

Circulating Biomarkers in Neuroendocrine Tumors of the Enteropancreatic Tract: Application to Diagnosis, Monitoring Disease, and as Prognostic Indicators

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KEYWORDS

- Neuroendocrine tumors • Prognostic indicators
- Circulating biomarkers • Disease monitoring

Neuroendocrine tumors (NETs) are difficult to diagnose. Their symptoms may be vague or intermittent, and are frequently associated with much more common diseases; many of the tumors may be asymptomatic. Therefore, diagnosis can be delayed for some years. Because most NETs are secretory, the measurement of circulating biomarkers is helpful not only for diagnosis but also for assessing tumor response to treatment, monitoring disease progression, and use as prognostic indicators.

Three technologies are used in the diagnosis of NETs: radiology, measurement of circulating tumor biomarkers, and tissue pathology. Each has advantages and disadvantages. Radiology (computed tomography [CT] and magnetic resonance imaging [MRI]) offers visualization, but only for lesions greater than 1.5 cm in diameter and, as many NETs are small (or both small and multiple) this technique has limitations. Liver and lymphatic metastases may be numerous but small resulting in an inability to assess metastatic spread at an early stage. In addition, radiological procedures are costly when used for frequent follow-up and in the cases of CT scans and somatostatin analogue scintigraphy or fluorodeoxyglucose (FDG)-positron emission tomography (PET) scans, accumulative

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radiation dose must be taken into consideration, particularly for the younger patient. Although radiological procedures are less sensitive than measurements of circulating tumor biomarkers or actual tissue pathology, they remain the gold standard by which therapeutic interventions are determined. Measurement of circulating biomarkers is noninvasive and relatively inexpensive but there are significant problems with false-positive results. Because of this, it is less useful as a diagnostic tool but much more useful for following disease progression and treatment and as a prognostic indicator.

Tissue pathology offers the definitive diagnosis and proliferative index from a needle biopsy or surgical specimen. The limitation is that this gives 1 time point only in the disease pathway and repeat specimens are usually difficult to justify clinically. In addition, there is concern that a specimen may not be representative of the whole tumor, or 1 metastatic deposit may be quite different to another or to the primary tumor.

Therefore, the 3 technologies are complementary for diagnosis but the measurement of circulating biomarkers is most informative when used after the initial diagnosis has been made.¹

LABORATORY DIAGNOSIS OF NETs: USING CIRCULATING TUMOR BIOMARKERS

General Biomarkers

There are several families of secretory proteins found in high concentrations in neuroendocrine cells and, in particular, neuroendocrine tumor cells. These proteins are used in the identification of NETs in the pathology laboratory. They include the granins, neuron-specific enolase (NSE), and synaptophysin. Both chromogranin A (CgA) and NSE are found in increased concentrations in the circulation of many patients with NETs and both have been used as general biomarkers for these tumors. CgA is well established as the most sensitive, indeed only general biomarker for NETs² and has been investigated extensively.³⁻⁶ In normal physiology, most CgA found in the circulation is derived from enterocromaffin-like (ECL) cells in the stomach, but in patients with NETs, the main source is the tumor itself and the level of CgA in the circulation may be increased as much as 300-fold. The level of CgA is not increased in patients with benign NETs, which include almost all appendiceal NETs, most insulinomas (75%), many pulmonary NETs, and a significant number of NETs in the duodenum and rectum. CgA may not be increased in patients with multiple endocrine neoplasia type 1 (MEN1) or in patients with poorly differentiated NETs. Therefore, CgA is not a universal biomarker for NETs.

Circulating CgA is increased in several common conditions, notable when there is little or no acid in the stomach. This includes patients with atrophic gastritis and those who are chronically treated with proton pump inhibitor (PPI) drugs. This is a significant proportion of the adult population. When gastric acid is absent, the negative feedback to gastrin is absent. Therefore, gastrin continues to be stimulated. This increased amount of gastrin in turn stimulates the ECL cell to secrete CgA. Therefore, CgA increases in the circulation.

CgA is also increased in patients with renal impairment when CgA cannot be cleared from the circulation. Therefore, false-positives in CgA assays are a significant problem. Many false-positives occur in the range 2 to 4 times the reference range but a significant number also occur in the range 5 to 20 times the reference range. Less than 1% of CgA tests that are more than 20 times greater than the reference range are false-positives. **Fig. 1** shows circulating CgA concentrations in 12 individuals who were being treated with PPIs and following withdrawal of the drug.

