Circulating chromogranin A in the assessment of patients with neuroendocrine tumours. A single institution experience


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Summary

Background: Chromogranin A (CgA) is a secretory protein present in dense-core vesicles of neuroendocrine (NE) cells. Its ubiquitous presence in NE tissues makes it a suitable circulating marker of neoplasms of NE origin.

Patients and Methods: Plasma CgA was determined in 178 patients with NE tumors and in 36 patients with non-endocrine malignancies. Circulating CgA was also serially evaluated in 39 NE cancer patients with advanced disease submitted to systemic therapy and in 14 patients with no evidence of disease (NED).

Results: Supranormal CgA values were found in 81% of patients with advanced NE tumors and in only 91% of NED cases. Plasma CgA in patients with well differentiated NE tumors, such as carcinoids, carcinoma of gastrointestinal tract, pheochromocytoma, pancreatic NE carcinoma (either functioning or not functioning), medullary thyroid carcinoma and NE tumors from various primary sites, was higher and more frequently elevated than in patients with small-cell lung cancer (P < 0.001). Plasma CgA did not discriminate patients with NE from those with non NE neoplasms since it was found elevated in 44% of the latter cases. Plasma CgA pattern correlated with the disease response in patients submitted to cytotoxic treatment and with changes in clinical symptomatology in patients receiving somatostatin analogs.

Conclusions: Our data confirm that CgA is the best circulating neuroendocrine marker available up to now available for the management of differentiated neuroendocrine malignancies irrespective of tumor location and functional status. CgA plasma levels could also identify the coexistence of neuroendocrine differentiation in the context of non-endocrine malignancies. Circulating CgA seems to be less useful in undifferentiated tumors such as small-cell lung cancer.

Key words: chromogranin A, neuroendocrine tumours

Introduction

Neuroendocrine tumors (NETs) represent a heterogeneous group of malignancies arising from the majority of organs and tissues of the human body. They encompass aggressive tumors such as small-cell lung cancer, low-growing tumors such as carcinoids, and tumors with intermediate aggressiveness [1, 2].

A neuroendocrine tumor is suspected when classical clinical symptoms occur but the large majority of NETs does not show any specific symptomatology [3]. Accordingly, the biochemical diagnosis is of great value. With the validation of radio-immunoenzymatic assays for various circulating peptide hormones in the last decade, clinical awareness of and ability to diagnose NETs increased [4-6]. However, due to the relative low incidence of NETs and the very large number of measurable hormones, clinicians need to know which measurable variables have an established clinical value and are cost-effective [7].

The glycoprotein Chromogranin A (CgA) is the best studied granin in humans. Its ubiquitous presence in the neuroendocrine tissue and its co-secretion with peptide hormones and neuropeptides makes it the more specific marker for tumors with neuroendocrine phenotype [8-11].

The possibility to measure CgA plasma levels by means of radio- or immunoenzymatic assay represents a tremendous step forward in the management of patients with NETs.

Plasma CgA levels were found elevated in a variety of NETs, including pheochromocytoma [12], carcinoid tumors, pancreatic islet cell tumors [13, 14], medullary carcinoma of the thyroid [15], small-cell lung cancer [16] and so forth [17-20].

The sensitivity and specificity of circulating CgA in any NETs vary between 70% and 95% [21]. The highest accuracy has been observed in tumors characterized by an intense secretory activity, but its specificity and sensitivity remain very high also in non-functioning tumors. Therefore, although CgA specificity cannot compete with that of the specific hormonal products secreted by many NETs, this molecule has very useful clinical applications in subjects with NETs for whom either no marker is available or the marker is inconvenient for routine clinical use [17-22].

The majority of studies published up to now involved
a rather small number of patients, and dynamic data during follow-up or treatment are rarely reported. In this paper we report the experience of our Institution on the usefulness of plasma CgA evaluation in the diagnosis and the follow-up of patients with NETs.

