Circulating biomarkers in gastroenteropancreatic neuroendocrine tumours

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Introduction

The clinical presentation of gastroenteropancreatic neuroendocrine tumours (GEP-NETs) varies according to the site and size of the primary tumour, the presence or absence of metastatic spread, whether associated features compatible with a hereditary syndrome exist or not, whether the tumour is functional or not and, if so, what syndrome is present (Rindi et al. 2000, Metz & Jensen 2008, Vinik et al. 2009). Early on in the disease process, patients present with vague symptoms associated with various hormonal symptoms that often are misdiagnosed for many years. In such a situation, early diagnosis depends on syndromic recognition by physicians and it is achieved by appropriate laboratory testing followed later by imaging studies and a tissue diagnosis (Metz & Jensen 2008, Vinik et al. 2009). A common exception to this rule occurs with midgut carcinoid syndrome-producing tumours which often present symptoms late in the disease course. Non-functional tumours also commonly present late in the disease course with imageable metastases identified per chance or when studies are ordered for symptoms attributable to tumour growth rather than hormone production (Metz & Jensen 2008, Vinik et al. 2009).

Biomarker testing

Neuroendocrine tumours (NETs) derive from neuroendocrine cells (highly specialised cells with both neural and endocrine characteristics) that upon specific stimulation secrete hormones regulating various functions (Rindi et al. 2000). The various cell types of the neuroendocrine cell system can secrete products, such as peptides and biogenic amines that are tumour specific and may serve as markers for the diagnosis and follow-up of treatment. Some tumour markers may have prognostic implications (Rindi et al. 2000, Turner et al. 2006, Vinik et al. 2009). Neuroendocrine cells can be confined to a specific gland, such as the adrenal medulla (phaeochromocytomas) or the thyroid (medullary thyroid carcinoma). These tumours will not be discussed in this review, the so-called ‘diffuse neuroendocrine cell system’ of the gastrointestinal tract is the largest endocrine organ of the body; 15 different neuroendocrine cell types of the gastrointestinal tract have been identified, and each of these has specific hormone products and regulatory function (Rindi et al. 2000). Today, we recognise more than 30 gut peptide hormone genes, which express more than 100 bioactive peptides. The amine- and peptide-producing cells utilise endocrine, paracrine, neurocrine or autocrine regulatory mechanisms (Rindi et al. 2000). The cytoplasm of the neuroendocrine cell is occupied by a large number of secretory granules of varying electron densities, size and shape, and is the storage site of secretory products (e.g. serotonin (5-hydroxytryptamine (5-HT)), tachykinins and gastrin). Upon specific stimulation, granules are translocated to the cell membrane and their content released by exocytosis. Peptide prohormones are synthesised in the rough endoplasmic reticulum, together with chromogranin A (CgA) and other granular proteins. Chromogranins may serve as substrates for proteolytic enzymes and thereby modulate this process (Eriksson et al. 1989, Winkler & Fischer-Colbrie 1992, Rindi et al. 2000, Taupenot et al. 2003). The products are then transported to the Golgi apparatus and packaged into secretory granules (large dense-core granules).
Amines might be stored in small synaptic vesicles. The presence of these secretory products in the serum can be exploited diagnostically as tumour markers for NETs and are divided into general markers and specific markers, depending on the cell type involved (Rindi et al. 2000, Modlin et al. 2008).

**General markers**

**Chromogranins**

The chromogranins constitute a whole family of glycoproteins, of which CgA and CgB are the most clinically interesting (Table 1; Eriksson et al. 1989, Winkler & Fischer-Colbrie 1992, Taupenot et al. 2003, Stridsberg et al. 2004, 2005, Modlin et al. 2008, Vinik et al. 2009). Chromogranins are found in neuroendocrine cells throughout the body, but are also located in the neuronal cells in the central and peripheral nervous systems (Winkler & Fischer-Colbrie 1992, Woulfe et al. 1999, Gustafsson et al. 2008). In adrenal chromaffin cells CgA and CgB are present in about equal amounts. Parathyroid cells and enterochromaffin cells in the stomach contain mostly CgA and very little CgB. CgA, a 439 amino acid glycoprotein, has multiple pairs of basic amino acids distributed along its length, these being more abundant in the carboxyl terminal part of the molecule (Fig. 1; Winkler & Fischer-Colbrie 1992, Stridsberg et al. 2004, Vinik et al. 2009). CgB has a similar chemical structure being a glycoprotein but otherwise different from CgA (Yoo et al. 2002, Stridsberg et al. 2005, O’Toole et al. 2009). Tumours of neuroendocrine origin usually present with increased plasma levels of CgA and sometimes also CgB (Eriksson et al. 1989, Gustafsson et al. 2008, Metz & Jensen 2008, O’Toole et al. 2009, Vinik et al. 2009).

