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RESEARCH ARTICLE

Limitations of Chromogranin A in clinical practice

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Abstract

Context: Usefulness of circulating Chromogranin A (CgA) for the diagnosis of neuroendocrine tumors (NEN) is controversial. The aim of the present study was to assess the actual role of this marker as diagnostic tool. *Methods*: Serum blood samples were obtained from 42 subjects affected with NEN, 120 subjects affected with non-endocrine neoplasias (non-NEN) and 100 non-neoplastic subjects affected with benign nodular goitre (NNG) . Determination of CgA was performed by means of immunoradiometric assay. *Results*: The CgA levels among NEN-patients were not significantly different from NNG and non-NEN subjects. The Receiver operating characteristic (ROC) curves analysis failed to identify a feasible cut-off value for the differential diagnosis between NEN and the other conditions. *Conclusion*: Serum CgA is not helpful for the first-line diagnosis of NEN.

Keywords: Chromogranin A, neuroendocrine tumor, false positive marker, chronic atrophic gastritis, proton pump inhibitors

Introduction

Chromogranin A (CgA) is a hydrophilic glycoprotein with a molecular mass of 49 Kd. It is the main component of a family of secretory proteins, the chromogranins, that are abundantly expressed in large dense core vesicles of neuroendocrine cells and plays a critical role in the mechanism of calcium-mediated exocytosis (Borges et al. 2010). If CgA has a well defined and central role for the histological diagnosis of neuroendocrine tumors (NEN) (Kloppel et al. 1999; Solcia 2000), nowadays, the diagnostic usefulness of circulating CgA is still controversial (Nobels et al. 1997; Goebel et al. 1999; Guignat et al. 2001; Campana et al. 2007; Faggiano et al. 2007; Zatelli et al. 2007; Ferolla et al. 2008).

The CgA is generally considered a helpful circulating marker for NENs, but its sensitivity and specificity are much lower than 100% (Goebel et al. 1999; Guignat et al. 2001; Campana et al. 2007; Nobels et al. 1997; Faggiano et al. 2007; Zatelli et al. 2007; Ferolla et al. 2008). The specificity of CgA assay for the diagnosis of NEN is limited by different factors. Several non-neoplastic conditions, such as chronic atrophic gastritis (CAG), Helicobater pylori-related gastritis (Granberg et al. 1999; Campana et al. 2007), renal or liver failure (O'Connor et al. 1989), cardiovascular diseases, such as acute coronary syndromes, heart failure, and arterial hypertension (Takiyyuddin et al. 1990; 1995), are associated with increased CgA levels. In addition, mildly increased CgA concentrations have been reported in inflammatory bowel diseases, airway obstruction, Parkinson's disease, rheumatoid arthritis, and endocrine diseases, such as hyperparathyroidism and hyperthyroidism (Nobels et al. 1993; Granberg et al. 1999; O'Toole et al. 2009). Treatment with proton pump inhibitors (PPI) or H2 receptor blockers generates high CgA levels by inducing hypergastrinemia and enterochromaffin-like (ECL) cell hyperplasia (Kuipers 2006). Non-endocrine neoplasias (non-NEN), such as breast cancer, lung cancer, gastrointestinal cancer, uterine cancer, genitourinary cancer, haematological cancer, head and neck cancer, are also frequently associated with an

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increased CgA levels (Nobels et al. 1997; Glinicki and Jeske 2010) as neuroendocrine cells have been demonstrated to be present within the mass of several non-endocrine tumors (Sundaresan et al. 1991; Scopsi et al. 1992; de Bruine et al. 1993).

Many of the cited conditions, particularly silent CAG, hypertension, heart failure, renal and liver dysfunctions, treatment with PPI, are extremely widespread, while non-NEN may represent a common finding in patients suspected for NEN.

The aim of the present study was to assess the diagnostic value of circulating CgA as diagnostic test for NEN by comparing NEN, non-NEN, and non-neoplastic patients at the diagnosis.

Materials and methods

Patients

From January 2008 to July 2011, serum blood samples from 162 consecutive subjects affected with neoplasia (86 males and 76 females; aged 60.1 ± 11.1 (mean ± ds)) afferent to a single Unit of Internal Medicine (Internal Medicine, San Gennaro Hospital, Naples) were collected at the time of diagnosis before performing any treatment and subjected to CgA determination: 42 subjects (32 males and 10 females; aged 58.4 ± 12.1 (mean ± ds)) had a diagnosis of NEN and 120 subjects (54 males and 66 females; aged 62.2 ± 15 (mean ± ds)) had a diagnosis of non-NEN. In all cases, diagnosis was definitively confirmed by histology. A total of 100 subjects affected with benign thyroid goiter (NNG) (12 males and 88 females; aged 60.5 ± 16.7 (mean ± ds)) were used as non-neoplastic controls.