Patients and methods

Patients

Between January 1997 and September 2000, plasma samples of 178 patients by many institutions of the Piemonte Region with histopathologically and immunohistochemically assessed NETs were sent for the CgA measurement to the laboratory of the Clinica Medica at the Azienda Ospedaliera San Luigi, Orbassano, Italy. All patients had serum creatinine within normality (≤ 1.2 mg/dl). Plasma samples were also obtained from 36 patients with a variety of advanced neoplasms of non-endocrine origin. In order to establish our own range of expected values we performed CgA measurement in a population of 89 healthy people: 42 males and 47 females. In addition, because renal failure can increase circulating CgA concentrations, this marker was measured in 10 patients with chronic renal failure with serum creatinine between 3 and 5 mg/dl.

Patients were considered with no evidence of disease (NED) when there was no evidence of metastases or local recurrence after surgery and when surgical margin status was judged negative by the pathologist.

Fifty-three NETs patients had circulating CgA assessed serially. The monitoring of CgA values was performed in 14 NED patients during follow-up in order to search for eventual progressive disease and in the remaining 39 cases with advanced disease with the aim to monitor the efficacy of systemic therapy. Of these latter patients, 15 were submitted to somatostatin analogues with long acting formulation, 17 received chemotherapy and 7 both somatostatin analogues and chemotherapy.

The patients without evidence of disease underwent CgA measurement every year and had a clinical follow-up of at least three years. Conversely CgA assessment was performed on baseline conditions and every three months in the patients with advanced disease submitted to chemotherapy and/or somatostatin analogues.

Antitumor activity was evaluated every three months on all measurable lesions. Tumor response was classified according to WHO criteria [23] and documented by two investigations at least four weeks apart. A complete response (CR) was defined as complete disappearance of all clinical and radiographic evidence of disease for a minimum of one month. A partial response (PR) required a 50% or greater reduction in the sum of the products of the longest diameter and its perpendicular. Stable disease (SD) indicated a decrease of less than 50% or an increase of less than 25% in the product of the longest perpendicular diameters of measurable lesions lasting three months. Progressive disease (PD) was defined as the appearance of new lesions or an increase of ≥ 25% in the sum of the products of the longest diameter and its perpendicular, as compared with the lowest value recorded.

CgA response was defined as greater than 50% decrease in plasma CgA compared to baseline, CgA stabilization was defined as 50% or less decrease, or 25% or less increase and CgA progression was defined as greater than 25% increase.

Specimen collection and analytical methods

Blood was collected in heparin-containing vials. Plasma separation was achieved by centrifugation (2500 revolutions per minute for 15 minutes at room temperature of 4°C). The supernatant fluids were divided into aliquots and stored at −20°C until the day of the assay.

Plasma CgA was measured by means of an enzyme-linked immunoassorbent assay (ELISA) kit purchased from DAKO A/S (Glostrup, Denmark). The antibodies used in this ELISA are developed in the rabbit and are directed to a 23-kDa carboxy-terminal fragment of human CgA. These antibodies may detect intact CgA and also some CgA-derived peptides. The assay had an analytical sensitivity of 2.0 U/l. The within and between-assay coefficients of variation were 3.8% and 6.6%, respectively. The measuring range was 5 to 450 U/l.

The upper normal level was defined as the mean levels plus two standard deviations (SDs) of the distribution in the normal population.

Results

Cross sectional evaluations

Patients with endocrine tumors were 81 males and 97 females, median age of 56 years, range 16–88. The primary tumor localizations were as follows: pancreas, gastrointestinal tract, lung, adrenal, skin and thyroid. NETs located in unusual sites included breast, prostate, cervix, ovary, bladder and ethmoid. One hundred twenty-three (69%) had residual disease at surgery or distant metastases and 55 (31%) were without any evidence of disease. The sites of metastases were: liver in 46 patients, lymph node in 22, bone in 10, lung in 8, peritoneal in 6, brain in 3 and skin in 3.