Eight of 12 patients returned to normal within 7 days.⁷ The longer they are on PPIs (especially the higher potency PPIs), the longer it takes the CgAs to normalize. This may be weeks.

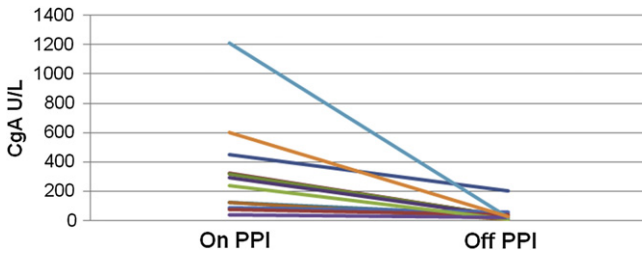


Fig. 1. Circulating CgA in patients on PPI and after withdrawal (N = 12). Reference range for CgA less than 30 units/L.

CgA has 439 amino acid residues with 11 pairs of dibasic residues and undergoes posttranslational processing that generates several products. Many of these products have been studied with respect to their potential as circulating biomarkers for NETs. The most interesting, clinically, is pancreastatin. Before the sequence of CgA was known and before there were any reliable assays that could measure the whole molecule, pancreastatin was used as a surrogate biomarker for CgA and was found to be significantly increased in the circulation of patients with NETs that had metastasized to liver. In some laboratories, pancreastatin measurement is preferred to whole molecule CgA measurement for the diagnosis and monitoring of NETs. Assays that measure midmolecule pancreastatin tend to cross-react strongly with CgA, whereas assays that detect the free C- or N-terminals of pancreastatin measure only this processed molecule; these assays are more interesting and have been recently shown to be clinically relevant.^{8,9} Pancreastatin is not increased in patients with gastric achlorhydria or hypochlorhydria as posttranslational processing of CgA differs in tumor patients. Therefore, false-positives are less problematic with the pancreastatin assay. It may well prove to be a very early biomarker for liver tumor activity, even when CgA is in the normal range.

Chromogranin B (CgB) is the second granin that has undergone investigation. It has 14 dibasic cleavage points but has been less well studied than CgA. CgB circulates in much lower concentrations than does CgA in most patients with NETs. However, in tumors where CgA is not found, CgB may be increased.¹⁰ Such patients include those with MEN1 and those with tumors in the duodenum or rectum. In these groups, CgB measurement may be useful.

SPECIFIC TUMOR BIOMARKERS FOR NETs OF PANCREATIC ORIGIN

About 6.5% of NETs occur in the pancreas. They may be benign or malignant and 15% to 20% are linked to an autosomal dominant gene mutation on chromosome 11 in MEN1.¹¹

The most common secretory NET of the pancreas that produces a symptomatic complex is insulinoma. More than 80% of insulinomas are benign. Insulinoma is uncommon in MEN1 although it is the second most common pancreatic NET to occur in patients with MEN1. Insulinomas secrete proinsulin, insulin and C-peptide intermittently, and, although insulin concentrations in the circulation may often be within reference range, insulin is at most times inappropriately high for the blood glucose concentration. Patients present with the symptoms associated with symptomatic hypoglycemia (palpitations, sweating pallor, anxiety, personality changes, and loss of consciousness). The latter symptom reflects both the severity and duration of hypoglycemia (see article elsewhere in this issue). Although these symptoms are profound,

they may be intermittent and diagnosis is not always straightforward. A carefully supervised 72-hour starvation usually precipitates hypoglycemia within the first 36 to 48 hours. The differential diagnosis is insulin abuse (eg, known insulin-requiring diabetes or factitious hypoglycemia), which is uncommon. With insulin abuse, circulating insulin is increased but not proinsulin or C-peptide, so the measurement of increased proinsulin or C-peptide secures the diagnosis of insulinoma.¹² On rare occasions, patients treated with oral hypoglycemic medication (sulfonylureas) in the setting of renal compromise may show symptoms that mimic insulinoma. As most insulinomas are benign, CgA is not increased in this population. However, CgA measurement can be a useful indicator of malignancy in insulinoma.

