

Original article

Clinical significance of blood chromogranin A measurement in neuroendocrine tumours

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Summary

Background: Tumour marker measurement gives clinicians useful information for the follow-up and management of patients with neuroendocrine tumours (NETs). The currently used tumour markers for NETs are neuron-specific enolase (NSE) and chromogranin A (CgA). The clinical accuracy of these biomarkers depends on histotype and disease extent. CgA is thought to be the optimal marker for most NETs, as it is independent of the biological characteristics of the tumour.

Aim of the study: In this study we investigated the value of CgA assessment with respect to the other biomarkers in the diagnosis and follow-up of patients with different types of NETs.

Patients and methods: We measured CgA, NSE, carcinoembryonic antigen (CEA) and urine 5-hydroxy-3-indoleacetic acid (5-HIAA) in 290 patients with 127 gastroenteropancreatic (GEP) tumours, 49 neuroblastomas, 36 lung tumours, 24 medullary thyroid carcinomas (MTCs), 15 pNETs, 12 paragangliomas, 7 Merkel's cell carcinomas (MCCs) and 20 NETs of unknown origin. CgA and 5-HIAA were quantitated by immunoenzymatic assays, while NSE and CEA were determined by radioimmunoassays.

Results: The biomarkers' specificity in GEP tumours was 86% for CgA, 100% for NSE, 91% for CEA and 100% for 5-HIAA. The corresponding sensitivity was 68% for CgA, 33%

for NSE, 15.4% for CEA and 35% for 5-HIAA. The sensitivity of CgA largely depends on disease extent or presence of functioning tumours and is highest in metastatic and syndromic patients. CgA determination in GEP tumour monitoring is useful to evaluate the response to therapy and to follow up patients with liver metastases. In neuroblastomas the overall specificity of NSE and CgA was 50% and 83%, respectively. In these tumours NSE sensitivity was close to 90% in all clinical stages, while the sensitivity of CgA depended on clinical stage (50% for stage I and II, 60% for stage III and 100% for stage IV tumours). Also in this type of tumour changes in CgA levels correlated with objective response. In paragangliomas CgA measurement may provide useful clinical information. Measurement of CgA is of use in the diagnosis of lung carcinoids, while its value in MTCs, pNETs and MCCs is very limited.

Conclusions: CgA was confirmed to be the best tumour marker currently available for identifying patients suffering from NETs of the GEP system, lung carcinoids and neuroblastomas. CgA evaluation is recommended in the follow-up of patients with such tumours.

Key words: carcinoid, chromogranin A, microcytoma, neuroblastoma, neuroendocrine tumours, paraganglioma, peripheral neuroectodermal tumours

Background

Tumour marker measurement provides the clinician with useful information for the follow-up and management of patients with neuroendocrine tumours (NETs). The hormones released by functioning tumours are currently used as biomarkers, but there is a need for accurate and sensitive markers also for biochemically silent tumours. In the latter group of NETs the currently used tumour markers are neuron-specific enolase (NSE) and the chromogranins [1–5]. Among the latter glycoproteins, chromogranin A (CgA) proved to be the most specific marker for the identification of NETs [1, 2, 5–8].

We previously reviewed the value of CgA measurement in NETs [9–11] and the fundamental role played by this marker in the follow-up of patients treated for gastroenteropancreatic (GEP) NETs [12, 13]. In the present study we investigated the value of plasma CgA assessment with respect to other biomarkers used for the

diagnosis of NETs in a large number of patients with different types of endocrine tumours. We studied circulating CgA levels for the diagnosis of GEP tumours, lung carcinoids, lung microcytomas, paragangliomas, medullary thyroid carcinomas (MTCs), neuroblastomas, Merkel's cell carcinomas (MCCs) and peripheral neuroectodermal tumours (pNETs). In addition, we evaluated blood CgA levels during follow-up and the role of this marker as a predictor of the response to therapy.

Patients and methods

Patients

We measured CgA, NSE, carcinoembryonic antigen (CEA) and urine 5-hydroxy-3-indoleacetic acid (5-HIAA) in 290 patients with histopathologically assessed NETs (127 GEP tumours, 49 neuroblastomas, 36 lung tumours, 24 MTCs, 15 pNETs, 12 paragangliomas, 7 MCCs and 20 NETs of unknown origin).

Biomarker sampling and assessment of marker alterations

A number of patients had multiple examinations and globally we measured the tumour markers in 411 samples. All these patients underwent three or more examinations and had a clinical follow-up of at least six months. Arbitrarily, we considered as variations only those changes that exceeded previous values by 25%.

With regard to 5-HIAA quantitation, patients were instructed not to eat bananas, pineapples or nuts or drink tea or coffee, and were not to be given drugs containing methyl dopa or sympathicomimetic amines at least three days before the start of urine sampling.