The group of non-endocrine tumors consisted of 19 males and 17 females, median age of 57 years, range 41–77. They were bearing non small cell lung cancer (no. = 8), pancreatic adenocarcinoma (no. = 9), liver carcinoma (no. = 5), colon rectum carcinoma (no. = 6), adrenal carcinoma (no. = 4), ovary carcinoma (no. = 2) and breast carcinoma (no. = 2).

The CgA concentration in healthy subjects ranged from 3 U/l to 35 U/l, with a median value of 8 U/l and a mean of 8 + SD of 5. So that the upper normal value was calculated as 18 U/l. As shown in Table 1, the plasma concentrations were elevated in 81% of the patients with advanced neuroendocrine disease, while 91% of NED patients had CgA values within normality. Supranormal CgA levels did not differ comparing patients with carcinoids/carcinomas of the gastrointestinal tract with pancreatic tumors (either functioning or not functioning), phaeocromocitomas/paragangliomas and neuroendocrine tumors arising from different sites. The above-mentioned patients bearing well differentiated neuroendocrine tumors had CgA more frequently elevated than patients with small-cell lung cancer (SCLC) (chi-square 7.2; P < 0.001). Patients with advanced gastrointestinal carcinomas displayed the highest CgA values that attained the statistical significance when compared to patients with merkel carcinoma (P < 0.04), medullary thyroid carcinoma (P < 0.05) and SCLC (P < 0.001). There was no statistically differences comparing patients with gastrointestinal neuroendocrine carcinomas to those with carcinoids, pancreatic tumors, phaeocromocitomas/paragangliomas and neuroendocrine tumors of other primary sites.

In the 36 patients with non-endocrine tumors, the CgA concentration ranged from 5 U/l to 91 U/l, with a median value of 21 U/l and a supranormal rate of 44% (Table 1). However, mean CgA levels and % of supra-
normal CgA values in these patients were significantly lower than those of well differentiated neuroendocrine tumors (Mann–Whitney U-test \( P < 0.001 \); chi-square \( 21.8 \ P < 0.0001 \)). In patients with chronic renal failure the values of CgA were extremely high: > 459 U/l in all of them.

### Dynamic evaluations

None of the 14 patients without evidence of disease who underwent serial CgA measurements developed disease recurrence during the follow-up performed. Among the 42 samples on the whole obtained from these patients, only three resulted slightly elevated for CgA: 20, 24 and 25 U/l, respectively. These borderline marker values promptly returned to normality at a subsequent assessment performed 15–20 days later. Of the 24 patients submitted to chemotherapy ± somatostatin analogues, CgA levels showed a decrease (>50% from baseline) in 12 cases, remained unchanged in three, while increased in nine. CgA plasma levels decreased in all patients who obtained a clinical disease response, but also in 2 out of 4 cases with SD and in 2 out of 12 with PD (Table 2). All four patients who showed a disease progression but a CgA decrease had received a somatostatin analog in addition to chemotherapy.

All patients submitted to long-acting somatostatin analogues have previously had clinical and/or symptomatic progressive disease to subcutaneous octreotide. Long-acting somatostatin analog administration did not obtain any tumor response. SD was observed in eight cases and PD in the remaining seven (Figure 1). Five out of fifteen cases suffered from symptomatic disease: diarrhea in three cases and flushing in two.