The most useful immunoassays for tumour detection have been those that measure the whole CgA molecule (O’Toole et al. 2009). Assays measuring specifically defined parts of the molecule (e.g. pancreastatin) usually have lower sensitivity in detecting patients with NETs (Tatemoto et al. 1986, O’Toole et al. 2009, Vinik et al. 2009). Commercial kits for determining CgA are available and measurement of CgA is routine in the management of NETs (Stridsberg et al. 2003, O’Toole et al. 2009). It should be noted, however, that there is a fair amount of variability between assays with sensitivities varying from 67 to 93% depending on which assay is used (Stridsberg et al. 2003, O’Toole et al. 2009). In most NETs, CgA is more abundant than CgB and thus CgA is usually a better circulating tumour marker than is CgB (Fig. 2). Other conditions with elevated levels of CgA are seen in patients with impaired kidney function or chronic atrophic gastritis type A, or in those treated with proton pump inhibitors (Table 2; Hsiao et al. 1990, Sanduleanu et al. 1999, Giusti et al. 2004, Peracchi et al. 2005, O’Toole et al. 2009). However, while none of these parameters affect the measurement of CgB, there are as yet no commercially available assays for CgB (Stridsberg et al. 2005, O’Toole et al. 2009).

Plasma CgA levels may be elevated in a variety of NETs, including phaeochromocytomas, paragangliomas, alimentary tract NETs, pancreatic islet cell tumours, medullary thyroid carcinomas, parathyroid and pituitary adenomas and also in a proportion of patients with small-cell lung cancer (Sobol et al. 1986, Gustafsson et al. 2008, Metz & Jensen 2008, O’Toole et al. 2009, Vinik et al. 2009). The highest CgA levels have been found in patients with metastatic small intestinal neuroendocrine (carcinoids) and islet cell tumours. Both tumour burden and the secretory activity reflect circulating CgA levels. The sensitivity and specificity vary between 60 and 100% and 70 and 100%, respectively, for different types of NETs, the highest values being for midgut carcinoids (Janson et al. 1997, Nobels et al. 1997, Baudin et al. 2001, Tomassetti et al. 2001, O’Toole et al. 2009, Vinik et al. 2009). CgA has been shown to be an independent prognostic factor for small intestinal NETs, because it correlates not only with the tumour burden, but also with the biological activity in the tumours (Fig. 3; Janson et al. 1997, Turner et al. 2006, Gustafsson et al. 2008). However, in patients treated with somatostatin analogues there is no correlation between circulating CgA levels and tumour mass. The reason for this is that somatostatin analogues are able to block the production and release of CgA in addition to also affecting tumour burden (Oberg & Stridsberg 2002, Rinke et al. 2009). Recent data are indicating that early response in CgA might indicate an anti-tumour effect by treatment with a target of rapamycin (mTOR) inhibitor (everolimus; Yao et al. 2010). The primary structure of human CgA contains ten pairs of basic amino acids, which are potential cleavage sites for specific endogenous proteases giving biologically active fragments such as vasostatins, pancreastatin and...

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**Table 1** The chromogranin family

<table>
<thead>
<tr>
<th>No.</th>
<th>Chromogranin</th>
<th>Name</th>
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<tbody>
<tr>
<td>1</td>
<td>CgA</td>
<td>Chromogranin A</td>
</tr>
<tr>
<td>2</td>
<td>CgB</td>
<td>Chromogranin B</td>
</tr>
<tr>
<td>3</td>
<td>CgC</td>
<td>Secretogranin II</td>
</tr>
<tr>
<td>4</td>
<td>1B1075</td>
<td>Secretogranin III</td>
</tr>
<tr>
<td>5</td>
<td>H1S2</td>
<td>Secretogranin IV</td>
</tr>
<tr>
<td>6</td>
<td>7B2</td>
<td>Secretogranin V</td>
</tr>
<tr>
<td>7</td>
<td>NESP55</td>
<td>Secretogranin VI</td>
</tr>
</tbody>
</table>
Chromogranin A related peptides

A1-15

77 114 208 248 322 338 373 400 409 437

Chromostatin

WE14

Catestatin

Chromacin I

Chromacin II

Vasostatins

Parastatin

Pancreastatins

Figure 1 Biochemical structure of the chromogranin A molecule with indicated splice products.

