Endocrine-Related Cancer (2003) **10** 459–462

The importance of the measurement of circulating markers in patients with neuroendocrine tumours of the pancreas and gut

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Abstract

The measurement of general and specific biochemical markers in patients with neuroendocrine tumours assists with diagnosis and gives an indication of the effectiveness of treatment and they may be used as prognostic indicators. There is much agreement that chromogranin A is the most universally helpful marker; it is found to be elevated in the circulation of about 90% of patients with metastatic neuroendocrine tumours and there are several excellent commercially available kits which give reliable estimations. Specific markers are useful for diagnosis also, and are helpful indicators of the effectiveness of treatment, particularly where tumour bulk may not change as much as tumour activity. Sporadic pancreatic neuroendocrine tumours may secrete more than one peptide and this indicates a worsening prognosis. Because of the wide variation in the progression of neuroendocrine tumours, a prognostic indicator gives a significant advantage to the clinician in order to facilitate optimum treatment at the optimum stage of disease. Both chromogranin A and neurokinin A have been used as powerful prognostic indicators for midgut carcinoid tumours.

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Introduction

The measurement of tumour markers in the circulation of patients with neuroendocrine tumours of the gastrointestinal tract is of threefold importance. First, it establishes the diagnosis, secondly, it is useful in monitoring progression of disease and response to treatment and thirdly, it may assist as a prognostic indicator. Neuroendocrine tumours may present early where there is an overt syndrome. In particular this is true of the pancreatic lesions, gastrinoma, insulinoma and VIPomas (tumours producing vasoactive intestinal polypeptide (VIP)). However, the majority of pancreatic neuroendocrine tumours are silent in the early stage and may eventually present with the burden of tumour mass, which is the case with non-functioning tumours. Carcinoid tumours of the appendix cause appendicitis and are removed at an early stage. They, almost invariably, are not metastatic at the time of resection and cause no further problem. A significant number of ileal carcinoids present by causing obstruction and are resected as an emergency. Many will have metastasised to adjacent lymph nodes or to liver at that time. Even those where there is no indication of metastatic disease at surgery will require monitoring, and this is most easily pursued using blood testing for the relevant biochemical markers. Almost all carcinoid tumours (with the exception of appendicial carcinoids) even after resection, progress eventually. The majority of carcinoid tumours, however, present with carcinoid syndrome, flushing and diarrhoea with or without carcinoid heart disease and bronchial constriction.

Because of the infrequency of neuroendocrine tumours, screening is not a consideration, but it is helpful to screen all patients who present with flushing or persistent, unexplained diarrhoea for carcinoid disease, gastrinoma and VIPoma. Patients with persistent peptic ulcer disease in the absence of *Helicobactor pylori* should be assessed for gastrinoma.

Biochemical diagnosis

The chromogranin (Cg) family of glycoproteins, CgA, CgB and CgC, are stored, along with peptide hormones, in the granular vesicles of neuroendocrine and endocrine cells. Raised concentrations of the Cgs, particularly CgA, are found in the circulation of patients with neuroendocrine tumours

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which have metastasised to liver (O'Connor & Deftos 1986). Wilander *et al*. (1989) reported that concentrations of CgA, pancreatic polypeptide (PP) and human chorionic gonadotrophin (hCG) are all found to be raised in the circulation of patients with carcinoid tumours and CgA and PP are raised in patients with endocrine pancreatic tumours. CgA is, however, considered to be the best general marker for neuroendocrine tumours, particularly the carcinoid group (Öberg 1997, Tomossetti *et al*. 2001). It is raised in pancreatic neuroendocrine tumours, where more than 92% of active gastrinomas have been shown to have elevated CgA (Goebel *et al*. 1999), and also in more than 60% of patients with multiple endocrine neoplasia type 1(MEN-1) (Öberg & Skogseid 1998). CgA is a large molecule and may be processed differently by different tumour cells. There is much discussion as to the most helpful measurement of the molecule in the circulation of tumour patients. Pancreastatin (CgA240–288), a mid-portion of the CgA molecule isolated by Tatemoto *et al*. (1986), was the first region of CgA to be assayed, but this measurement has been superseded by assays directed to other regions of the molecule. There are numerous assay kits for CgA. The DAKO Chromogranin A ELISA Kit is directed towards a 23 kDa fragment of C-terminal human CgA and is widely used. EURIA-CHROMOGRANIN A, an RIA that uses an antibody raised to CgA116–439 (Stridsberg *et al*. 1993) is also widely used. A comparison of three commercially available CgA assays has been made by Stridsberg *et al*. (2003) with the conclusion that in a group of 77 patients all three kits that were tested were satisfactory but with some variation of sensitivity and specificity (Table 1). An international reference standard would help to achieve uniformity between centres and methods.

