CLINICAL STUDY

Plasma chromogranin A in incidental non-functioning, benign, solid adrenocortical tumors

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Abstract

Objective: To evaluate whether adenomas arising from the adrenal cortex, a tissue of epithelial origin, are associated with high chromogranin A (CgA) levels and whether such tumors may express and release this protein. In addition, to investigate whether high CgA levels imply a neuroendocrine differentiation of the adrenocortical adenomas and, therefore, represent a humoral marker of malignant transformation of these tumors.

Design: Plasma CgA of 80 patients with non-functioning, benign adrenocortical adenomas was compared with that of 137 tumor-free subjects. In 15 patients, the masses were surgically removed and CgA was measured 2 months later. The other 65 patients with adrenocortical adenomas underwent clinical and radiological follow-up (range 24–36 months).

Methods: CgA was evaluated by immunoradiometric assay in peripheral blood and by immunohistochemistry in adrenal tissue specimens.

Results: An increase in plasma CgA ($P < 0.001$) was observed in patients with adrenocortical adenomas (83.4 ± 7.5 ng/ml) in comparison with tumor-free subjects (43.1 ± 1.5 ng/ml). The prevalence of high CgA levels was 25% in the former and 0.7% in the latter. By multiple regression analysis, an increase (49%) in the expected median CgA value was estimated for adrenocortical adenomas ($P < 0.001$). Receiver operating characteristic analysis showed a good diagnostic performance of CgA in identifying patients with adrenocortical adenomas (pure accuracy = 0.78, 95% CI = 0.71–0.84). In the operated patients, CgA levels did not change before (80.6 ± 16.5 ng/ml) and after (74.3 ± 16.3 ng/ml) surgery and in no case was CgA immunoreactivity found in adenoma tissues. The non-operated patients did not develop signs or symptoms of disease and showed no features of malignant transformation of the masses.

Conclusions: Our data showed a strong association between adrenocortical adenomas and high CgA levels. CgA hypersecretion was not due to adenoma tissue, which did not show immunoreactivity for CgA. Finally, elevated CgA levels did not represent a humoral marker of malignant transformation of cortical adenomas.

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Introduction

In several endocrine and neuroendocrine cells, chromogranin A (CgA), a 48 kDa acid glycoprotein, is co-stored in secreting granules and co-released with resident hormones (1–3). For this reason, CgA is currently used as a tissue marker for many tumors with endocrine activity (4–7). However, growing evidence suggests that blood CgA measurement is likewise very useful for identifying and following-up patients with neuroendocrine tumors (8–14).

Recently, elevated levels of CgA have been reported in patients with cancers of epithelial origin, such as prostate, breast, ovary, pancreas and liver cancer (15–19).

The ability to synthesize CgA implies a neuroendocrine differentiation of these epithelial tumors. Such an event is a key step in tumor biology, since it is frequently associated with a poor prognosis (8, 15, 20, 21). The presence of serum markers of this transformation might therefore identify more aggressive tumors.

The adrenal cortex is of epithelial origin and cortical adenomas are benign lesions and the risk of malignant transformation, albeit low, is present (22). Our previous study (12), while confirming the utility of plasma CgA measurement in the diagnosis of pheochromocytomas and gastroenteropancreatic tumors, unexpectedly showed elevated CgA levels in six of 25 patients with benign adrenal cortex tumors.

In order to verify this finding in a larger cohort of patients, we compared plasma CgA levels in 80 patients with non-functioning, benign, adrenocortical tumors with levels found in 137 tumor-free subjects. Furthermore, to investigate whether CgA may be synthesized and released by adrenocortical adenomas, plasma CgA was measured before and after surgery in 15 patients. CgA immunoreactivity in the removed adenoma tissues was then evaluated by immunohistochemistry.

Materials and methods

Subjects

In this observational study patients were selected from among those referred to our unit (Department of Internal Medicine) from 1999 to 2000 for the presence of adrenal masses.