### Table 1. Chromogranin A plasma levels in 178 patients affected of neuroendocrine tumours.

<table>
<thead>
<tr>
<th>Neuroendocrine gastroenteropancreatic tumours</th>
<th>Mean U/l</th>
<th>DS</th>
<th>Median U/l</th>
<th>Range U/l</th>
<th>CgA &gt; 18 U/l (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carcinoids</strong></td>
<td></td>
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<tr>
<td>Advanced (pts 30)</td>
<td>289 ±522</td>
<td>67</td>
<td>5–2020</td>
<td>93 (28/30)</td>
<td></td>
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<tr>
<td>NED (pts 36)</td>
<td>11 ±9</td>
<td>8</td>
<td>5–52</td>
<td>11 (4/36)</td>
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<tr>
<td><strong>Carcinomas</strong></td>
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<tr>
<td>Advanced (pts 23)</td>
<td>640 ±1077</td>
<td>277</td>
<td>9–4480</td>
<td>78 (18/23)</td>
<td></td>
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<tr>
<td>NED (pts 2)</td>
<td>11 ±6</td>
<td>11</td>
<td>7–16</td>
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<tr>
<td><strong>Neuroendocrine tumours of pancreas</strong></td>
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<tr>
<td>Non-functioning</td>
<td></td>
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<tr>
<td>Advanced (pts 12)</td>
<td>225 ±305</td>
<td>57</td>
<td>7–918</td>
<td>83 (10/12)</td>
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<tr>
<td>Functioning (insulinoma/gastrinoma)</td>
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<tr>
<td>Advanced (pts 6)</td>
<td>262 ±236</td>
<td>279</td>
<td>5–522</td>
<td>83 (5–522)</td>
<td></td>
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<tr>
<td>NED (pts 2)</td>
<td>16 ±5</td>
<td>16</td>
<td>12–20</td>
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<td><strong>Medullary thyroid carcinoma</strong></td>
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<tr>
<td>Advanced (pts 6)</td>
<td>103 ±191</td>
<td>32</td>
<td>5–492</td>
<td>66 (4/6)</td>
<td></td>
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<tr>
<td>NED (pts 7)</td>
<td>11 ±5</td>
<td>12</td>
<td>4–16</td>
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<tr>
<td><strong>Pheochromocytoma/paraganglioma</strong></td>
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<tr>
<td>Advanced (pts 11)</td>
<td>197 ±153</td>
<td>259</td>
<td>16–459</td>
<td>90 (10/11)</td>
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<tr>
<td>NED (pts 4)</td>
<td>8 ±6</td>
<td>5</td>
<td>5–17</td>
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<td><strong>Merkel cell carcinoma</strong></td>
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<tr>
<td>Advanced (pts 4)</td>
<td>24 ±13</td>
<td>26</td>
<td>7–37</td>
<td>75 (3/4)</td>
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<tr>
<td>NED (pts 3)</td>
<td>9 ±8</td>
<td>5</td>
<td>5–18</td>
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<td><strong>Small-cell lung cancer</strong></td>
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<tr>
<td>Advanced (pts 22)</td>
<td>42 ±48</td>
<td>23</td>
<td>4–194</td>
<td>60 (13/22)</td>
<td></td>
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<tr>
<td><strong>Neuroendocrine tumours of other sites</strong></td>
<td></td>
<td></td>
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<tr>
<td>Advanced (pts 9)</td>
<td>107 ±176</td>
<td>41</td>
<td>12–566</td>
<td>77 (7/9)</td>
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<tr>
<td>NED (pts 1)</td>
<td>–</td>
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</table>

### Table 2. Relationship between response to treatment and CgA level modification.

<table>
<thead>
<tr>
<th>Response to therapy</th>
<th>Plasma CgA levels</th>
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<tbody>
<tr>
<td></td>
<td>Stable</td>
</tr>
<tr>
<td>CR+PR</td>
<td>8/8</td>
</tr>
<tr>
<td>SD</td>
<td>2/4 (50%)</td>
</tr>
<tr>
<td>PD</td>
<td>1/12 (8%)</td>
</tr>
</tbody>
</table>

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

\( P < 0.01 \) two-tailed Fisher exact test.
Three of these patients obtained symptomatic benefit. As far as CgA is concerned, this marker decreased in the three patients who obtained a symptomatic improvement, while remained unchanged in seven and increased in five (Figure 1).