In recent years, assays have been developed which generate specific fragments of CgA using an enzyme, generally trypsin (Hogg 1998). These assays have the advantage of measuring total CgA in the circulation, independently of the processing within the tumour cells, but have the disadvantage of being technically laborious.

Specific markers for pancreatic tumours

Insulinomas, gastrinomas and VIPomas frequently present with circulating concentrations of the relevant peptide raised only marginally above the reference range. Larkin *et al*. (1998) reported almost 10% of gastrinomas presented with fasting gastrin concentrations raised by less than 30% above those in normal healthy controls. Tartaglia *et al*. (2003) also observed a number of these normally 'loud' tumours that have presented with fasting concentrations of peptide within the normal reference range. In such instances it is necessary to use a provocative test to stimulate the concentration of the specific hormone. A secretin stimulation test confirms gastrinoma in 95% of cases (McGuigan & Wolfe 1980). A supervised 72 h fast is used for the diagnoses of insulinoma, where hypoglycaemia in the face of rising insulin concentrations is diagnostic. In the vast majority of insulinomas, gastrinomas and VIPomas the relevant peptide circulates at a concentration several fold above the normal range.

Table 1 Comparison of three commercially available kits for the measurement of CgA (Stridsberg *et al.* 2003)

Commercial chromogranin kit	Sensitivity (%)	Specificity (%)
CGA-RIA CT, CIS Bio International France	67	96
DAKO Chromogranin A Elisa, DAKO A/S Denmark	85	85
CGA-RIA, EuroDiagnostica Sweden	93	88

Specific markers for carcinoid tumours

Midgut carcinoid tumours are characterised by the production of serotonin. Serotonin, however, has proved difficult to measure reliably in the circulation. Traditionally the excretory products of serotonin, 5-hydroxy-indoleacetic acid (5HIAA) and 5-hydroxy-tryptamine have been measured in a 24 h urine collection from patients suspected of carcinoid. Patients must exclude items that are rich in serotonin from their diet before and during the urine collection. Three-quarters of patients with midgut carcinoid excrete urinary 5HIAA (U-5HIAA) as do approximately one-third of patients with foregut carcinoid. Patients with hindgut carcinoid do not excrete these products. An assay for serum 5HIAA has been described which shows similar specificity, sensitivity and diagnostic potential to the urinary assay but has the advantage of being more acceptable and convenient for the patient (Degg *et al*. 2002).

A wide range of peptides that are found in the circulation of patients with carcinoid tumours have been described (Table 2) (Öberg *et al*. 1999). Raised neurokinin A (NKA) has been found in the circulation of 81% of metastatic midgut carcinoid patients (Turner *et al*. 2002).

Assessment of the progression of neuroendocrine tumours

Tumour imaging is frequently used to assess the success of treatment. However, the volume of tumour may remain unchanged while the activity is decreased or indeed increased. CgA or specific markers are useful indicators of change in tumour activity (Eriksson & Öberg 1991). Table 3 illustrates the effect of treatment on circulating gastrin in two patients with sporadic gastrinoma, over a period of 6 years. Only a small number of the plasma estimations are included in the Table for simplification.

Table 2 Circulating peptides in carcinoid disease: percentage positivity

Peptides and markers	Foregut	Midgut	Hindgut
U-5HIAA*	31	76	0
CGRP*	45	0	0
GRP*	52	0	0
PP*	30	25	25
PYY*	0	0	30
Somatostatin*	0	0	35
hCGα*	35	11	100
N Peptide K*	15	46	25
Neurokinin A**	—	81	—
CgA pancreastatin**	50	53	80
CgA (DAKO)***	—	85	—
CgA (EURIA)***	—	93	—
CgA (CIS)***	—	67	—

CGRP, calcitonin gene-related peptide; GRP, gastrin releasing peptide; N peptide K, neuropeptide K; PYY, peptide YY.
*Öberg *et al.* (1999).
**Turner *et al.* (2002).
***Stridsberg *et al.* (2003).