All enrolled subjects underwent a detailed anamnestic, clinical, and biochemical assessment to define the existence of the main interfering factors associated with CgA increase (CAG, interference by PPI, arterial hypertension, and liver failure). The blood pressure was evaluated by either single measurement and 24 h holter, heart function by ECG and echocardiography, renal and liver function by biochemical parameters, and abdominal ultrasonography. Each patient was screened for the presence of CAG by searching for the related symptoms (heartburn) and by determining serum gastrin levels. Patients suspected for CAG were advised for endoscopy. All patients had serum creatinine levels within the normal range. None of the enrolled subjects had heart dysfunctions. An accurate pharmacological evaluation was done for each patient.

The CAG was defined by histology obtained at gastric endoscopy; arterial hypertension was defined by a systolic blood pressure above 140 mm/Hg and/or a diastolic blood pressure above 90 mmHg; liver failure was defined by employing Child–Pugh score A-B in patients with chronic hepatic disease. Interference by PPI was considered in all subjects taking one of the PPIs available for medical treatment (omeprazole, lansoprazole, esomeprazole, and pantoprazole) at the time and 30 days prior CgA determination.

Table 1. Clinicopathological features of NEN-patients.

Patients	*N.* (%)
Total	42
Primary tumor	
Pancreas	17 (40.5)
Stomach	5 (11.9)
Ileum	2 (4.8)
Appendix	2 (4.8)
Lung	7 (16.6)
Larinx	4 (9.5)
Adrenal	4 (9.5)
Non-functioning NEN	26 (61.9)
Functioning NEN	16 (38.1)
Insulin	4 (9.5)
Gastrin	3 (7.1)
Glucagon	1 (2.4)
Serotonin	8 (19)
Grade	
Low grade (G1)	21 (50)
Intermediate grade (G2)	16 (38.1)
High grade (G3)	5 (11.9)
Stage	
I	11 (26.2)
IIA	5 (11.9)
IIB	0 (0)
IIIA	0 (0)
IIIB	18 (42.8)

NEN: neuroendocrine neoplasm; *N.*: Number; %: proportion

Clinicopathological features of patients affected with NEN are reported in Table 1. Tumors were localized in pancreas in 17 patients (40.5%, 9 non-functioning and 8 functioning tumors), stomach in 5 patients (11.9%, all type-1 gastric carcinoids), ileum in 2 patients (4.8%), appendix in 2 patients (4.8%), lung in 7 patients (16.6%, 4 typical carcinoids, 1 atypical carcinoid, 1 small cell carcinoma and 1 large cell carcinoma), larynx in 4 patients (9.5%), and 4 subjects were affected with malignant pheocromocytoma (9.5%). A total of 16 subjects (38.1%) had a functioning disease. According to WHO and ENETS classifications (Klimstra et al. 2010), 21 NEN were classified as low grade (G1) (50%), 16 as intermediate grade (G2) (38.1%), and 5 as high grade (G3) (11.9%). According to the recent guidelines of the American Join Committee on Cancer (AJCC), TNM staging manual (Edge et al. 2010), 11 NEN were classified as stage I (26.2%), 5 as stage IIA (11.9%), 18 as stage IIIB (42.8%), and 8 cases (19%) as stage IV.

Among non-NEN patients, 38 subjects (31.6%) were affected with prostate cancer, 30 subjects (25%) with colorectal cancer, 21 subjects with lung cancer (17.5%), 17 subjects (14.2%) with hepatocellular carcinoma, 11 subjects with gastric cancer (9.2%), and 3 subjects with papillary thyroid cancer (2.5%). A total 60 subjects affected with NNG had multiple nodules and 40 subjects had solitary nodules with a diameter between 10 mm and 45 mm. All nodules larger than 1 cm and subcentimetric nodules with suspicious echographic features (increased

intranodular vascularity, irregular infiltrative margins, presence of microcalcifications, absence of halo sign, and a shape taller than the width measured in the transverse dimension) were advised to cytological examination and malignancy was excluded. Serum fT3, fT4, TSH, calcitonin, and CEA levels as well as catecholamines and metanephrines urinary levels were within the normal range in all NNG-patients.

Methods

All blood samples were collected after overnight fasting in tubes containing EDTA and centrifuged at 6.000 rpm with an ALC 4235A Centrifuge (ALC International, Milan, Italy). Serum was stored at –80°C until assay.

