General review

Chromogranin A assay in clinical practice

Dosage de chromogranine A en pratique clinique

M. d’Herbomez a,∗, b, C. Do Cao c, D. Vezzosi d, F. Borzon-Chasot e, E. Baudin f, et le groupe des tumeurs endocrines (GTE France)

a Département de médecine nucléaire, centre de biologie pathologie, CHRU, 59037 Lille cedex, France
b Faculté de médecine, université de Lille 2, Lille, France
c Clinique d’endocrinologie, hôpital Huriez, centre hospitalier de Lille, Lille, France
d Service d’endocrinologie, hôpital Larrey, 24, chemin de Pouvourville, TSA 30030, 31059 Toulouse cedex 9, France
e Fédération d’endocrinologie, hôpital neurocardiologique, 59, boulevard Pinel, 69677 Bron cedex, France
f Institut Gustave-Roussy, 39, rue Camille-Desmoulins, 94805 Villejuif cedex, France

Available online 9 June 2010

Résumé

Les chromogranines sont une famille de protéines localisées exclusivement dans les granules de sécrétion des cellules endocrines, neuroendocrines et des neurones. La chromogranine A (CgA) en est la principale protéine. La CgA se comporte comme une prohormone qui subit un processus de dégradation par protéolyse donnant naissance à des peptides actifs. Elle possède des fonctions auto, para, endocrine. C’est un marqueur très utilisé en immunohistochimie pour affirmer l’origine endocrine d’une tumeur. En dépit de l’absence de standardisation internationale, du défaut de définition précise des seuils diagnostiques, certains dosages sériques ou plasmatiques de CgA sont fiables. De nombreuses études cliniques ont montré l’intérêt du dosage de la CgA circulante dans l’approche diagnostique et pronostique des tumeurs neuroendocrines. Ce marqueur général présente majoritairement des concentrations sériques proportionnelles au volume tumoral. L’interprétation des taux de CgA tiendra des capacités sécrétoires propres aux différents types de tumeurs endocrines variables selon leur niveau de différenciation cellulaire et leur siège anatomique. Seront aussi à considérer les éventuelles sécrétions hormonales associées, l’existence d’une insuffisance rénale et/ou d’une hypergastrinémie. De nouvelles applications cliniques du dosage de CgA apparaissent dans l’évaluation du stress en réanimation et aussi dans l’appréciation du risque cardiovasculaire ainsi que de nouveaux dosages de peptides actifs.

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Mots clés : Tumeurs neuroendocrines ; Chromogranine A ; Dosage

Abstract

Chromogranins belong to the family of secretory chromogranin and secretogranin proteins. They are found in secretory vesicles throughout the neuroendocrine system. Chromogranin A (CgA) is the main component. CgA acts as a prohormone submitted to processes of degradation through which active peptides are generated. CgA has auto, para and endocrine functions. It is widely used as an immunohistochemical marker. Despite the lack of international standardization, and the lack of an accurate definition of the diagnostic cut-off levels, some CgA assays are reliable. Numerous studies have suggested that CgA determination may be of interest for the diagnosis and the follow-up of various endocrine tumors. Plasma levels of this general marker are proportional to tumor mass. The localization of the primitive tumor, the presence of associated hormonal secretions and possible renal failure and/or hypergastrinemia must be taken into consideration for proper interpretation of CgA levels. New clinical indications are emerging for the evaluation of stress in intensive care units and the assessment of cardiovascular risk. New assays estimating the concentration of active peptides are under development.

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Keywords: Neuroendocrine tumors; Chromogranin A; Assay

Abbreviations: NET, Neuroendocrine tumors; Cg, Chromogranin; CgA, Chromogranin A; CgB, Chromogranin B; CEA, Carcino embryonic antigen; NSE, Neuron specific enolase; PSA, Prostatic specific antigen; ACTH, Adenocorticotropic hormone; 5HIAA, 5-Hydroxyindol acetic acid; VIP, Vasoactive intestinal peptide; PP, Pancreatic polypeptide; SMS, Somatostatin; IMA, Immunometric assay; MEN, Multiple endocrine neoplasia; MN, Metanephrines; PPI, Protons pump inhibitors; CRP, C reactive protein; CT, Calcitonin; AA, Amino acids.

∗ Corresponding author.
E-mail address: m-dherbomez@chru-lille.fr (M. d’Herbomez).

0003-4266/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ando.2010.04.004
1. Introduction

NET constitute a heterogeneous group of rare tumors with a straightforward diagnosis, as long as it is systematically evoked. When investigating a clinical syndrome suggestive of a NET, the biological work-up must include not only the hormonal assays indicated by functional signs, but also a systematic assay of general markers. The pathology report then establishes the final diagnosis of NET. General markers of neuroendocrine differentiation include NSE and the adhesion marker (CD56), but the most widely ordered tests are for CgA and synaptophysin.