Analyte measurements

Plasma CgA was measured by means of an enzyme-linked immunosorbent assay (ELISA) purchased from Dako A/S (Glostrup, Denmark). This ELISA uses rabbit antibodies to a 23 kDa carboxy-terminal fragment of human CgA and the analyte measuring range was 5 to 450 U/l. The analytical sensitivity of the assay was 2 U/l. The analytical precision of the ELISA was evaluated by calculating within- and between-assay coefficients of variation (CVs) on 10 replicates and 5 runs, respectively. Within-assay CVs ranged from 6.2% to 8.2% and between-assay CVs from 7.8% to 10.3% for samples with mean CgA concentrations ranging from 6.2 U/l to 312 U/l. ELISA's accuracy was assessed by means of linearity and recovery tests. The former test was performed on 10 serially diluted samples and the ratios between observed and expected values ranged from 92% to 110%. Recovery was calculated on 10 samples by adding different amounts of CgA and varied from 87% to 121%.

Serum neuron-specific enolase (NSE) evaluation was performed by an immunoradiometric assay (IRMA) supplied by AB Sangtec Medical (Bromma, Sweden). The monoclonal antibodies used in this assay are directed to the γ subunit of the enzyme, allowing the detection of both $\alpha\gamma$ and $\gamma\gamma$ enzyme dimers. The minimum detectable analyte dose was 0.5 $\mu\text{g/l}$, and the within- and between-assay CVs were 3.0% and 5.0%, respectively.

Serum CEA was measured by an IRMA purchased from Techno Genetics (Cassina de Pecchi, Italy). This assay had an analytical sensitivity of 0.1 $\mu\text{g/l}$, and the within- and between-assay CVs were 4.0% and 5.8%, respectively.

In the patients with carcinoid syndrome 5-hydroxy-3-indoleacetic acid (5-HIAA) was evaluated in 24-hour urine samples by an ELISA supplied by ICN Biomedicals (Opera, Italy). The lowest measurable level was 0.2 mg/l in 24-hour urine samples. The within- and between-assay CVs were 7.7% and 9.4%, respectively.

Biomarker cut-off values

We measured CgA and NSE in the blood of 103 blood donors (76 males and 27 females, median age 47 years, range 31 to 65) in order to establish our own range of expected values. The values corresponding to the 95th percentile of our series were taken as biomarker cut-off levels (34 U/l and 12.5 $\mu\text{g/l}$ for CgA and NSE, respectively).

The CEA cut-off value was 5.0 $\mu\text{g/l}$ as established on the basis of the 95th percentile of a non-smoking population. For 5-HIAA we assumed a cut-off level of 10.0 mg/l in 24-hour urine samples.

Statistical analysis

Simple descriptive statistics were used in describing the variables. Whenever the obtained data were not normally distributed, the non-parametric Mann Whitney U-test was used for between-group comparisons. When the relationship between discrete values was examined, chi-squared analysis was performed.

In all statistical tests a 5% level of significance was used. The software SPSS[®] for Windows[™] release 6.0 by SPSS Inc. (Chicago, Illinois) was used for data management and statistical elaboration.

Results

Gastroenteropancreatic tumours

To assess the clinical specificity and sensitivity of CgA we examined 127 patients, 21 of whom without any evidence of disease and 106 with locoregional or metastatic disease (16 and 90 cases, respectively). Tumour-related syndromes were demonstrable in 38 patients.

Statistical analysis showed that CgA and NSE levels were significantly different in patients with and without disease (Mann–Whitney U-test, $P = 0.00003$ for CgA and $P = 0.00240$ for NSE, respectively). The CEA concentration did not differ in the two patient subgroups.

When the above-mentioned cut-off values were adopted, the clinical specificity was 86% for CgA, 100% for NSE, 91% for CEA and 100% for 5-HIAA. With the same cut-off levels the sensitivity of the markers in identifying patients with GEP tract NETs was 68% for CgA, 33% for NSE, 15.4% for CEA and 35% for 5-HIAA. It is worth noting that the sensitivity of CgA largely depends on disease extent or presence of functioning tumours, being highest in metastatic and syndromic patients (Table 1).

We evaluated plasma CgA also in 46 patients treated with bio- or chemotherapy and the biomarker reflected the clinical outcome very well since most of the patients experiencing an objective tumour response or stable disease had decreasing or stable CgA levels. CgA assessment is particularly useful in the follow-up of patients with liver metastases inasmuch as 100% of patients with progressive disease showed an increase in blood CgA. Table 2 summarises these interesting findings.

Neuroblastomas

CgA and NSE were assessed in 49 patients with neuroblastomas, six of whom without any evidence of disease and 43 with stage I–IV tumours; no patient had stage IVs disease.

The overall specificity of NSE and CgA were 50% and 83%, respectively. The sensitivity of NSE was close to 90% in all patients and independent of tumour stage, while the ability of CgA to identify patients with neuroblastoma largely depended on clinical stage. The sensi-

Table 1. Chromogranin A sensitivity in patients with gastroenteropancreatic tumours according to disease extent.

Patients (number)	Positive/total	Sensitivity (%)
Locoregional (16)	6/16	37
Metastatic (90)		
Liver	46/59	78
Lung	4/5	80
Skeletal	6/9	67
Multiple sites	11/17	65
Syndromic (38)	26/38	68
Nonsyndromic (89)	47/89	53

Table 2. Chromogranin A changes in the follow-up of patients with gastroenteropancreatic tumours.