The following inclusion criteria were adopted: (a) incidental discovery of the adrenal mass by radiological investigation performed for unrelated reasons; (b) absence of symptoms and signs of endocrine and neuroendocrine disease, except for hypertension, disturbed glucose tolerance and obesity; (c) no abnormalities in glucocorticoid (urine cortisol, plasma adrenocorticotropin and cortisol before and after 1 and 2 mg dexamethasone suppression tests), mineralocorticoid (upright plasma renin activity and plasma and urine aldosterone), androgen (plasma free and total testosterone, androstenedione, dehydroepiandrosterone sulphate and 17-hydroxyprogesterone) or catecholamine (plasma and urine) and methanephine levels; (d) radiological and/or scintigraphic features of non-malignancy. On computed tomography (CT), inclusion criteria required adrenal masses to be solid, small (usually <3.5 cm), rounded, well-marginated, homogeneous, with lower than water density (<0–15 Hounsfield Units) and absent or mild homogeneous CT enhancement after i.v. contrast, without hemorrhage, necrosis or calcification. ¹³¹I-6β-norcholesterol scintigraphy had to show concordant or symmetrical uptake of the radiotracer; (e) no mass size increase and no development of specific signs and symptoms of disease on adequate follow-up (range 8–14 months).

On the basis of these inclusion criteria, we excluded patients with adrenal pheochromocytoma and selected only 80 patients (62 hypertensive and 18 normotensive) with incidental, asymptomatic, non-functioning, solid and benign tumors of the adrenal cortex (45 right sided, 12 left sided and 23 bilateral with maximum diameter of the mass of 26.3 ± 1.26 mm, means $\pm$ S.E.).

One hundred and thirty-seven tumor-free subjects (83 essential hypertensive and 54 normotensive subjects) were also investigated after careful exclusion of the presence of adrenal masses on CT, magnetic resonance or ultrasonography.

Overall, 80 patients with adrenocortical tumors and 137 tumor-free subjects were investigated. In both groups, normotensive and hypertensive subjects were analysed together as in our previous reports (12, 23) we showed that, at least with this method, CgA levels in normotensive and essential hypertensive subjects are superimposable. Patients with serum creatinine >1.5 mg/dl were not enrolled to avoid the well-known false-positive CgA levels for hormone-secreting tumors in subjects with renal failure (8, 24). Patients taking omeprazol were also excluded since this drug increases CgA levels (25). Demographic and clinical data for our subjects are given in Table 1.

Experimental design

All subjects were maintained on their usual diet without salt restriction, except for essential hypertensive subjects who observed a salt-controlled diet (80–100 mmol/day sodium and 60–80 mmol/day potassium). Patients taking drugs (anti-hypertensives or other drugs) suspended medication for at least 2 weeks prior to the study. On the test day, all subjects collected 24-h urine samples for creatinine determination and underwent blood sampling (between 0800 and 0900 h) after overnight fasting and in the sitting position. An indwelling 21 gauge scalp vein needle was inserted in an antecubital vein and was then used, after 15–20 min, for creatinine, catecholamine and CgA measurements. Blood pressure was recorded by mercury sphygmomanometer in accordance with recent guidelines (26).

Table 1 Demographic and clinical data of tumor-free subjects and of patients with adrenocortical tumors. Values are means $\pm$ S.E. with range in parentheses.

Variables	Tumor-free subjects (n = 137)	Adrenocortical tumors (n = 80)	P value
Gender (male/female)	98/39	38/42	<0.001 ^a
Age (years)	41.8 $\pm$ 1.1 (17–75)	57.3 $\pm$ 1.1 (23–79)	<0.001
Systolic blood pressure (mmHg)	138.9 $\pm$ 1.6	147.0 $\pm$ 2.3	0.003
Diastolic blood pressure (mmHg)	89.7 $\pm$ 1.0	90.0 $\pm$ 1.2	0.836
Plasma creatinine (mg/dl)	0.90 $\pm$ 0.01	0.97 $\pm$ 0.03	0.024
Noradrenaline (pg/ml)	248.6 $\pm$ 9.5	335.1 $\pm$ 17.9	<0.001
Adrenaline (pg/ml)	31.2 $\pm$ 1.7	36.4 $\pm$ 2.1	0.058

P, by unpaired Student's t-test; ^aOne degree of freedom chi-squared test.