Chromostatin (see Fig. 1; Winkler & Fischer-Colbrie 1992, Taupenot et al. 2003, O’Connor et al. 2005, Belloni et al. 2007, Vinik et al. 2009). Several CgA-related peptides have been identified in the tissues and blood circulation. Many of the biological effects attributed to CgA seem to be mediated by these peptides. Concentrations of individual CgA-related peptides are generally lower than the concentration of total CgA. Different NETs seem to process CgA differently. However, CgA is a general marker, not specific for a particular NET type. A member of the chromogranin family, neuroendocrine secretory protein 55 (NESP55) is a 241 amino acid polypeptide that has been localised to the large dense-core secretory granules of neuroendocrine cells. NESP55 seems to be specific for pancreatic endocrine tumours (PETs) and phaeochromocytomas, whereas ileal (midgut) NETs are negative for NESP55, but positive for other members of the chromogranin family. So far, this member of the CgA family has been used only in immunohistochemistry. No plasma or serum assay has been published. These observations might be of help in localising small PETs (Sobol et al. 1986, Tatemoto et al. 1986, Hsiao et al. 1990, Janson et al. 1997, Nobels et al. 1997, Sanduleanu et al. 1999, Baudin et al. 2001, Tomassetti et al. 2001, Oberg & Stridsberg 2002, Yoo et al. 2002, Stridsberg et al. 2003, Giusti et al. 2004, Peracchi et al. 2005, O’Toole et al. 2009, Rinke et al. 2009, Modlin et al. 2010a).

Pancreatic polypeptide

Pancreatic polypeptide (PP), a 36 amino acid linear peptide, is another general tumour marker secreted by PP cells, which are located in the gut mucosa and pancreas. Other members of the same family are

Figure 2 Chromogranins A and B levels in patients with different neuroendocrine tumours compared with healthy individuals. EPT, endocrine pancreatic tumours; MEN1, multiple endocrine neoplasia type 1; PHEO, phaeochromocytomas.
peptide tyrosine–tyrosine (PYY) and neuropeptide Y (NPY). The release of PP is caused by ingestion of meals, particularly those containing protein. It has been found to be elevated in NETs of the gastrointestinal tract and pancreas, with a sensitivity of about 50–80% (Metz & Jensen 2008, Vinik et al. 2009). However, there are a lot of clinical conditions where falsely elevated levels are noted, such as diarrhoea, laxative abuse, high age, inflammatory processes in the gut and chronic renal disease (Metz & Jensen 2008, Vinik et al. 2009). A combination of CgA and PP has been useful in patients with non-functional PETs, with a sensitivity of almost 95% (Panzuto et al. 2004, Metz & Jensen 2008, Vinik et al. 2009). A specific meal stimulatory test (mixed meal) has been particularly useful in patients with multiple endocrine neoplasia type 1 (MEN1) and PETs (Oberg & Stridsberg 2002).

Neuron-specific enolase

Neuron-specific enolase (NSE) is present in the cytoplasm of neurons and neuroendocrine cells and can serve as a circulating marker for NETs. NSE is most frequently elevated in patients with small-cell lung cancer, but has also been found to be elevated in 30–50% of patients with intestinal NETs, medullary thyroid carcinoma, PETs and phaeochromocytomas. In patients with poorly differentiated NETs NSE might be elevated despite normal CgA. Increased levels of NSE are also roughly correlated with tumour size, although the specificity is lower than that of CgA (Baudin et al. 1998, Bajetta et al. 1999, O’Toole et al. 2009, Vinik et al. 2009). The combination of CgA and NSE has a higher sensitivity than either parameter separately (Oberg & Stridsberg 2002). Recently, an early response in NSE is related to therapeutic response with mTOR inhibitor (everolimus; Fig. 4; Yao et al. 2010).