CgA assessment	Objective response		
	CR+PR	SD	PD
<i>Patients given bio- or chemotherapy (46 patients)</i>			
Stable levels	75% ^a	54%	17%
Diminished levels	25%	27%	–
Increased levels	–	19%	83%
Chi-squared test, probability	$\chi^2 = 20.56, P = 0.00039$		
<i>Patients with liver metastases (29 patients)</i>			
Stable levels	33%	69%	–
Diminished levels	67%	19%	–
Increased levels	–	12%	100%
Chi-squared test, probability	$\chi^2 = 25.84, P = 0.00003$		

Abbreviations: CR – complete response; PR – partial response; SD – stable disease; PD – progressive disease.

^a Percentage of patients.

tivity of CgA was 50% for stage I and II, 60% for stage III and 100% for stage IV tumours.

In addition, we measured CgA and NSE in 28 patients during treatment for their disease. The value of the two markers in monitoring response to therapy was highly similar (chi-squared test, $P = 0.92788$) and the observed circulating level variations correlated well with the patients' clinical status (Table 3).

Lung tumours

We measured circulating CgA in 36 patients with lung NETs (20 carcinoids and 16 microcytomas) and the overall biomarker sensitivity was 67%. The sensitivity of CgA in identifying patients with lung NETs was shown to be dependent on histotype: plasma CgA was elevated in 75% of patients with carcinoids and in only 56% of patients with microcytomas. For the latter neoplasms NSE remains the best tumour marker for diagnosis, as its level exceeds the cut-off value in about 70% of patients.

Paragangliomas

Blood CgA and NSE were also evaluated in 12 patients with paragangliomas, two of whom being disease-free and 10 having metastases or locally advanced disease. In both patients without any evidence of paragangliomas, CgA and NSE were below the cut-off levels. NSE is the best tumour marker for paraganglioma, being elevated in 80% of patients with this disease. CgA on the other hand did not seem a reliable marker since its level was raised in only 40% of the cases.

CgA measurement may give additional information during the monitoring of patients treated with ¹³¹I-meta-iodo-benzylguanidine (¹³¹I-mIBG) since, in our experi-

Table 3. Chromogranin A and neuron-specific enolase changes during the treatment of patients with neuroblastoma.

Biomarker assessment	Objective response		
	CR+PR	SD	PD
<i>Biomarker assessment (28 patients)</i>			
Chromogranin A	–50% ^a	–14%	+171%
Neuron-specific enolase	–66%	–4.3%	+109%
Chi-squared test, probability	$\chi^2 = 0.15, P = 0.92788$		

Abbreviations: CR – complete response; PR – partial response; SD – stable disease; PD – progressive disease.

^a Percentage of variation with respect to baseline.

ence, blood CgA concentrations correlate well with urine catecholamines.

Other tumours

Lastly, we assessed the clinical value of CgA measurement in other NETs including 24 MTCs, 15 pNETs, 7 MCCs and 20 metastatic NETs of which the primary sites were unknown. The clinical sensitivity of the biomarker was as follows: 24% for MTCs, 27% for pNETs, 43% for MCCs and 75% for tumours of unknown primary sites.

Conclusions

In our experience CgA is the best biomarker currently available to confirm the diagnosis of GEP NETs and its measurement is very helpful in monitoring patient with these neoplasms. As previously reported, circulating CgA levels accurately reflect the clinical status of patients with advanced disease, especially in the presence of liver metastases. No other tumour markers (NSE or CEA) were shown to be as accurate as CgA in identifying GEP NETs. It is worthy of note that CgA's sensitivity as a biomarker seems to depend in part on the secretory behaviour of the tumour, being highest in patients with syndromes. These findings are in agreement with our previous results [9–11, 13].

The accuracy of CgA for the diagnosis of neuroblastomas depends on the clinical stage of the disease. In fact, its sensitivity is quite low for stage I and II and considerably higher for stage IV tumours. Although NSE is a good biomarker for the diagnosis and prognosis of neuroblastomas [14], the measurement of CgA during chemotherapeutic treatment may be useful since changes in marker levels correlate with objective response.

As far as lung tumours are concerned, CgA assessment is useful only for carcinoids and its indication is similar to that for tumours of the GEP system. CgA measurement in microcytomas has no additional value with respect to NSE assessment.

In the diagnosis of paragangliomas CgA determination does not seem to add more information than NSE or catecholamine quantitation, but circulating CgA determination may play an ancillary role in evaluating the response to therapy in biochemically active tumours.

CgA's diagnostic sensitivity for MTCs is very low and its measurement should not be recommended for the management of patients with these tumours. Calcitonin and CEA are the most accurate markers for this type of neoplasm [15, 16].

Up to now no specific tumour marker for MCCs and pNETs has been identified, and plasma CgA assessment may therefore be utilised in the small number of patients having elevated levels of CgA.

Finally, the diagnostic value of CgA maintains its importance in the presence of metastases from NETs whose primary site is unknown. Seventy-five percent of patients in this condition have CgA levels above the cut-off.

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