Fifteen patients with adrenocortical adenomas underwent surgery by voluntary choice ($n = 12$) and for tumor size over 35 mm ($n = 3$). In the operated patients, the above-described protocol was repeated 2 months or more after operation. The other 65 patients underwent clinical and radiological follow-up (range 24–36 months) in order to detect signs or symptoms of possible malignant transformation of the mass.

Patients and controls gave their informed consent to the study which was approved by the local ethical committee.

Assays

Serum creatinine was measured by a standard technique. Plasma catecholamines and CgA were measured using the same blood samples collected into chilled anti-coagulant (EGTA plus L-glutathione reduced for catecholamines; heparin and trasylol for CgA) glass tubes. Blood was spun down in a refrigerated centrifuge and the plasma was stored at -80°C until the assay. Catecholamines and CgA were then assayed in duplicate and in the same run.

Catecholamines were analyzed by high-performance liquid chromatography, as previously described (27). Within-run precision was 10%, while the reproducibility of peak height was 1.2% and day-to-day precision of quantitative results was 0.7%. Intra- and interassay coefficients of variation (C.V.) for noradrenaline were 14% and 20% respectively. Intra- and interassay C.V. values for adrenaline were 17% and 22% respectively. The reference range for noradrenaline was <600 pg/ml, and for adrenaline <80 pg/ml in a sitting position.

CgA was measured by a solid-phase two-site immunoradiometric based on monoclonal antibodies that bind to two distinct contiguous epitopes within the 145–245 region of CgA (CgA-RIA CT; Cis Bio International, Gif-Sur Yvette Cedex, France) (28). The first antibody was coated onto the solid phase and the second, used as a tracer, was radiolabeled with ^{125}I . CgA (molecules or fragments) present in the standards (recombinant human CgA) was sandwiched between the two antibodies. Thus, by using antibodies directed against the median part of the protein, only the region of CgA unexposed to proteolytic processes was involved, allowing detection of the intact form and major fragments of the molecule. Intra- and inter-assay C.V. values were 2.5% and 7.0% respectively, while assay sensitivity was 1.5 ng/ml. Values obtained for anti-coagulated plasma in 162 normal subjects were: median 41.6 ng/ml; range 19–98.6 ng/ml.

Specimens

Specimens ($n = 15$) of adrenal tissue were formalin-fixed, paraffin-embedded and stained with hematoxylin–eosin for histological processing.

Immunohistochemistry

Tissue sections (4 μm thick) were dewaxed and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 15 min. After rinsing in distilled water, the sections were then immersed in 0.03 mM citrate buffer (pH 6.0) and incubated at 300 watts for 30 min in microwaves.

Monoclonal anti-chromogranin antibody, clone LK2H10, were purchased from Ventana Medical Systems (Novocastra Laboratories Ltd, Newcastle, UK). Ventana medical systems' anti-chromogranin primary antibody is an antibody which binds to chromogranin in paraffin-embedded tissue sections. The specific antibody was localized by biotin-conjugated secondary antibody formulation that recognized rabbit and mouse immunoglobulins. This step was followed by addition of an avidin–streptavidin–enzyme conjugate that binds to the biotin present on the secondary antibody. The specific antibody–secondary antibody–avidin/streptavidin–enzyme complex was then visualized using a precipitating enzyme reaction product which is readily detected by light microscopy. Each step was incubated for a precise time and temperature. At the end of each incubation step, a Ventana automated slide stainer washed the sections to stop the reaction and remove unbound material that would hinder the desired reaction in subsequent steps. It also applies liquid coverslip, which minimizes evaporation of the aqueous reagents from the specimen-containing slide. The peroxidase reaction was visualized by incubating the sections with 3,3'-diaminobenzidine; the sections were counterstained with hematoxylin.

Positive controls consisted of medullary thyroid carcinoma known to express CgA antigen. Negative controls were obtained by omitting primary antibodies.