Gastrinoma is the second most common secretory pancreatic NET with just more than half malignant at presentation. Approximately 25% to 35% of gastrinomas are associated with MEN1. Gastrinoma is the most common gastroenteropancreatic tumor associated with MEN1. Gastrinomas secrete gastrin but, because gastrin circulates in numerous forms, this is not exclusively gastrin 17. Progastrin, gastrin 34, gastrin 17, and C-terminally extended gastrins may all circulate in high concentrations in patients with gastrinoma. Patients usually present with hyperchlorhydria, which results in peptic ulceration that may extend throughout the stomach, the duodenum, and into the jejunum. When untreated, this results in severe pain and hematemesis. Before the development of acid-controlling drugs, gastrinoma was a life-threatening disease. Since the advent of H₂ antagonists and PPIs, the syndrome is controlled by these drugs in the early stages, which results in a delayed diagnosis. Suspicion of gastrinoma should be raised with any patient who has recurrent ulcer in the absence of *Helicobacter pylori* infection. In addition to gastrin and CgA, pancreatic polypeptide (PP) may be increased in 35% to 40% of patients with gastrinoma. The increase in circulating gastrin may be small. In 20% of cases, circulating gastrin may be only 5% to 20% more than the reference range at presentation.¹³ This is particularly evident when gastrin 17 and gastrin 34 are the predominant products secreted, as acid secretion is powerfully stimulated and severe symptoms develop rapidly.

The diagnosis of gastrinoma is not without difficulty in the laboratory. All patients with peptic ulcer symptoms receive acid-suppressing therapy, usually PPIs. In any patient receiving PPIs, both gastrin and CgA are increased to within the range of that recorded in many patients with gastrinoma.¹⁴ When the negative feedback of acid is absent because of acid-suppressing therapy, gastrin and CgA continue to be stimulated and circulating concentrations of these cell products increase. This is also true for patients with atrophic gastritis. In patients in whom there is a suspicion of gastrinoma, PPIs should be withdrawn under careful supervision, as hematemesis and perforation may be a significant risk. A fasting blood specimen taken 5 to 7 days after withdrawal of PPI therapy will show a significant reduction in circulating gastrin and CgA in patients who do not have gastrinoma. In patients not on PPIs, autoimmune atrophic gastritis must also be excluded. Even considering the problems associated with the laboratory diagnosis of gastrinoma, the measurement of gastrin and CgA remains important in these patients.

Gastrinomas may be small and multiple, smaller than can be visualized by any current radiological method. Therefore, circulating gastrin remains a useful tool for diagnosis. Because of the problems associated with multiple small lesions in the pancreas for both gastrinoma and insulinoma, intraoperative venous sampling with rapid assay for the relevant peptides may be helpful to ensure that adequate surgery has been performed.

In addition, the secretin stimulation test may be used for the diagnosis of gastrinoma. Highly purified porcine secretion (2 units/kg body weight bolus) remains the

best test for detecting the existence of gastrinoma. A paradoxical release of gastrin of 200 pg/mL from basal is diagnostic for the presence of gastrinoma in more than 90% of cases.¹⁵

VIPoma is much less common than insulinoma and gastrinoma with an incidence of approximately 0.02 per 100,000 per year. VIPoma is characterized by watery diarrhea, hypokalemia and achlorhydria (WDHA syndrome). The clinical features are caused by vasoactive intestinal peptide (VIP), which is a potent stimulator of intestinal secretion and inhibitor of gastric acid secretion. Diarrhea may be watery and may escalate to 15 to 20 L per day causing the control of fluid and electrolytes (especially K^+ and HCO_3^-) to be critically compromised. Relatively small chronic increases in VIP in the circulation result in a profound VIPoma syndrome and patients frequently present when circulating VIP is no more than 20% to 50% more than the reference range. VIP acts as a neuromodulator and not a hormone in normal physiology; thus, it circulates in low quantities unless associated with a pathologic condition as seen in VIP-secreting NETs. There are several excellent VIP assays available in Europe and the United States.