Some NET are classified as hereditary diseases (MEN type I and II, Von Hippel Lindau disease, Recklinghausen disease, tuberous Bourneville’s sclerosis). The treatment depends on the location of the tumor, its extent, its degree of differentiation [1–5].

In this paper written a decade after the emergence of CgA assays in clinical practice, we propose a review of their performances for diagnosis and as markers providing prognostic information for the follow-up of patients with NET. Potential avenues of development will also be discussed.

2. Granins

Granins are proteins found in dense core secretory granules of most endocrine and neuroendocrine cells as well as central and peripheral neurons. There are two subfamilies: Cg, mainly A and B, and secretogranins II to VI. Their number continues to grow. Secretogranins I and II are also known as CgB and CgC. In the research literature, 1B1075, HISL-19, 7B2 and NESP55 are headings used to designate secretogranins III to VI [6].

Granins act as osmotic stabilizers and can bind a considerable amount of calcium. They play an important role in intracellular calcium homeostasis [7]. Cg have auto, endo and paracrine functions [6].

2.1. Chromogranin A: prohormone

It was in 1965 that Banks and Helle proved that acetylcholine stimulation leads to cosecretion of a specific protein from the chromafin granules together with catecholamines from the adrenal gland [7]. This high molecular mass soluble protein identified with immune sera was called CgA by Herman Blaschko’s group [8].

In the human genome, the gene that codes for CgA is located on chromosome 14. There are eight exons and seven introns. CgA has a molecular mass of 49 kDa and is made up of 439 residues of which 25% are acid AA. Numerous dibasic sites are potential points for cleavage of the molecule. The N-terminal end of CgA has a structure composed of two cysteine residues (positions 17 and 48) linked by an S-S loop which plays a functional role [6,9,10].

Many studies have shown that CgA is a precursory molecule of biologically active peptides which means that it has a prohormonal function [11,12]. The N- and C-terminal ends of this molecule are subject to considerable proteolysis due to the action of convertases or specific endoproteases [13]. Cg degradation is tissue-specific, depending on cell type within each tissue. The main peptides stemming from the CgA cleavages are depicted in Fig. 1. The first peptide discovered was pancreastatin, a 49-AA peptide isolated from porcine pancreas having the capacity to inhibit insulin secretion stimulated by glucose in the endocrine pancreas. Pancreastatin controls hepatic metabolism of glycogen [6,14]. The other established peptides are chromostatin, catestatin, parastatin, peptide WE-14 and vasostatins I and II. Vasostatins I and II, like chromostatin, inhibit the vasoconstriction induced by endothelin-1. Catestatin inhibits the release of catecholamines via stimulation of the acetylcholine receptor of the chromafin cells in the adrenal medulla [15]. Parastatin inhibits the secretion of parathormone [16]. In rats, the highly preserved WE-14 peptide enhances the release of histamine by mast cells. Aunis and Metz-Boutigue discovered antibacterial and antifungal activities associated with peptides issuing from the degradation of Cg [6,10]. Vasostatin I is relevant as it possesses both antibacterial properties against Gram + bacteria and antifungal qualities against yeast. Prochromacin/ chromatocin also appears to have these qualities. These peptides could contribute to innate immunity by creating a natural defensive barrier during infections or in states of stress [6,7]. These peptides are already present in the secretory granules and are cosecreted with the intact CgA as well as other hormones. To date, assays of these peptides are not advised for routine clinical investigations.

2.2. CgA: general marker of endocrine tumors

Cellular distribution of Cg is ubiquitous [6]. CgA has the widest physiological distribution and will therefore be potentially found in all endocrine tumors; hence, its role as a general marker for all endocrine tumors. CgA is found in the pituitary gland (except in cells secreting prolactin), in the thyroid and parathyroid glands, in the digestive tract in all A, β, C, and D type cells; enterochromafins EC and ECL, G, L, and PP are found in the adrenal medulla and in paraganglions, neurons, skin, prostate and breasts. Anti-CgA antibodies can thus be used for immunohistochemistry tests to reveal the neuroendocrine nature of tumors.