The positivity index was obtained by counting almost 500 cells for lesions in pathological tissue on ten $\times 250$ fields and calculating the percentage of cells with cytoplasmatic CgA immunoreactivity. Specimens were considered positive when at least 10% of cells showed distinct cytoplasmatic staining.

Statistical analysis

To evaluate the relationship between CgA and the presence/absence of adrenal mass (disease status), univariate and multivariate statistical methods were used. Paired and unpaired *t*-tests were used to assess inter- and intra-group differences. Descriptive analyses were also carried out by creating and examining the conditional distribution of CgA by covariates (tumor diameter, age at examination, gender, hypertensive status, systolic and diastolic blood pressure, creatinine, noradrenaline and adrenaline). In general, continuous covariates were *a priori* categorized according to quartiles (25°, 50° and 75° percentiles) of their observed distributions. Given the small number of patients with adrenocortical masses, tumor diameter was categorized

by tertiles (33.3° and 66.7° percentiles). Missing data on creatinine (28 values) were gathered into a specific category. Univariate differences in CgA levels by covariates were assessed by a non-parametric procedure (two-tailed median test). The joint effect of disease status and all other covariates on CgA levels were estimated by multiple regression modeling (29). In view of the right-skewed distribution of CgA, data were log-transformed. In the regression analysis, log-transformation of response variable leads to an estimate a statistical index referred to as the median ratio (MR) (30): as a categorical predictor, MR represents the expected median value of CgA in a category divided by the same index as the reference category. For each MR, 95% confidence intervals (95% CI) were calculated and, to show the explanatory power of each model, the index R² (coefficient of determination = model-explained variability/total variability) was computed. Leverage, residual and influence measures were applied to assess the adequacy of the modeling. The statistical significance of each model and each covariate in the model was evaluated by two-tailed F-test. Furthermore, in order to estimate the discriminant power of CgA and all other continuous covariates on the study group, a non-parametric receiver operating characteristic (ROC) analysis was carried out using disease status as the classification factor (31). In particular, the area under a ROC curve (AUC) was used to estimate the pure accuracy, which represents the probability that a (randomly selected) patient with an adrenocortical mass has a CgA value higher than a (randomly selected) tumor-free subject. Data were analyzed by Stata statistical software (32).

Results

Patients with adrenocortical tumors were older, with higher systolic blood pressure values and with greater plasma noradrenaline and creatinine levels than tumor-free subjects (Table 1). Descriptive analysis of the CgA distribution (Table 2 and Fig. 1) showed a statistically significant increase in CgA levels for patients with adrenocortical tumors as compared with tumor-free subjects. Accordingly, individual data (Fig. 1) indicated that in only one out of 137 tumor-free subjects (0.7%) was plasma CgA above our cut-off for normal (100 ng/ml). In contrast, the cut-off was exceeded in 20 of 80 patients with adrenocortical tumors (25%). An increase in plasma CgA was also found in the older subjects and in those with more elevated plasma creatinine and noradrenaline levels. No significant variation was found for gender, blood pressure or adrenaline levels (Table 2). The joint effect on CgA levels of disease status and other covariates estimated through multiple log-normal regression modeling is shown in Table 3. Using tumor-free subjects as a reference category, a non-linear (parabolic) trend in CgA

median levels was seen as a function of tumor diameter. In particular, the maximum excess (MR = 1.49, 95% CI = 1.21–1.84) was found for patients with a lesion diameter ranging from 20 to 30 mm. On average, i.e. irrespective of tumor diameter, patients with an adrenocortical mass showed an estimated 38% median CgA excess (MR = 1.38, 95% CI = 1.16–1.63) in comparison with the same reference (data not shown). A substantial contribution appeared to be made by age at examination (for patients aged over 57 years: MR = 1.46, 95% CI = 1.17–1.85) and plasma creatinine (for values greater than 1.10 mg/dl: MR = 1.52, 95% CI = 1.19–1.93), while no significant effect of gender, blood pressure or catecholamines was noted.