Glucagonoma occurs at approximately the same frequency as VIPoma. In normal physiologic conditions, glucagon and its associated gene products are secreted from the alpha cells of the islets of Langerhans and from the L cells in the intestinal mucosa. From these 2 sites, proglucagon is processed differently. In the pancreas, proglucagon is processed to produce glucagon, glycentin-related peptide, intervening peptide, and the major glucagon fragment. Intestinal proglucagon undergoes alternative posttranslational processing that generates glycentin, sometimes referred to as gut glucagon, glucagon-like peptide 1 (GLP1), and glucagon-like peptide 2 (GLP2). Glycentin contains the glucagon sequence but is extended at the C-terminus.¹⁶ Considering the importance of glucagon in the control of blood glucose one would expect a glucagon-secreting tumor to produce a profound syndrome. However this is not the case and glucagonoma usually presents late with extensive metastatic spread, mild diabetes, and a characteristic rash (necrolytic migratory erythema). Circulating glucagon concentrations are typically more than 5-fold higher than the reference range. Both pancreatic glucagon and glycentin are measured in high concentrations. When the tumor products are processed according to the pattern seen in the intestinal cells, then GLP1 and/or GLP2 may also be significantly increased. When secreted under tumor conditions, GLP2 results in the development of giant intestinal villi. In glucagon-secreting tumors, the measurement of CgA, glucagon, glycentin, GLP1, and GLP2 may be helpful. Although pancreatic glucagon measurements are readily obtainable in Europe and the United States, GLP1 is more difficult to obtain commercially. An excellent GLP1 radioimmunoassay is available in Denmark.¹⁷

Pancreatic somatostatinoma is uncommon and symptoms are vague thus diagnosis is frequently delayed. Patients may present with mild diabetes and steatorrhoea but more frequently with symptoms associated with tumor bulk. Circulating somatostatin concentrations may be more than 100 times the reference range.

PP-secreting pancreatic tumors are not associated with clinical signs and remain undetected in the early stages, becoming apparent because of symptoms associated with tumor bulk as disease progresses, or as an incidental finding. At the time of diagnosis, circulating PP may be more than 100 times higher than the reference range.

CgA is also increased in patients with glucagonoma, VIPoma, somatostatinoma, and PPoma when disease is metastatic, which is usual at diagnosis. In a significant number of patients with a pancreatic NET (35%), PP may be raised in addition to the specific syndrome tumor biomarker (Table 1).¹

Tumor Type	Syndrome	Symptoms	Circulating Biomarkers
Insulinoma	Whipple's triad	Hypoglycemia, dizziness, sweating	CgA and CgB, insulin inappropriate for blood glucose level, proinsulin, C-peptide
Gastrinoma	Zollinger-Ellison	Epigastric pain, peptic ulcer, diarrhea	CgA, gastrin, PP (35%)
VIPoma	WDHA	Watery diarrhea	CgA, VIP
Glucagonoma	None	NME	CgA, glucagon, glycentin
Somatostatinoma	None	Mild diabetes	CgA, somatostatin
PPoma	None	None	CgA, PP
Nonsecreting	None	None	CgA

CgA is raised only in metastatic tumors.

Abbreviations: NME, necrolytic migratory erythema; WDHA, watery diarrhea hypokalemia, and achlorhydria.

A significant number of pancreatic NETs are termed nonsecretory because they do not secrete any peptide or it may be that they secrete 1 or several peptides that do not cause observed imbalance to normal physiology and as yet these potential biomarkers have not been identified. These tumors normally secrete CgA when they are malignant.

Patients with MEN1 possess the potential to produce several different tumors of endocrine origin including the potential of these tumors to change tumor cell type. Commonly in the pancreas, a tumor that secretes 1 peptide progresses to the situation where there are some cells, clusters of cells or indeed discrete tumors develop that produce other peptides. Similarly, in malignant sporadic pancreatic NETs, the same potential exists and may be manifest in 50% of patients with metastatic gastrinoma. In sporadic pancreatic NETs, this is associated with a worsening prognosis, and with the secretion of some products such as adrenocorticotrophic hormone (ACTH), the prognosis becomes very poor.¹⁸ Early identification of any such change is important as more aggressive treatment options must be followed promptly. A regular annual screen for a variety of these circulating biomarkers is recommended for both patients with MEN1 and those with sporadic metastatic pancreatic NETs. This should include insulin, gastrin, VIP, glucagon, somatostatin, PP, calcitonin, prolactin, and ACTH (Fig. 2).