The CgA determination was performed by immunoradiometric assay (IRMA) (IRMA, CGA-RIA CT, CIS-bio international-Schering, Gif-sur-Yvette, France). The IRMA was based on two monoclonal antibodies directed forward the central domain of the human CgA (CgA 145–245). The method allows sensitive detection of total human CgA. Recombinant human CgA was used as calibrator and the standard curve concentrations ranged from 22 to 1200 ng/mL, with a minimal detectable level of 10 ng/mL. Inter-assay coefficients of variation were 4.4% and 4.9% at 129 and 359 ng/mL, respectively. Intra-assay coefficients of variation were 5.9%, 4%, and 8.1% for the following ranges 15–25, 90–110, and 500–700 ng/mL, respectively. The method was standardized by performing CgA determination in 30 healthy controls with exclusion of the main interfering conditions (CAG, renal or liver failure, cardiovascular diseases, and use of PPI). In all cases, serum CgA was within the normal range (19–98 ng/mL). The method was performed according to the manufacturer's instruction. All samples were assayed three times by the same physician and the mean value was considered for statistical analysis.

Statistical analysis

Statistical analysis was performed using SPSS Version 17.0 for Windows (SPSS Inc, Chicago, IL, USA). The distribution of CgA levels was assessed by one sample Kolmogorov–Smirnov test. Given the abnormal distribution of CgA levels, Kruskall–Wallis test was used to compare the different groups of study (NEN, NNG, and non-NEN). A $p < 0{,}05$ was considered significant in all tests. Receiver operator characteristic (ROC) analysis was performed to identify a feasible CgA cut-off value that could discriminate NEN from NNG and non-NEN.

Results

Distribution of CgA levels

Considering the overall population, CgA levels showed a wide variability and were not normally distributed ($p < 0.001$). These data were confirmed in each of the three groups under study (NEN ($p < 0.001$), NNG ($p < 0.001$), and non-NEN ($p < 0.001$)) (Figure 1). A total of

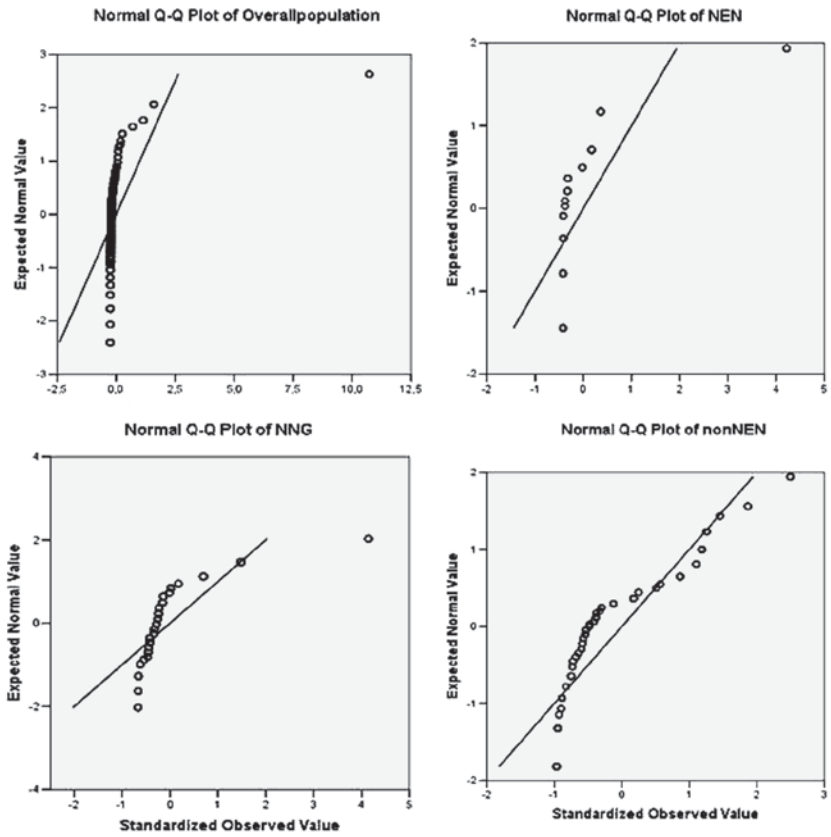

Figure 1. Distribution of CgA levels within the overall population and in the different groups of study.

34 NNG-subjects without any interfering condition were selected: considering this subgroup CgA levels showed a normal distribution ($p = 0.294$).