Table 4 shows pure accuracy values estimated through ROC analysis performed on all continuous covariates. As expected, CgA showed a very good test performance (AUC = 0.78, 95% CI = 0.71–0.84), though less than that shown by age at examination (AUC = 0.81, 95% CI = 0.75–0.87) (Fig. 2).

Patients (*n* = 65) who underwent clinical and morphological follow-up, both with normal (75%) and high (25%) CgA levels, did not develop signs or symptoms of disease and exhibited no features of malignant transformation of the masses. In the 15 patients with adrenocortical tumors who underwent surgery, plasma CgA did not change significantly (80.6 ± 16.5 before and 74.3 ± 16.3 ng/ml after removal of the mass), a finding observed in both patients with normal (73%) and patients with high (27%) CgA values. In particular, in the four cases with elevated CgA, the following were found: patient no. 1 = 84 vs basal 107 mg/dl; patient no. 2 = 212 vs basal 119 mg/dl; patient no. 3 = 189 vs basal 153 mg/dl; patient no. 4 = 255 vs basal 262 mg/dl.

Immunohistochemical analysis of the removed tumors failed to show CgA immunoreactivity in any of the specimens examined (Fig. 3). These results were obtained by comparing CgA expression in medullary thyroid carcinoma (our positive control) with that in the tissues. In no case was distinct cytoplasmatic staining observed and, according to our criteria, all specimens were considered negative.

Since creatinine is a crucial parameter for CgA values, we compared plasma creatinine in the operated (0.95 ± 0.06 mg/dl) and non-operated (0.97 ± 0.03 mg/dl) patients. No statistical difference was found. Similarly, in the 15 patients with adrenocortical tumors who underwent surgery, plasma creatinine did not change significantly (0.95 ± 0.03 before and 0.98 ± 0.06 mg/dl after removal of the mass), a finding observed in both patients with normal and high CgA values. In particular, in the four cases with elevated CgA, the following values in plasma creatinine were found: patient no. 1 = 0.7 vs basal 0.9 mg/dl; patient no. 2 = 1.2 vs basal 1.0 mg/dl; patient no. 3 = 1.1 vs basal 1.1 mg/dl; patient no. 4 = 1.2 vs basal 1.1 mg/dl.

Table 2 Conditional distributions of CgA levels according to factors taken into consideration for analyses.

Factors and levels	Subjects (n)	Mean	s.d.	Median	Minimum	Maximum	P
Disease status							<0.001
Tumor-free subjects	137	43.13	17.60	38.51	17.58	102.06	
Adrenocortical tumors ^a	80	83.41	67.56	62.59	23.42	392.61	
< 20 (diameter, mm)	26	99.16	97.81	61.91	32.75	392.61	
20–30	29	81.71	38.98	73.67	44.64	246.62	
> 30	21	72.67	57.11	46.26	23.42	261.87	
Age at examination (years)							<0.001
≤ 36	52	39.14	14.95	34.80	17.58	78.69	
37–49	54	54.04	47.34	44.03	21.29	353.90	
50–57	57	53.39	24.56	40.77	24.91	119.24	
> 57	54	84.91	70.20	60.31	26.70	392.61	
Gender							0.958
Male	136	55.31	36.98	44.16	20.35	285.96	
Female	81	62.47	60.98	44.69	17.58	392.61	
Hypertensive status							0.336
Normal	72	52.27	41.57	41.05	17.58	353.90	
Hypertensive	145	60.81	49.89	45.65	20.35	392.61	
Systolic blood pressure (mmHg)							0.061
≤ 128	52	55.27	47.72	45.82	17.58	353.60	
129–142	57	48.96	26.13	40.29	20.35	142.02	
143–154	55	56.08	39.10	40.04	21.72	261.87	
> 154	53	72.32	66.74	53.03	24.77	392.61	
Diastolic blood pressure (mmHg)							0.726
≤ 80	59	54.54	45.88	40.70	17.58	353.90	
81–90	49	60.95	56.32	47.50	21.72	392.61	
91–98	56	56.69	38.78	45.16	24.77	261.87	
> 98	53	60.43	49.25	44.69	20.35	285.96	
Creatinine (mg/dl)							<0.001
≤ 0.80	37	50.26	54.86	38.51	17.58	353.90	
0.81–0.90	72	46.74	21.78	40.01	21.29	142.02	
0.91–1.10	37	51.85	30.96	36.95	20.35	153.30	
> 1.10	43	88.24	76.17	63.81	26.15	392.61	
Unknown	28	58.72	21.44	55.96	29.13	115.90	
Noradrenaline (pg/ml)							0.028
≤ 176.90	55	53.08	45.87	40.29	17.58	285.96	
176.91–257.80	54	54.38	40.17	40.26	20.35	261.87	
257.81–351.50	54	53.19	29.03	45.96	21.79	162.05	
> 351.50	54	71.36	65.59	56.07	21.29	392.61	
Adrenaline (pg/ml)							0.241
≤ 16.7	54	48.85	22.81	43.39	23.42	142.02	
16.8–31.2	55	48.95	25.86	37.64	17.58	119.24	
31.3–44.8	54	67.81	61.56	47.03	20.35	353.90	
> 44.8	54	66.47	61.76	49.09	17.99	392.61	
Whole sample	217	57.98	47.37	44.69	17.58	392.61	