Simple tumors secrete gastrin only. Complex tumors may begin as simple gastrinomas but progress, secreting additional peptides including glucagon, insulin VIP, calcitonin, somatostatin prolactin, and ACTH.

SPECIFIC BIOMARKERS OF NETS OF GASTROINTESTINAL ORIGIN

Stomach

There are 3 types of NETs that occur in the stomach, and the incidence of these tumors is much higher than was believed some years ago.

Type 1 is associated with atrophic gastritis and is related to the absence of negative feedback resulting from the achlorhydric stomach. Increased gastrin in the circulation continuously drives the ECL cell resulting in the development of ECL adenomas.

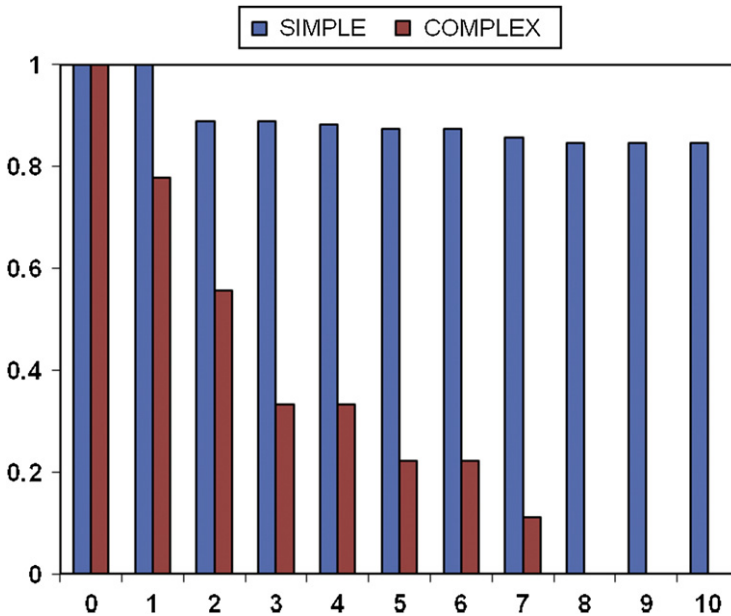


Fig. 2. Sporadic malignant gastrinomas and 10-year survival.

These tumors are small, multiple, and almost always benign. Gastrin is increased in the circulation of these patients as is CgA, similar to what can be observed with chronic potent PPI therapy (see earlier discussion).

Type 2 NETs of the stomach occur because of increased gastrin secretion caused by gastrinoma, most often MEN1. Gastrinomas in MEN1 are frequently multiple and small, and are often treated conservatively. Therefore, gastrin remains high in the circulation on a permanent basis. These patients are treated with PPIs to maintain a neutral pH in the stomach and remove the risk of ulcers and the associated problems. The drive to the ECL cell is increased through the lack of negative feedback in addition to the drive from the gastrinoma. In these patients both gastrin and CgA are increased in the circulation.

Type 3 NETs of the stomach are sporadic, usually solitary, and have a high proliferative index. Gastrin and CgA are not increased in the circulation of these patients although histologic examination confirms neuroendocrine tumor cell characteristics. Prognosis is poor for these patients, and the chance for metastatic spread to the liver is high at the time of diagnosis.

NETs of the Duodenum

Neuroendocrine tumors of the duodenum are rare and usually associated with MEN1. They most often secrete gastrin and, if malignant, may also secrete CgA. Very rarely, tumors of the duodenum may secrete PP or somatostatin or the tachykinins. These tumors may be benign and slow growing.

NETS of the Ileum and Proximal Colon

These tumors are the most common NETs located in the gastrointestinal (GI) tract and are generally small, often solitary, but multifocal in many cases. They secrete serotonin and are commonly referred to as midgut carcinoid (MGC) tumors. As

well as serotonin, they secrete CgA and the tachykinins, especially neurokinin A (NKA) and substance P (SP). As the primary tumor may be small, they often go undetected and the patients may present with metastatic disease, which is associated with the typical carcinoid syndrome of flushing and diarrhea, which may be accompanied by palpitations, night sweats, bronchoconstriction, abdominal pain, pellagra, and over time, right-sided heart disease. These symptoms are a result of the high concentrations of serotonin and the tachykinins that circulate when disease has metastasized to the liver. A significant number of patients, however, present with early disease as the secretory products from the tumor causes local fibrosis and a desmoplastic reaction in the mesentery. This may result in intermittent or acute obstruction even when the primary lesion remains very small. These patients present for surgery without having had circulating biomarkers measured and only postoperative testing is possible. Biomarkers may decrease to within the reference range for CgA, serotonin, and the tachykinins giving a false sense of security of surgical cure when there is no evidence of hepatic spread and local lymph nodes have been dissected with good results. However, it should be noted that surgical cure in these patients is rare and long-term follow-up is essential.