Human chorionic gonadotropin subunits

Human chorionic gonadotropin (HCG), a glycoprotein hormone consisting of α and β subunits, can be ectopically produced by neoplasms. Assays for the intact glycoprotein and its α and β subunits have been used as markers to screen for a number of different tumours including NETs (Grossmann et al. 1994, Shah et al. 2008). In particular, HCGα and β subunits have been found to be increased in patients with malignant pancreatic NETs (pNETs).

Specific markers and related tumours

Carcinoid tumours have traditionally been divided according to the presumed embryological origin of the precursor cell. Thus, they were divided into foregut (lung, thymus, stomach and duodenum), midgut (jejunum, ileum, appendix and caecum) and hindgut (distal colon and rectum) carcinoids and together with pNETs collectively considered as gastroenteropancreatic NETs (Gustafsson et al. 2008, Metz & Jensen 2008, O’Toole et al. 2009, Vinik et al. 2009). An alternative to this classification is the WHO classification, which also takes into consideration the tumour biology and biochemical profile of the tumour (Gustafsson et al. 2008, O’Toole et al. 2009). The main categories of tumour according to the WHO classification are well-differentiated endocrine tumours (WHOI; G1 tumours) characterised by a low grade of proliferation. Well-differentiated endocrine cancer with Ki-67 > 2 > 20% (WHOII, G2 tumours) and poorly differentiated endocrine carcinomas with a high grade of proliferation Ki-67 > 20% WHO III and mixed exocrine–endocrine tumours (Gustafsson et al. 2008, O’Toole et al. 2009). However, from a

<table>
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<th>Table 2 Non-tumour associated increases of chromogranin A</th>
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Figure 3 Chromogranin A levels in patients with malignant carcinoid tumours and relation to tumour mass.
clinical point of view, the older classification might still be of value. Specific markers for various types of NETs using the earlier anatomical classification are summarised in Table 2. These markers include both structural and functional products which form the basis of yet another classification system for NETs (i.e. functional tumours which produce a clinical hormone related syndrome versus non-functional tumours which might present symptoms related to tumour growth (Metz & Jensen 2008, Vinik et al. 2009).

Foregut carcinoid tumours (bronchial, gastric and duodenal NETs)

Foregut carcinoid tumours display a variety of clinical symptoms and hormone production (Table 3). These tumours can produce almost any of the described hormones or amines in the body. The carcinoid syndrome, including flushing, diarrhoea, cardiac fibrosis, wheezing and dysphonia, occurs with both foregut and midgut carcinoids. This syndrome is related to the production of serotonin (5-HT) and tachykinins (NPK, substance P; Kema et al. 1995, 2000, Turner et al. 2006, Gustafsson et al. 2008, Vinik et al. 2009). A small number of patients with lung NETs develop an atypical syndrome with prolonged flushing, headache, palpitations and bronchoconstriction. In this syndrome histamine is one of the main mediators as well as 5-hydroxytryptaphan (5-HTP). Lung NETs may also produce Cushing’s syndrome due to ectopic ACTH production or acromegaly due to ectopic GH-releasing hormone (GHRH) secretion (O’Toole et al. 2009, Vinik et al. 2009). Duodenal tumours capable of secreting gastrin, give rise to the Zollinger–Ellison syndrome (ZES), with recurrent ulcers, diarrhoea and abdominal pain (Metz & Jensen 2008). Duodenal somatostatinomas are recognised histologically by the presence of psammoma bodies rather than the presence of the somatostatinoma syndrome which is characterised by glucose intolerance, gallstones and steatorrhea (Vinik et al. 2009).

Gastric NETs may derive from histamine-producing ECL cells in the corpus fundus region followed by

Table 3 Biomarkers in neuroendocrine tumours (NETs)