P, significance level of the non-parametric median test.

^a Missing tumor size value in four cases.

Discussion

Since neuroendocrine cells synthesize and secrete CgA, this protein is generally utilized as a tissue and serum marker of chromaffin system tumors (4–14). However, some epithelial tumors may also acquire the ability to produce CgA (15–18), a neuroendocrine transformation associated with poor prognosis (19–21).

The aim of our study was to evaluate whether adenomas arising from the adrenal cortex, a tissue of epithelial origin, are associated with high CgA levels and whether such tumors may express and release this protein. For this reason, plasma CgA levels were investigated in a large selected cohort of patients with benign, non-functioning adrenocortical tumors after

careful exclusion of tumors of medulla origin. While the majority of the patients were followed-up, 15 were operated on and the specimens used for immunohistochemical investigations.

Our results showed that the presence/absence of the adrenal mass, age and plasma creatinine affect CgA status, with a clear positive correlation between CgA levels and these parameters. Log-CgA multiple regression on all available covariates confirmed that patients with adrenal masses have up to 49% greater median CgA values than tumor-free patients, and that the relation between mass and CgA values was positive and statistically significant. Accordingly, ROC analysis indicated that the probabilities of finding high CgA levels are 78% greater in the presence than in the

Figure 1 Means $\pm$ S.E. of plasma CgA in tumor-free subjects and in patients with adrenocortical tumors. The statistical significance is shown (upper graph). Individual data of CgA values in both groups are given. The solid line represents the upper limit of normal values (lower graph).

absence of the adrenal mass, so that CgA appears to be a strong discriminant for the presence/absence of a mass. However, given the small number of significant predictors and the modest value of R^2 (0.38), it is possible that the estimated difference and non-linear trend in CgA levels in relation to the subjects' disease status and stage might be biased by the lack of other predictors not considered in the present analysis.

The biological significance of this strong association is, at present, unclear. It is possible that CgA was produced and released in large amounts by some adrenocortical adenomas, as occurs for other tumors of epithelial origin (15–18). This could imply neuroendocrine transformation of the tumor and thus be considered a marker of malignancy. However, such a hypothesis is unlikely for a number of reasons. In patients who underwent surgery, both with high and normal CgA values, removal of the mass was not associated with significant modifications of this protein. Furthermore, adrenal adenomas of these patients never expressed CgA immunoreactivity, even in patients with high CgA levels. Finally, at present no patient has shown morphological modifications of the adrenal masses or has developed signs or symptoms of diseases. Taken together, these data indicated that the elevated CgA levels in patients with adrenal masses are not due to the presence of the tumor and cannot therefore be considered as markers of tumoral neuroendocrine transformation.