Comparison between groups

The CgA levels among NEN patients were not significantly different from subjects affected with NNG ($p = 0.204$) and non-NEN ($p = 0.737$). Surprisingly, CgA levels were significantly higher in patients with non-NEN than NNG subjects ($p < 0.001$) (Table 2).

Then, 34 NNG-patients and 30 healthy controls without interfering conditions were compared. In both groups, CgA levels were normally distributed ($p = 0.294$ and $p = 0.251$, respectively). In this case, one way analysis of variance (ANOVA) was performed to compare the groups and no significant difference in CgA levels was found ($p = 0.534$).

Influence of interfering factors on CgA levels

The different groups (NEN, NNG, and non-NEN) were divided in four subgroups considering the following conditions: CAG, treatment with PPI, arterial hypertension, and liver failure. All interfering factors were associated with significantly higher levels of CgA in NNG patients, while no one among CAG, PPI, arterial hypertension, and liver failure was associated with significantly higher levels of CgA in NEN and non-NEN patients (Table 3).

Table 2. Serum levels of Chromogranin A in the different groups and comparison between groups.

CgA	NEN	Non-NEN	NNG
No.	42	120	100
Mean (ng/mL)	2098*f	327**	148
SD (ng/mL)	4908	310	187
Range (ng/mL)	22769	1070	898

Comparison between groups has been performed by Kruskall–Wallis test. CgA: Chromogranin A; NEN: neuroendocrine neoplasm; Non-NEN: non-endocrine neoplasm; NNG: non-toxic nodular goiter; *$p = 0.204$ versus NNG; $fp = 0.737$ versus non-NEN; **$p < 0.001$ versus NNG

ROC analysis

Two ROC curves were constructed comparing CgA levels from 42 NEN with those from 100 NNG and 120 non-NEN, considering NEN as state of disease at the moment of blood sampling. The test failed to identify an effective cut-off of CgA value for the differential diagnosis between NEN and the other two conditions (Figure 2). The areas under the ROC curves (AUC) were 0.568 and 0.483, indicating that CgA assay has a poor ability in distinguishing NEN from NNG and non-NEN, respectively.

Discussion

The CgA is the most available and most frequently applied marker for the histological diagnosis of NEN (Kloppel et al. 1999; Milione and Seregni 2010). However, clinical value of serum CgA in the diagnosis of NEN is debated. Indeed, the sensitivity of this marker is strikingly related

Table 3. Comparison of serum levels of Chromogranin A according to groups (NEN, Non-NEN, and NNG) and according to interfering factors (CAG, PPI, HYP, and LF).

	NEN (no.) Mean ± SD	Non-NEN (no.) Mean ± SD	NNG (no.) Mean ± SD
CAG+	(5) 921 ± 1647	(18) 280 ± 256	(30) 202 ± 136
CAG–	(37) 2758 ± 6077	(102) 312 ± 283	(70) 116 ± 203
p	*0.483*	*0.424*	***<0.001***
PPI+	(24) 843 ± 1197	(81) 321 ± 294	(56) 216 ± 228
PPI–	(18) 2951 ± 6482	(39) 259 ± 278	(44) 61,5 ± 32,7
p	*0.536*	*0.141*	***<0.001***
AH+	(19) 1327 ± 1893	(71) 505 ± 404	(42) 242 ± 260
AH–	(23) 2329 ± 5987	(49) 480 ± 456	(58) 79,4 ± 39,4
p	*0.704*	*0.207*	***0.008***
LF+	(13) 759 ± 1182	(53) 339 ± 312	(8) 535 ± 445
LF–	(29) 480 ± 757	(67) 222 ± 216	(92) 114 ± 103
p	*0.343*	*0.168*	***0.005***

NEN: neuroendocrine neoplasm; Non-NEN: non-endocrine neoplasm; NNG: non-toxic nodular goiter; CAG: chronic atrophic gastritis; PPI: interference by proton pump inhibitors; AH: arterial hypertension; LF: liver failure.