Table 3 Circulating plasma gastrin concentrations (ng/l) in two gastrinoma patients across several treatments over a 6-year period

Treatment	Patient 1	Patient 2
—	4355	340
—	4550	695
—	4750	1575
Streptozotocin	Yes	Yes
	1800	23
	3600	950
Hepatic embolisation	Yes	Yes
	180	540
	1060	5760
Hepatic embolisation	Yes	—
	450	—
	560	—

Prognostic indicators for neuroendocrine tumours

Although it is generally considered that neuroendocrine tumours progress comparatively slowly, the course of disease is unpredictable. Some tumours, even with considerable metastatic spread, are indolent while others follow a more aggressive course with rapid spread and short survival. Prognostic indicators allow clinicians to decide when treatments are appropriate. The importance of treating rapidly progressing tumours as quickly and aggressively as possible cannot be overestimated.

Carcinoid tumours

The concentration of circulating CgA has been used as a prognostic indicator for midgut carcinoid where CgA has been shown to reflect tumour mass (D'Herbomez & Gouze 2002). It has been shown that where CgA concentrations are greater than 5000 μg/l (75 nmol/l) prognosis is significantly poorer than in patients with CgA levels lower than this threshold ($P < 0.01$). The survival at 5 years in the group with high CgA is 22%, whereas it is 63% in the group with lower CgA (Janson & Öberg 1996).

Turner has shown in a retrospective study using a clinical database comprising 117 midgut carcinoid patients that NKA is an excellent prognostic indicator. In the patients who presented with NKA > 50 ng/l (45 pmol/l) survival at 5 years was 18%, whereas in the group who presented with NKA levels lower than this threshold the survival at 5 years was 70% ($P < 0.005$).

Pancreatic tumours

Pancreatic endocrine tumours fall into two groups, MEN-1 and sporadic tumours. MEN-1 patients frequently present with several different tumours in different organs, and also with the production of more than one peptide from within a single tumour. In this group of patients this is not indicative of poor prognosis. Sporadic neuroendocrine tumours also possess the ability to produce more than one peptide or hormone, and in contrast to the MEN-1 group this indicates a worsening prognosis. In a group of 50 malignant gastrinoma patients in Northern Ireland, 20% were MEN-1 and presented with a median fasting gastrin of 600 ng/l. The median fasting gastrin at presentation for the sporadic gastrinoma group was 590 ng/l. Twenty-two per cent of the sporadic group progressed to secrete additional peptides and hormones beyond the general tumour markers (CgA and PP) which included insulin, glucagon, VIP, adrenocorticotrophin and prolactin. These patients initially presented with a median fasting gastrin of 1100 ng/l. The survival at 5 years was 22% for these patients, whereas the survival at 5 years for the group who continued to secrete only gastrin and the general tumour markers was 87% (Table 4).

Standardisation and quality control of assays

There is a necessity for standardisation of the assays and laboratories that offer the measurement of plasma and serum markers for neuroendocrine tumours. This is particularly true for the assay of CgA, where assays are directed to specific regions of a large protein, which may or may not be processed by tumour cells, and may give quite different results depending on the particular assay used. At present, until international reference standards are available it must be recommended that the same laboratory, or at the very least the same assay, should be used for monitoring any individual patient over a period of time. This may not always be

Table 4 Survival data relating to patients with gastrinomas who progressed to produce additional peptides along with gastrin and the general markers contrasted with those who did not

	MEN 1 gastrinoma	Spontaneous gastrinoma	Spontaneous gastrinoma
Plasma gastrin at presentation (ng/l)	600	430	1100
Production of CgA and/or PP	100%	100%	100%
Production of additional hormones	60%	0%	100%
Five-year survival	90%	87%	22%

straightforward as many patients with neuroendocrine tumours may survive for many years.

Conclusion

In conclusion, the measurement of general and specific markers offers important information for the clinician treating patients with gastrointestinal neuroendocrine tumours. Not only does this information assist with the initial diagnosis but it also offers an easy means of monitoring patients who have inactive disease or who are undergoing treatment. Several of the markers are good prognostic markers for both carcinoid and pancreatic disease.

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