Fig. 1. Main peptides issued from cleavages of chromogranin A.
of certain tumors. CgA are rare or absent in muscles, the liver and adipose tissue [6,17]. To a lesser extent, CgB are found in larger amounts than CgA in some prolactinomas, insulinomas, and NET of the rectum. CgA immunoreactivity is by far greatest in the adrenal gland [17]. Setting the CgA concentration in the adrenal gland at 100%, the concentration in the pituitary is 25% and only 5% in the pancreas; under physiological conditions, it reaches 2.5% in the stomach and jejunum. Western blot techniques allow an assessment of the tissue concentration of CgA. CgA assays in serum and/or plasma have been available as routine clinical tests for the last 10 years. CgA has been put forward as the general marker of endocrine neoplasia type 1 and 2 [18,19]. An elevated level of CgA would be suggestive of gastrinoma in a context of type 1 MEN and of pheochromocytoma in a context of type 2 MEN or von Hippel-Lindau disease.

3. Assessment of CgA levels in serum or plasma

3.1. Assays

The first competitive CgA assay was described by O’Connor and Bernstein in 1984 [20]. These authors created polyclonal antibodies directed against CgA extracted from human pheochromocytomas, which they then marked with radioactive iodine. Research assays were also developed to measure unaltered CgA as well as its biologically active peptide derivatives in various animal species.

In clinical practice, several kits are currently commercialized. Competitive and IMA are available, using enzymatic, luminescent or radioactive tracers. Not all kits possess analytical capacities [21] or clinical equivalents [22,23]. With no international standard, other than a positive or negative test, comparison between series is hazardous. The results are expressed in nmol/L or ng/mL or in U/L. The cutoff for positivity ranges from 10 nmol/L to 130 ng/mL. Some researchers, desiring high specificity, have maintained high diagnostic thresholds [24]. According to the test subject (patients and control subjects), 10 to 36% of patients can be alternatively classified (normal versus pathological) depending on the assay used [24–29]. The main characteristics of commercialized CgA assays used in clinical practice are given in Table 1.

CgA levels are robust. They remain unaffected by sex, age, daily stress and cytolyis.

3.2. Interferences in assays

The two major interferences are kidney failure and hypergastrinemia. The rise in CgA levels observed in both instances can mimic levels observed in some endocrine tumors. CgA is eliminated via hepatic and renal metabolism. In the event of kidney failure, the CgA level increases proportionally to the severity of the kidney failure due to retention of low-molecular-mass CgA fragments observed for creatinine clearance below 70 ml/min [6,30–33]. Theoretically, CgA levels can be corrected for creatinine clearance.

Hypergastrinemia is the second cause of false positive CgA assays. The main causes of hypergastrinemia are Zollinger-Ellison syndrome or hyperplasia of the antral G cells related to atrophic gastritis such as in Biemer’s anemia [2,34]. In the general population, 16 to 20% of subjects are taking PPI. When PPIs are discontinued, the CgA level drops to nearly normal limits by the 5th day and becomes strictly normal by the 7th [35].

SMS inhibits cell growth, as well as the hormonal secretion process, directly and indirectly. In most cases, a decrease in the CgA level corresponds to an improvement in clinical signs and also to a decrease in the secretion of other hormones. CgA assays must therefore be standardized for the prescription of SMS analogs [36,37].

Moderate elevations in blood CgA levels (less than three times the normal range) have been observed in inflammatory bowel diseases [38] as well as in Graves’ disease hyperthyroidism [39] although the mechanism remains unclear. Levels fall during treatment. All known causes of interferences in CgA assays, whether positive or negative, and even the rarest [40], are laid out in Table 2. All CgA assays should be associated with systematic screening for the two principal causes of false positives at the least suspicion of an isolated rise in CgA, with an evaluation of creatinine clearance and gastrinemia.

<table>
<thead>
<tr>
<th>False positives</th>
<th>False negatives</th>
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</thead>
<tbody>
<tr>
<td>Renal and heart failure</td>
<td>Somatostatin analogs</td>
</tr>
<tr>
<td>Hypergastrinemia</td>
<td>Assay’s antibody</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Poorly differentiated tumors</td>
</tr>
<tr>
<td>Inflammatory bowel diseases</td>
<td></td>
</tr>
<tr>
<td>Graves’ disease</td>
<td></td>
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<tr>
<td>Heterophilic antibodies (IMA)</td>
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</table>
4. Clinical performance

4.1. The best general marker of NET

The diagnostic superiority of CgA assays over NSE as a general marker for NET has been confirmed by multiple studies [41–44]. A significant linear relation between marker level and tumor mass has not been proven for NSE, whereas it has been for CgA. The tumor secretion/volume correlation is true for all NET except for gastrinomas. Diagnostic sensitivities are dependent on the secreting nature of the probed tumors. In the study from Lyon [44], excellent specificity (98.4%) was compared with diagnostic sensitivity: 73% for secreting tumors and 45% for non-secreting tumors. Sensitivity also varies according to tumor location [41,42].