Another possibility is that CgA was primarily elevated, causing and/or contributing to development and/or growth of at least some adrenal tumors. This hypothesis

Table 3 Joint effect on CgA levels on disease status and other covariates estimated through multiple log-normal regression modeling.

Factors and levels	MR	95% CL	F-test	P value
Constant	29.71	23.07–38.29	—	—
Disease status			6.56	<0.001
Tumor-free subjects	1.00	Ref.		
Adrenocortical tumors				
< 20 mm	1.45	1.16–1.80		
20–30 mm	1.49	1.21–1.84		
> 30 mm	1.12	0.87–1.44		
Gender			0.10	0.758
Male	1.00	Ref.		
Female	1.03	0.88–1.20		
Age at examination (years)			4.22	0.007
≤ 36	1.00	Ref.		
37–49	1.10	0.91–1.32		
50–57	1.18	0.98–1.42		
> 57	1.46	1.17–1.85		
Hypertensive status			0.48	0.489
Normal	1.00	Ref.		
Hypertensive	0.95	0.82–1.10		
Creatinine (mg/dl)			4.02	0.004
≤ 0.80	1.00	Ref.		
0.80–0.90	1.10	0.90–1.35		
0.91–1.10	1.13	0.89–1.44		
> 1.10	1.52	1.19–1.93		
Unknown	1.23	0.96–1.56		
Noradrenaline (pg/ml)			0.46	0.708
≤ 176.90	1.00	Ref.		
176.91–257.80	1.05	0.89–1.25		
257.81–351.50	1.10	0.92–1.31		
> 351.50	1.09	0.91–1.30		
Adrenaline (pg/ml)			1.87	0.136
≤ 16.7	1.00	Ref.		
16.8–31.2	0.92	0.77–1.10		
31.3–44.8	1.09	0.92–1.30		
> 44.8	1.12	0.93–1.35		

Overall F-test = 6.66; P value < 0.001; R^2 = 0.38.

95% CL = 95% confidence limits; P value = significance value of the F-test; Constant = estimated baseline median value; Ref. = reference category; MR, median ratio.

derives from the fact that the CgA molecule usually behaves as a prohormone, generating various biologically active peptides (33) able to influence growth, morphogenesis and progression of endocrine tumors (34–40). Unfortunately, our study was not planned to verify this aspect which, however, deserves further investigation.

Table 4 Results of non-parametric ROC analysis performed on each continuous covariate.

Variables	AUC	95% CL
Age at examination (years)	0.81	0.75–0.87
Chromogranine (ng/ml)	0.78	0.71–0.84
Noradrenaline (pg/ml)	0.65	0.56–0.74
Systolic blood pressure (mmHg)	0.63	0.54–0.71
Adrenaline (pg/ml)	0.58	0.50–0.67
Creatinine (mg/dl)	0.58	0.49–0.67
Diastolic blood pressure (mmHg)	0.47	0.39–0.56

AUC, area under the curve; CL, confidence limit.

Figure 2 Non-parametric ROC curve applied to CgA. AUC = 0.78 and 95% CI = 0.71–0.84 estimate the pure accuracy which is the diagnostic performance of the study biomarker.

Figure 3 Total absence of immunostaining in adenoma tissue of one of the patients (upper) in comparison with clearly detectable CgA immunoreactivity in medullary thyroid carcinoma (lower). Magnification $\times 10$.

Nevertheless, even though we cannot offer a clear explanation for the strong association between high CgA levels and the presence of adrenocortical adenomas, our work may constitute a basis for further investigations in this field. In addition, it may also be clinically useful by indicating that, in patients with an adrenocortical mass, elevated CgA values do not represent a marker of potential malignant transformation of the lesion.

In conclusion, our data show a strong association between benign adrenocortical tumors and elevated CgA levels, a finding not entirely explicable by the older age and higher creatinine levels of patients with an adrenal mass. CgA hypersecretion is not due to adenoma tissue, which never shows immunoreactivity for CgA. Finally, elevated CgA is not a marker of neuroendocrine transformation of cortical adenomas and therefore of a possible shift toward malignancy.

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