Serotonin has been difficult to measure reliably in serum. Therefore the measurement of its metabolite, 5-hydroxyindole acetic acid (5HIAA) in urine, is the method of choice for assessing serotonin concentration, especially if liver metastasis is suspected, and a 24-hour collection of urine is made. Foods that are high in serotonin and tryptophan must be excluded from the diet for 24 hours preceding and during urine collection. Drugs that affect tryptophan metabolism interfere in the assay also making results unreliable. Some centers measure serum serotonin, but restriction with certain foods and drugs remains important. Recently, more reliable and reproducible serotonin assays have made it possible to monitor midgut carcinoid activity in whole blood.

There are 3 tachykinins that are secreted in humans: neuropeptide K, NKA, and SP. These are produced and secreted in high concentrations by NETs of the ileum and proximal colon.^{19,20} As tachykinins circulate in very low concentrations in normal healthy individuals and are increased significantly only in this condition, this offers an excellent alternative method of diagnosis for these tumors.¹⁹ Sensitive assays have been developed for NKA, which is much more stable in blood than SP.

NETs of the Appendix

NETs of the appendix are not uncommon but present at an early stage with appendicitis. With few exceptions, they are benign, are cured surgically, and do not require follow-up (except for those >2 cm in diameter or those sited on the lip of the appendix or invading the mesoappendix). For the small percentage of patients who do require follow-up, then the same range of biomarkers used to diagnose and follow tumors (MGC, urinary 5HIAA, CgA, and NKA) are suggested.

NETs of the Distal Colon and Rectum

It has become apparent that NETs of the distal colon and rectum are much more common than was originally believed. They are small, usually solitary, often benign, and are slow growing. Where a tumor is malignant and small with no metastatic spread to the liver, then again the circulating CgA is not significantly increased. A proportion of these tumors secrete pancreatic polypeptide tyrosine (PPY). PPY assays are commercially available in Europe and in the United States. Some PP assays cross-react with PPY and indicate increased concentrations in these patients. Some NETs of the distal

bowel may secrete somatostatin. When a biomarker is found for a particular patient, then this remains a helpful tool to monitor disease.

Pancreastatin is increased when tumors have metastasized to the liver only.⁸ It seems to reflect early liver tumor activity.⁹

NETs of Meckel diverticulum are surprisingly common in those individuals who have this particular anatomic abnormality. By the time of diagnosis these tumors have usually metastasized. They secrete CgA and more than half also secrete gastrin.

Secretory products of NETs of the GI tract are shown in **Table 2**.

MEASUREMENT OF CIRCULATING BIOMARKERS FOR NETS

Regulatory peptides have traditionally been measured by radioimmunoassay. This method has offered specificity and sensitivity. With the endeavor to minimize the use of radiochemicals in the laboratory, enzyme-linked immunoassay (ELISA) has been used increasingly and these assays have improved in sensitivity in recent years. There are many commercial assays available for CgA and for the specific peptides biomarkers; many diagnostic laboratories use in-house assays. There are no international standards available and few quality assurance systems, except for insulin and gastrin, thus very strict internal standardization and quality assurance must be in place and only accredited laboratories should be used. There are numerous assays available for the measurement of circulating tumor biomarkers for NETs and many reliable commercial kits.^{20,21}

Specimens should be collected a minimum of 6 hours post prandial for the measurement of insulin, gastrin, glucagon, and PP. When CgA and/or gastrin are assayed, it is important that information about PPI therapy is available. Some additional clinical information accompanying the request may be helpful for the diagnostic laboratory as this assists with interpretation of results. In particular, it is important to know if the patient is being treated with somatostatin analogues as this therapy suppresses the secretion of CgA and the regulatory peptides (**Fig. 3**).²²