Other markers, such as CEA, 5-HIAA and the subunit α, have showed lower diagnostic performances [45].

At the present time, NSE assays are only performed in patients with poorly differentiated forms of NET, as a marker of the cytolytic process.

4.2. Tumors with high potential for CgA secretion

In the physiological state, most secreted CgA comes from the adrenal medulla [6,17]. CgA assays give excellent diagnostic results in patients with secreting pheochromocytomas and paragangliomas [46,47]. Other tumors with a high frequency of secretion are neuroblastomas and especially gastrinomas. Therefore, after excluding causes for false positives and in the absence of metastasis, both diagnoses become a priority.

4.2.1. Pheochromocytomas and paragangliomas: biological diagnosis

The biological diagnosis of pheochromocytoma and/or paraganglioma is based on finding an excess amount of catecholamine secretion or better yet an increase in catecholamine metabolites, MN. Since a potentially lethal condition has to be excluded, the existence of several metabolic pathways has led to the development of several assay methods, but no one parameter has provided 100% precision [48–50]. At the present time, it is recommended to first carry out MN assays, using either plasma or urinary samples or both [51]. Free plasma MN levels mirror continuous tumor secretion, but such assays require specialized equipment which is not always available. Eisenhofer et al. estimated that around 15% of free plasma MN assays produce false positives, half of which can be explained by drug interference [52]. The drugs usually involved are tricyclic antidepressants, phenoxynbenzamine and even paracetamol.

CgA levels are proportional to tumor mass [53] and in some reports have been the only positive result for paragangliomas with little or no secretion [54]. Although no guidelines have been published concerning CgA assays, CgA tests coupled with MN assays are widely used in clinical practice. CgA testing can be useful because of its excellent predictive negative value (98% in the Lille experience) [46], especially when a modest rise in MN is observed. Furthermore, CgA assays and MN assays are not susceptible to the same drug interferences [50]. Using the strategy adopted by Algericas-Schimnich et al. in a recent study [55], CgA assays could be a second line exploration for the initial biological work-up for pheochromocytomas. CgA assays could be carried out in patients with an elevated plasma MN (tested first), either to confirm the diagnosis or on the contrary to recognize drug interference in the first assay.

4.2.2. Neuroblastomas: biological diagnosis

CgA is a specific and sensitive marker exhibiting a good correlation between tumor volume and clinical stage. CgA assay performances are superior to NSE or ferritin assays [56,57].

4.2.3. Gastrinomas and CgA levels

Several clinical studies have found a rise in CgA levels in 100% of gastrinomas [41]. These results are explained by the secretion of CgA by antral G cells and ECL fundic (enterochromafin-like) cells and ectopic G cells. The measured CgA concentrations are therefore the result of two secretions: that of the ECL cells rendered hyperplastic by the hypergastrinemia and that of the tumor. It is impossible to differentiate the amount due to each particular secretion. CgA and gastrin assays in this context lack the sensitivity and specificity to ascertain whether the tumor volume is stabilizing and/or increasing [34,58,59].

4.3. Tumors with intermediate CgA secretion potential

These are essentially midgut (ileum) and hindgut tumors as well as non functional pancreatic tumors. The CgA assay sensitivity ranges between 60 and 100% in the metastatic stage depending on the amount of secretion, but between 10 and 50% in the localized stage. Depending on the clinical symptoms, CgA assays can be associated with other specific markers (Tables 2 and 3).

CgA assay is suggested as a factor for prognosis of differentiated metastatic NET [60–64] for CgA levels between three and ten times the normal range. In the absence of a precise analysis of the tumor volume in most studies apart from one [61], this prognostic function of CgA needs to be confirmed. CgA levels vary depending on the size of the hepatic metastasis [60]. The CgA kinetics can be used in order to predict recurrences and relapses in some studies [65]. To date, CgA assays have not been validated

<table>
<thead>
<tr>
<th>Primitive tumor</th>
<th>General markers</th>
<th>Specific markers</th>
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<tbody>
<tr>
<td>Thymus</td>
<td>CgA, calcium</td>
<td>ACTH, cortisol</td>
</tr>
<tr>
<td>Bronchus</td>
<td>CgA, calcium</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>CgA, calcium</td>
<td>Gastrin</td>
</tr>
<tr>
<td>Pancreas</td>
<td>CgA, calcium</td>
<td>Insulin, gastrin, PP Glucagon, SMS, VIP</td>
</tr>
<tr>
<td>Duodenum</td>
<td>CgA, calcium</td>
<td>Gastrin, 5HIAA, SMS</td>
</tr>
<tr>
<td>Ileon</td>
<td>CgA</td>
<td></td>
</tr>
<tr>
<td>Appendice</td>
<td>CgA</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>CgA</td>
<td>5HIAA, Platelet Serotonin</td>
</tr>
<tr>
<td>Rectum</td>
<td>CgA</td>
<td></td>
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</tbody>
</table>

Table 3
Biological evaluations according to the location of the tumor.

