, LH, and erythropoietin (43).

Summary

NETs are rare tumours, with an annual incidence estimated as 2–5 cases per 100 000 population. They are able to produce non-specific and specific biomarkers. Presently the most frequently used biomarker in GEP-NETs is CgA, although it is a non-specific biomarker and has its limitations. More specific markers can be used in functioning NET. The use of NEN transcripts is promising, but its application of this PCR-based blood test in patients with a NET is still in research.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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