Tumor Site	Syndrome	Symptoms	Biomarkers
Gastric (type 1)	None	Upper GI	CgA gastrin
Gastric (type 2)	Usually	Upper GI	CgA gastrin
Gastric (type 3)	None	Upper GI	CgA ^b
Duodenal	Zollinger-Ellison (G)	Epigastric pain (G), peptic ulcer (G), diarrhea (G)	CgA gastrin (>50%), PP (35%), Som (<10%)
Ileal	Carcinoid	Diarrhea, flushing, sweating	CgA, serotonin, NKA, and SP
Appendix	Carcinoid ^a	Diarrhea, flushing, sweating ^a	CgA, ^a serotonin, ^a NKA, and SP ^a
Rectal	None	None	CgA, ^a PYY(10%)
Meckel diverticulum	Zollinger-Ellison (G)	Epigastric pain (G), peptic ulcer (G), diarrhea (G)	CgA, gastrin (>50%)

Abbreviations: G, when gastrin is secreted; Som, somatostatin.

^a Only if metastatic (appendiceal tumors are rarely metastatic).

^b CgA may not be secreted if tumor is very poorly differentiated.

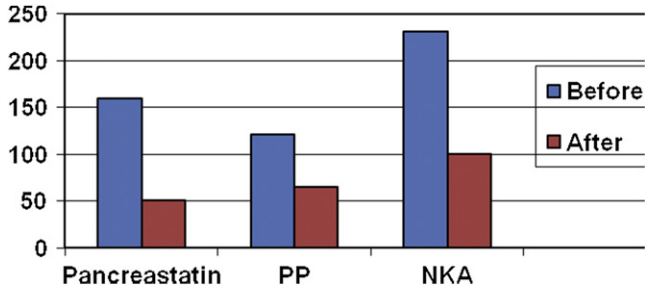


Fig. 3. Circulating biomarkers (ng/L) in MGC before and after octreotide (50 µg).

As CgA and regulatory peptides (with the exception of insulin) are processed through the kidney, patients with renal impairment have increased concentrations of these molecules in the circulation. This makes interpretation of results from patients with renal impairment almost impossible.

LABORATORY DIAGNOSIS, MONITORING TREATMENT, AND DETECTING RECURRENT DISEASE

There has been some discussion already regarding the limitations of CgA and regulatory peptides in the diagnosis of NETs. CgA is the only general circulating biomarker for NETs but is poor because of the problems with false-positives. However, it is important to establish a baseline concentration so that treatment and recurrence may be monitored. Gastrin measurement is also inadequate as a sole diagnostic tool for gastrinoma but pretreatment values are required to assess success of treatment or to detect recurrent disease.

There are many advantages to using circulating biomarkers to monitor treatment or detect early recurrence of disease for NETs. The ease of frequent repeat testing, the noninvasive nature of testing and the relatively low cost are persuasive arguments for the ready use of biomarkers for follow-up.

Fig. 4 shows circulating gastrin response to surgery, streptozotocin, and 5-fluorouracil repeated for 3 treatments and then followed by hepatic artery embolization on 2 occasions in a single patient.

The sensitivity of circulating biomarkers to detect changes is most important. As liver metastases may be small and multiple, measurement of circulating biomarkers

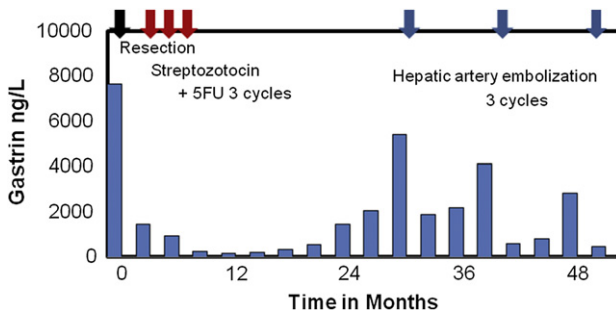


Fig. 4. Gastrin monitoring in a single patient with gastrinoma in a 4-year period during several treatments.

offers earlier indication of advancing disease than radiological techniques. With the increasing options for treatment of liver metastases and disseminated disease, early indication of progression is important.