<table>
<thead>
<tr>
<th>Tumour marker</th>
<th>GI-NET</th>
<th>pNET</th>
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<tr>
<td><strong>Plasma markers</strong></td>
<td></td>
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<tr>
<td>Chromogranin A (CgA)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Chromogranin B (CgB)</td>
<td>X</td>
<td></td>
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<tr>
<td>Neuron-specific enolase (NSE)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Pancreatic polypeptide</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>α subunit of glycoprotein hormones</td>
<td>X</td>
<td>X</td>
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<tr>
<td>HCGβ</td>
<td>X</td>
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<tr>
<td>Gastrin</td>
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<td>Glucagon</td>
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<td>Insulin</td>
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<tr>
<td>Proinsulin</td>
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<tr>
<td>Somatostatin</td>
<td>X</td>
<td></td>
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<tr>
<td>Ghrelin</td>
<td>X</td>
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<tr>
<td>Substance P</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Neuropeptide K (NPK)</td>
<td>X</td>
<td></td>
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<tr>
<td>Vasoactive intestinal polypeptide (VIP)</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>X</td>
<td></td>
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<tr>
<td><strong>Urinary markers</strong></td>
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<tr>
<td>5-hydroxyindolacetic acid (5-HIAA)</td>
<td>X</td>
<td></td>
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<tr>
<td>Tele-methylimidazoleacetic acid (MelmII)</td>
<td></td>
<td>X</td>
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GI-NET, gastrointestinal NET.
gastrin-producing G-cell tumours of the antrum. Those types of gastric carcinoid are those producing gastrin, ghrelin and serotonin (Corbetta et al. 2003, Tsolakis et al. 2004, O’Toole et al. 2009, Vinik et al. 2009). Three clinical pathologic subtypes of ECL cell tumours are recognised. The type 1 ECL tumour is associated with diffuse corpus-restricted chronic atrophic gastritis. Type 2 is associated with MEN1, ZES and hypertrophic gastropathy. Type 3 tumours are sporadic and are not associated with any distinctive gastric pathology. Types 1 and 2 tumours are sharing common hypergastrinaemia whereas type 3 tumours are independent of any overt hormonal imbalance. All three subtypes of ECL tumours present elevated plasma CgA levels.

Midgut carcinoid tumours (small intestinal NETs)
Small intestinal NETs, also called classical carcinoids, have a more uniform clinical presentation, where up to 60% can present with the carcinoid syndrome (see above). These symptoms are related to secretion of 5-HT, tachykinins, kallikrein and prostaglandins. The urinary excretion of 5-hydroxyindole acetic acid (5-HIAA), the breakdown product of 5-HT, is an important marker for midgut carcinoids. Patients with the carcinoid syndrome usually have liver metastases and present with 5-HIAA levels of 100–3000 μmol/24 h (reference <50 μmol/24 h). Assays for urinary 5-HIAA include HPLC with electrochemical detection, and colorimetric and fluorescence methods (Kema et al. 1995, 2000, Turner et al. 2006, Gustafsson et al. 2008, O’Toole et al. 2009, Vinik et al. 2009). Various foods and drugs can interfere with the measurement of urinary 5-HIAA and patients should avoid these agents for 24 h before sampling. Normally, two 24 h urine collections are recommended (O’Toole et al. 2009). The sensitivity and specificity of these assays in midgut carcinoids are 60–70 and 100% respectively (Kema et al. 2000, O’Toole et al. 2009). Some studies have demonstrated higher sensitivity (100%) for the measurement of platelet poor plasma 5-HT compared with urinary 5-HIAA (90%) in patients with small intestinal NETs. However, there are large fluctuations in the plasma levels of 5-HT depending on food ingestion, time of the day, stress and sampling procedures (Kema et al. 1995, 2000, O’Toole et al. 2009). In addition, certain malabsortive conditions including Whipple’s disease and celiac sprue may be associated with elevated urine 5-HIAA levels (Kema et al. 1995, 2000, O’Toole et al. 2009). Plasma 5-HT might be of value in the early detection of recurrence of a midgut carcinoid after surgery (Oberg & Stridsberg 2002).

Hindgut carcinoid tumours (colorectal NETs)
Colorectal NETs generally present clinically as non-functional tumours (Gustafsson et al. 2008, Modlin et al. 2008). Patients develop abdominal pain, gastrointestinal bleeding and an enlarged liver with metastases with tumour growth. In recent years, more and more hindgut carcinoids are being discovered at an earlier asymptomatic stage during routine screening for colorectal cancer. They might secrete PP, somatostatin, PYY and CgA.