Évaluation biologique en fonction de la localisation de la tumeur.
as a substitute marker for morphological tumor progression. The sensitivities and specificities for the diagnosis of progression are between 64 to 89% and 54 to 100% respectively in the five studies published on the subject [60–64]. These variable results can be explained, at least in part, by the many causes affecting the interpretation of CgA levels (false positives as well as the use of SMS analogs) and the difficulty in analyzing changes in tumor morphology with conventional and functional imaging.

4.4. Tumors with little CgA secretion

These are medullary thyroid cancers, pituitary adenomas, insulinomas, and small-cell lung cancers.

- medullary thyroid cancer. CgA levels only rise in the most advanced stages of medullary thyroid cancer (around 20% of cases), for CT concentrations above 700 to 1000 pg/ml. Therefore, a standardized ratio of CT/CgA greater than one implies a medullary thyroid cancer while the inverse implies a gastro-enteropancreatic NET of another origin. Pentagastrin stimulates CgA very little. There is therefore no point in the assay in this context [66,67];
- pituitary tumors. Whilst physiologically, the pituitary gland is the second most CgA secreting organ, and in immunohistochemistry, the marking is clear except for cells secreting prolactin, the results from serum CgA assays have been disappointing [41,68,69]. The low tumor mass is a probable explanation in light of the sensitivity of the assay used. Therefore, the existence of Cushing’s syndrome or acromegaly associated with a strong rise in the CgA level brings about questions on an ectopic origin;
- insulinomas give an irregular positivity in relation to CgA in immunohistochemistry. This is shown by the low success of the serum CgA assays raised in 10 to 50% of cases [41].

4.5. Mixed tumors

Some tumors are observed as having a certain amount of endocrine cells, hence their classification as mixed tumors when this second contingent is significant. This occurs in prostate cancer and breast cancer since there is structural homology between the short sequence of BRCA1 and CgA and some lung cancers. Prostate cancers are the most studied. In this context, CgA assays are supplementary to PSA as a diagnostic marker as well as a follow-up or prognostic one. The rise of CgA levels predicts tumor aggressiveness, both in highly and averagely differentiated forms, tumor progression, and escape from treatment [70–72]. This correlation is not found in all forms of mixed cancer.

5. Recent data

5.1. Differentiation between ectopic ACTH secretion and Cushing syndrome

Zemskova et al. have confirmed the results previously obtained by Nobels et al. A high CgA level with a normal MRI scan implies often metastatic ectopic ACTH secretion [73,74].

5.2. CgA Stress hormone . . .

A prospective study in Strasbourg compared the performances of CRP, procalcitonin and CgA response to acute stress in the intensive care unit. CgA was observed to be a powerful independent prognosis indicator in the intensive care context. Assays associating CgA and salivary cortisol are being considered [75].

5.3. CgA, catestatin and cardiovascular risk

Catestatin operates a negative feedback on catecholamine secretion. Elevated levels of CgA, associated with diminished levels of catestatin are seen in genetically determined hypertension. A change in the CgA/catestatin ratio can be an early element in evaluating cardiovascular risk. CgA is an independent long-term predictive factor for mortality in coronary patients [76–82].

5.4. Pancreatic assays

Pancreastatin is a negative regulator of sensitivity to insulin. It plays a part in glucose homoeostasis. Its level rises with age and in diabetic patients. In CgA knock-out mice, loss of pancreastatin can protect against potential hypertension induced by metabolic disorders [83].

6. Conclusion

To conclude, the story of granin has not yet been completely written. CgA is the most ubiquitous and sensitive general marker for the diagnosis of endocrine tumors. Some CgA assays are reliable but require an international calibration and a better definition of diagnostic thresholds. CgA diagnostic performances are in general proportional to tumor volume but equally dependent on the primary location of the tumor, the existence or not of hormonal secretions associated with possible kidney failure and/or hypergastrinemia. CgA assays still need to be recognized as a marker for the prognosis and follow-up of NET. New approaches to CgA assays, associated or not with an assessment of active peptides, are being put forward in intensive care units and for the evaluation of cardiovascular risk.

Conflict of interest

No major conflicts of interest of the authors in connection with the subject developed in this paper.

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