Discussion

In the present single institution experience we confirm the great diagnostic accuracy of CgA in the diagnosis and follow-up of patients with neuroendocrine tumors. Plasma concentrations were measured in a large group of patients with neuroendocrine neoplasms arising from various primary sites and compared with those in a control group with non-endocrine neoplasms. The upper limit of normality (18 U/l) that we have calculated in our group of healthy subjects was the same as that proposed by the manufacturer. Circulating CgA was shown to be very sensitive and very specific for various types of NETs. A supranormal rate was found in about 80% of patients with local recurrence, distant metastases or residual disease to surgery, while it was found in less than 10% in NED cases. In these latter patients, the slight, borderline increased values returned to normal at subsequent measurements. The majority of our advanced cases had metastases and this could account for the higher sensitivity encountered in comparison to some published series [17–20]. When considering the subgroup with pancreatic neuroendocrine tumors, elevated plasma CgA levels were found irrespective to their functional status. These data confirm that this marker is of great value in tumors that are either not able to secrete hormonal products or release products that cannot be detected by current techniques. The very frequent elevation of CgA in patients with pheochromocytomas/paragangliomas confirms that it may be the marker of choice for these rare diseases, being more convenient than catecholamines either measured in plasma or in urine [22].

The highest CgA levels were noted in patients with metastatic carcinoid tumors and neuroendocrine carcinomas of gastrointestinal origin, and this confirms previous studies [6, 7, 17–20]. Conversely, the lowest values were found in patients with advanced SCLC. These data support the notion that CgA is less useful in undifferentiated neuroendocrine neoplasms [16].

It is noteworthy that elevated plasma CgA levels cannot differentiate between neuroendocrine and non-neuroendocrine neoplasms. Slightly elevated CgA levels, in fact, were identified in 44% of patients with advanced non-endocrine tumors, a proportion that was not so different from that of patients with SCLC. This finding was also observed in other studies [18, 20]. Neuroendocrine cells within non-endocrine tumors are often detected by immunohistochemistry. They are usually scattered distributed or multifocally located in small nests. The detection of elevated plasma CgA in non-endocrine tumors mainly indicates that there is a neuroendocrine differentiation and a proliferation of neuroendocrine cells at advanced stage of many carcinomas. The concept of neuroendocrine differentiation in non-endocrine tumors has been extensively studied in prostate cancer [24, 25], but it was less extensively investigated in other primary malignancies. The availability of a simple and specific circulating marker of neuroendocrine phenotype could facilitate clinical studies in this field.

There are some pitfalls in the interpretation of CgA levels. Among them, renal impairment is one of the most important. In our experience all the patients with chronic renal failure presented very high levels of CgA, thus suggesting that serum creatinine should always be measured concomitantly with plasma CgA.

In the present study, circulating CgA was found to be a reliable marker for the follow-up of patients with neuroendocrine tumors. CgA levels were within normality in almost all plasma samples obtained from NED patients. In advanced cases submitted to systemic treatment, a clear relationship was found between changes in CgA levels and disease response. This marker decreased in all patients showing a tumour shrinkage after cytotoxic treatment, increased in the great majority of patients showing progressive disease, and did not change in most cases depicting a disease stabilization. Discrepancies between tumor and biochemical changes in non-responding patients are attributable to the concomitant administration of somatostatin analogs. The correlation between CgA levels and tumor mass is lost during treatment with somatostatin so that CgA cannot be used as a marker of tumor response when a cytotoxic regimen...
is administered in combination with a somatostatin analog.

Our patients submitted to long-acting somatostatin analogs were previously treated with daily administration of subcutaneously somatostatin analog. This is the reason why CgA decreased only in 3 out of 15 treated cases. It should be noted that the changes in CgA paralleled the clinical outcome of the disease, as it decreased in patients obtaining symptomatic improvement and increased when tumor mass increased and/or clinical symptomatology worsened.

In conclusion, our data confirm the general view that CgA is the best circulating neuroendocrine marker available up to now. Its clinical application involves all differentiated NETs, irrespective of tumor location and functional status. This marker seems to be less useful in undifferentiated tumors such as SCLC. Supranormal CgA plasma levels allow the identification of the coexistence of neuroendocrine differentiation in the context of non-endocrine malignancies and this could have diagnostic, prognostic, and possibly therapeutic implications. A dynamic evaluation of this marker in the follow-up of NETs provides useful information on the disease recurrence in NED cases or on the treatment efficacy in advanced cases submitted to cytotoxic or biologic therapy.

References


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