Pancreatic endocrine tumours
PETs present with a wide variety of well-known clinical syndromes such as the ZES (gastrinoma; characterised by abdominal pain, diarrhoea and peptic ulceration), the insulinoma syndrome (characterised by hypoglycaemia-induced neuroglycopenic and sympathetic overdrive symptoms), the glucagonoma syndrome (characterised by glucose intolerance and a specific rash called migratory necrolytic erythema), the Verner–Morrison or VIPoma syndrome (characterised by watery diarrhoea, hypokalaemia and achlorhydria), the somatostatinoma syndrome (as described above) and others (Metz & Jensen 2008, Vinik et al. 2009). In each case, identification of the specific elevated serum level of the syndromic product is useful for diagnosis and provocative testing may be useful too (secretin testing for gastrinoma, fasting-induced hypoglycaemia for insulinoma, etc; Metz & Jensen 2008, Vinik et al. 2009). However, a large proportion (at least 40%) are so-called non-functional islet cell tumours, which do not present with hormone-related clinical symptoms. Non-functional PETs can either be truly non-functional (in that no measurable peptide is produced at all) or they may produce PP (i.e. a PPoma) which is clinically silent (despite the fact that exogenous PP causes diarrhoea when administered i.v. to normal individuals; Metz & Jensen 2008, Vinik et al. 2009). Ghrelin, a 28 amino acid peptide that was first identified in the rat stomach, has been identified in human gastric mucosa, but has also been detected in the lung, endocrine cells of the pancreas, pituitary gland, hypothalamus, heart, adipose tissue and the reproductive system (Corbetta et al. 2003, Tsolakis et al. 2004, Vinik et al. 2009). Recently, pancreatic tumours containing ghrelin-producing cells have been identified by immunohistochemistry or by mRNA expression. In one report high circulating levels of ghrelin were reported, though a specific clinical syndrome was not apparent (Tsolakis et al. 2004).
**Laboratory testing recommendations**

Plasma CgA is the most important general tumour marker and it should be measured in every patient with a suspected NET (Metz & Jensen 2008, O’Toole et al. 2009, Vinik et al. 2009). PP may be useful to distinguish between PETs and other gastroenteropancreatic NETs though specific studies to address the sensitivity and specificity of a combination of CgA and PP in making this distinction are lacking. On the other hand, by combining PP and CgA testing overall sensitivity for NETs in general is enhanced (Metz & Jensen 2008, Vinik et al. 2009). There is probably little to be gained clinically by measuring other general markers in addition. NSE might sometimes be of value particularly in poorly differentiated NET. However, both the anatomical and biochemical diagnosis of a specific NET can be narrowed significantly by testing for specific markers as well.

The choice of which specific markers to test for should be predicated by the clinical presentation and tumour primary site (if this is known). For example, ACTH or GHRH may be useful in patients with known foregut carcinoid tumours exhibiting symptoms compatible with Cushing’s syndrome or acromegaly whereas serotonin or urinary 5-HIAA may be useful in patients with mid- or foregut tumours exhibiting symptoms compatible with the carcinoid syndrome (O’Toole et al. 2009, Vinik et al. 2009). For patients with PETs, gastrin levels supplemented by gastric acid analysis and secretin stimulation testing are useful to diagnose gastrinoma, glucose, insulin and proinsulin levels obtained during a 48–72 h fast are useful to diagnose the insulinoma syndrome, vasoactive intestinal polypeptide levels are useful to diagnose the VIPoma syndrome and glucagons levels are useful to diagnose the glucagonoma syndrome (Metz & Jensen 2008, O’Toole et al. 2009, Vinik et al. 2009). In addition, a meal stimulatory test with measurement of plasma PP and gastrin may be useful in the early diagnosis of PET patients with MEN1 (Oberg & Stridsberg 2002). Recently, a NET nomogram has been suggested including biomarkers, histopathology and therapy to generate a score which might indicate the degree of malignancy (Modlin et al. 2010b).

**Conclusion**

NETs provide unique possibilities to analyse peptides and amines released from the tumours, which can be used as circulating biomarkers. The best general biomarker today is CgA, which can be applied in most NETs but should be complemented with specific markers related to localisation of the primary tumour as well as clinical symptoms. Although CgA serve as a good clinical biomarker, we are in deep need of new biomarkers for detection of early recurrence after surgery with curative intent but also early diagnosis. Such markers are now in development besides circulating tumour markers new tissue markers are also in development and also studies on circulating tumour cells that might provide important information for treatment decisions in the future.

**Declaration of interest**

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