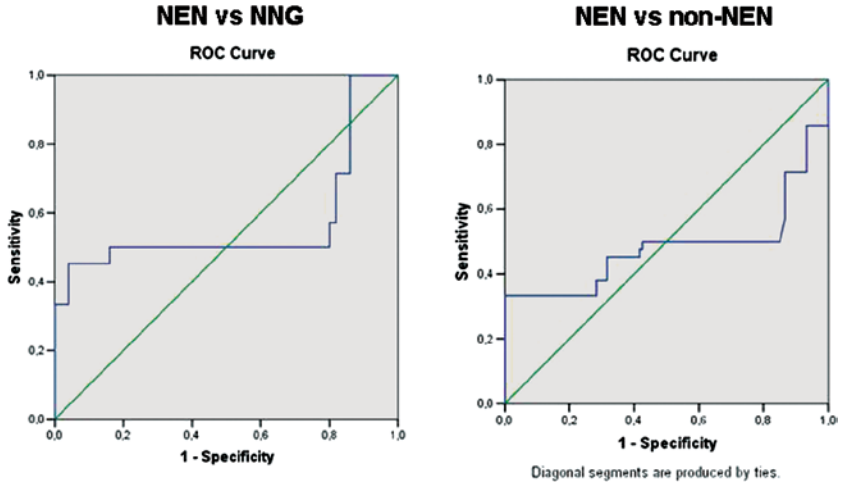

Figure 2. ROC analysis.

to several factors, such as histotype, total tumor load, degree of differentiation, secretory activity (Seregni et al. 2001), while its specificity could be heavily affected by several neoplastic and non-neoplastic conditions related to increased CgA levels.

A study performed by Nobels et al. (1997) assessed the diagnostic accuracy of serum CgA as marker of NEN, comparing to other circulating markers (neuron-specific enolase and α-subunit of glycoprotein hormones). Patients with endocrine tumors and patients with non-endocrine neoplasia were enrolled in the study. The authors found that CgA was the best serum marker of NEN showing a sensitivity and specificity of 53% and 93%, respectively. In other words, the conclusions were that CgA has not to be considered an optimal marker but it is the best available. From that study, not much has changed. Recently, Campana et al. (2007) compared serum CgA levels between NEN patients, CAG patients and healthy subjects. The main finding was that CAG strongly reduces the specificity of CgA as serum marker of NEN. In 2007, Zatelli et al. (2007) compared serum CgA between NEN patients and healthy subjects with exclusion of the main conditions inducing an increase in CgA levels (CAG, renal or liver failure, hypertension, and treatment with PPI). The authors found that, even excluding interfering factors, CgA determination showed a limited diagnostic power. A recent study performed by Vezzosi et al. (2011) assessed the accuracy of serum CgA as marker of morphological evolution of disease in an population of well-differentiated gastroenteropancreatic NEN. The authors demonstrated that CgA levels were strongly affected from interferent conditions and sensibility and specificity of the marker were so low that it cannot be used as surrogate marker of tumor progression even in such a homogenous group of NEN.

The aim of the present study was to clarify the actual role of serum CgA as first-line tool for the diagnosis of NEN. To achieve this purpose, and in contrast with previous similar studies, in the current study an unselected internistic population was considered. Several interfering factors related to increased CgA levels were not considered as exclusion criteria but were assessed as variables of study.

The crucial finding of the study was that CgA levels were not significantly different in NEN patients than NNG and non-NEN subjects. As expected ROC analysis failed to indentify an effective cut-off value that can distinguish NEN from the other two conditions. Another relevant data was that CgA levels showed an abnormal distribution within the overall population and even when the three different groups of study (NEN, NNG, and non-NEN) were considered separately. However, when excluding interfering factors, the distribution of serum CgA was normal.

Then, the impact of CAG, treatment with PPI, hypertension, and liver failure on CgA levels within the different groups was considered. The data demonstrated that interfering factors significantly impacted on serum CgA levels only in NNG patients and not in NEN and non-NEN patients.

This finding strongly confirms the hypothesis that the high variability of CgA levels among NEN patients is mainly related to intrinsic features of the disease. Indeed, NEN represent a heterogeneous group of diseases with a broad variability of clinicopathological features, as demonstrated by the characteristics of our unselected group of NEN-patients. This is the main cause of the limited sensitivity of serum CgA as marker of NEN. Conversely, the findings that CgA levels were significantly affected from interfering factors in NNG and that the subgroup of NNG patients without interferent conditions showed a normal distribution of CgA levels further confirm the relevance of interfering factors in reducing the specificity of serum CgA as marker of NEN.

Another relevant finding was that non-NEN subjects showed higher levels of CgA than NNG subjects. As non-NEN may represent a common finding in patients suspected for a NEN, many neoplasias other than NEN further affect the specificity of serum CgA.

In conclusion, the clinical value of serum CgA as marker of NEN is strongly limited. This marker is not helpful for the diagnosis of such disease and should not be used as first-line screening of patients suspected for a NEN.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. This study was partially supported by a grant from the Italian Minister of Research and University in Rome (no. 2008LFK7J5).

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