When disease is inoperable, then circulating biomarkers can indicate stable disease and controlled secretion of active peptides. Retesting at frequent intervals reassures the physician of their choice of treatment. **Fig. 5** illustrates control of MGC achieved for a 3-year period in a single patient with advanced disease who did not respond to somatostatin analogues (SST) alone, but was controlled for more than 7 years when alpha interferon was administered concomitant with SST.

PROGNOSTIC INDICATORS

Because of the variability of tumor progression in NETs from indolent to aggressive and the unpredictable nature of these tumors, prognostic indicators are sought. This makes it possible to avoid over-treating slow-growing tumors and not neglect tumors that will progress rapidly. The window of opportunity for treatment options in aggressive tumors may be narrow.

Several studies have analyzed retrospective data that have resulted in the accumulation of information about several potential prognostic indicators. Age, depth of penetration into the mucosa for tumors in the lumen, metastatic spread, metastatic volume or number of metastases,^{23–25} proliferative index,²⁶ and circulating biomarkers²⁷ including CgA, pancreastatin and for tumors of the ileum and proximal colon, serotonin concentrations, urinary 5HIAA excretion, and circulating NKA have all been identified as prognostic indicators for tumors of the midgut.¹⁹

Prognostic indicators identified at surgery or by biopsy relate to a single point in time and it is not possible to assess changing prognosis.²⁶ The proliferative index (Ki67 or MIB⁻¹) may change with time, and it may not be possible to perform repeat biopsies. Circulating biomarkers, therefore, remain an attractive option for assessing prognosis and for reassessing a potential change in prognosis after treatment. Chromogranin A has been identified as a prognostic indicator with a CgA level greater than 1000 indicating poor prognosis. Pancreastatin has also been used as a prognostic indicator and rapidly increasing pancreastatin measured in an assay that does not cross-react with total molecule CgA has been identified as an indicator of active progressive liver

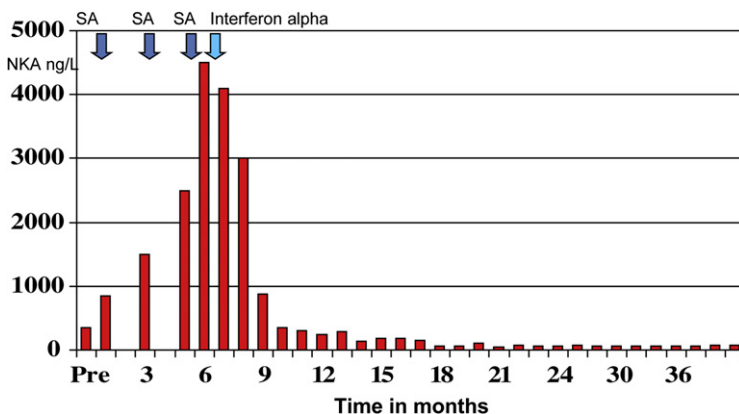


Fig. 5. Circulating NKA used to monitor disease in a single patient with MGC during treatment with somatostatin analogues and alpha interferon.

activity.^{8,9} This may be more sensitive than CgA in most patients with extensive metastatic disease. Circulating CgA remains high, more than 30 times more than the reference range.

For the relatively common tumors of the ileum and proximal colon, which frequently have a proliferative index less than 2% yet have a very variable disease progression, both increased urinary 5HIAA and increased NKA have been identified as independent indicators of poor prognosis. NKA is sensitive and specific, and it has also been shown, in a retrospective study, that the most recent NKA estimation is the most accurate indicator of prognosis.¹⁷ Early reports from a prospective study indicated that decreasing NKA in patients with MGC offers longer survival times and it is postulated that circulating NKA not only reflects tumor bulk but also tumor activity.²⁸

Circulating biomarkers offer a useful diagnostic tool in conjunction with radiology and tissue pathology for NETs. However, these biomarkers are more reliable when used to monitor disease progression, response to treatment, and for early indication of recurrence after treatment. The increasing use of CgA and pancreastatin as prognostic indicators for NETs in general and the use of NKA for tumors of the midgut in particular offers great potential to assist in decision making for earlier treatment with newly presenting patients and for those with recurring or advancing disease. Although the list of circulating biomarkers that are useful to the clinician who manages these patients is small, they offer readily available and potentially frequent information with noninvasive technology. Additional circulating biomarkers should be sought, particularly in the light of new cellular pathways being explored as therapeutic targets and the potential of personalized treatments becoming a reality.

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