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Source / Izvornik: **Neuroendocrinology, 2018, 107, 284 - 291**

Journal article, Accepted version
Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

https://doi.org/10.1159/000492934

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:609482

The role of acute octreotide suppression test in detecting patients with neuroendocrine neoplasms

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Abstract

Background: Serum chromogranin A (CgA) is routinely used as a biomarker in patients with neuroendocrine neoplasms (NENs). Several conditions and comorbidities may be associated with falsely elevated CgA, often leading to extensive diagnostic evaluation, which may be costly and harmful. The aim of this study was to analyze the effectiveness of acute octreotide suppression test (AOST) in differentiating falsely elevated serum CgA.

Methods: Our prospective study enrolled 45 patients from two different patient cohorts: 1) 29 patients with suspicion or presence of NEN (extensive work-up and subsequent biopsy confirmed 16 NENs); 2) 16 consecutive patients admitted via emergency department without NEN (nonNENs). AOST was performed after an overnight fast. Baseline CgA was measured, after which 0.25 mg of octreotide was administered subcutaneously. CgA was measured 3 hours and 6 hours after administration.

Results: Baseline CgA were similar in NENs and nonNENs. At the end of AOST, CgA decreased by a median of 83.3% (41.0-127.4) in nonNENs and 13.8% (0.0-43.6) in NENs ($P<0.001$). In patients with increased baseline CgA, decrease in CgA at 6^{th} hour by <51.3% had 90.0% sensitivity and 88.9% specificity in detecting NENs. In patients with normal baseline serum CgA, decrease in CgA at 3^{rd} hour by <17.6% had 83.3% sensitivity and 81.8% specificity in detecting patients with NENs. The diagnostic accuracy of AOST in the entire study population was 86.7%.

Conclusions: AOST is a promising tool to increase the diagnostic accuracy of serum CgA.

Key words: octreotide; suppression; test; chromogranin A; diagnosis; neuroendocrine neoplasm

Introduction

Chromogranin A (CgA) is routinely used only in the diagnosis and follow-up of patients with neuroendocrine neoplasms (NENs)[1]. NEN tumor cells secrete CgA, which has a diagnostic, predictive and prognostic role in this population of patients[1]. However, increased serum CgA can be found in patients with sepsis[2], acute exacerbation of rheumatoid arthritis[3], inflammatory bowel disease[4], various metastatic malignancies[5], heart and renal failure[67], complicated myocardial infarction[8], arterial hypertension[9] and chronic atrophic gastritis[10]. Moreover, the use of proton pump inhibitors (PPIs) may also increase CgA serum levels[11].

Due to its non-specific nature, the exact diagnostic accuracy of CgA as a screening method in detecting patients with NENs remains controversial. Sensitivity of CgA ranges from 53% to 85%, while specificity ranges from 84% to 96% in detecting patients with NENs in the general population[12]. This greatly differs between studies, depending mainly on the control group. Studies focusing on the diagnostic accuracy of CgA may be distinguished to those which exclude or include subjects with interfering factors. Studies that used healthy blood donors had highest diagnostic accuracy of CgA in detecting NENs, while the diagnostic accuracy substantially decreased when control group constited of patients using PPI or patients with other malignant diseases[1,12]. Moreover, in a study by Marotta V et al. that included 42 subjects affected with NEN, 120 subjects affected with non-endocrine neoplasias and 100 non-neoplastic subjects affected with benign nodular goiter, serum CgA had no diagnostic value in detecting patients with NENs[13].

The role of acute octreotide suppression test (AOST) in predicting long-term disease control has been extensively studied in patients with acromegaly, but with conflicting results. The dose of octreotide used in AOST varied between 0.05 and 0.1 mg. AOST seemed useful in predicting response to long-acting somatostatin analogue if the nadir growth hormone concentrations were

use to interpret the AOST, but it showed no predictive value when the test was interpreted as relative change in serum growth hormone levels[14].

So far, only three studies have analyzed the role of AOST as a prognostic factor in patients with NENs[15–17]. The most representative was a study by Massironi et al., which showed that the decrease in serum CgA >30% during AOST with 0.2 mg of subcutaneous octreotide, was associated with greater overall survival and more favorable response to long-term octreotide treatment[17].

Serum CgA is influenced by wide variety of both physiological and pathological factors. It is often hard to determine the etiology of increased CgA in real patient: a consequence of comorbidities or "autonomous" hypersecretion from NENs? Dynamic testing is the cornerstone of modern endocrinology designed to deal with diagnosis of diseases caused by hormone hypersecretion. Although it seems logical that the AOST might help in differentiating the cause of increased serum CgA, neither one study has analyzed the difference in response to AOST between patients with falsely increased serum CgA and patients with NENs.

We hypothesized that patients without NENs (nonNENs) have more pronounced decrease in serum CgA during AOST, and that AOST is superior to single CgA measurement in detecting patients with NENs.

Methods

This prospective study included two different patient cohorts.

The first cohort consisted of consecutive patients referred to our institution due to suspicion or presence of NEN (N=29). All these patients underwent computed tomography (CT) and/or magnetic resonance imaging (MRI) and 99mTc-Tektrotyd scintigraphy. 18F-fludeoxyglucose

positron emission tomography (18F-FDG PET) was performed in 2 patients. CT protocols included early arterial phase sequences and MRI protocols included both contrast enhanced and diffusion-weighted sequences. After the following diagnostic work-up and subsequent biopsy, NEN was confirmed in 16 patients and the other 13 patients were labeled as controls.

The second cohort included 16 randomly selected patients admitted via emergency department due to various diseases. AOST was performed within the last two days of hospitalization. Extensive aforementioned diagnostic work-up has not been performed in the second cohort, due to low incidence of NENs in general population and consequent low probability that some of these randomly selected patients from emergency department had NEN.

Patients who underwent curative surgery and did not have radiological evidence of recurrence or metastases were excluded from the study.

Patients with NENs were classified based on ENETS guidelines of 2012[18]. Medical history was analyzed in details for nonNEN patients. Based on previous reports regarding comorbidities that may affect serum CgA levels, they were labeled with one of the following which was considered to have the highest impact on serum CgA: acute infection, malignant disease apart from NEN, autoimmune disease, other chronic noninfectious diseases (2 or more: diabetes mellitus, arterial hypertension, chronic obstructive pulmonary disease, chronic renal failure, chronic heart failure) and PPI use.

AOST was performed after an overnight fast and all patients were required to fast during the test. Plasma CgA was measured at baseline, after which octreotide 250 μg was administered subcutaneously. In order to lower costs of the study, we have divided one ampule of Sandostatin 0.5 mg and performed the test simultaneously in two different patients. Venous blood samples were drawn 3 and 6 h after octreotide administration. CgA level was measured via ELISA using

a commercially available kit (Demeditec Diagnostics GmbH, Germany) [26]. Blood samples were collected by venipuncture into serum-separator tubes without anticoagulant. Serum was separated by centrifugation and immediately stored at −20 °C until analysis, which was performed as described in the manufacturer's instructions. Normal range was considered to be 12.5 – 100 μg/L, as provided by the manufacturer. Elevated serum CgA was considered > 100 μg/L.

The study was conducted according to the Declaration of Helsinki and approved by the ethics committee of the University Hospital Center Sisters of charity. All patients gave their written informed consent to the study.

Statistical analyses

Patient characteristics were analyzed with descriptive statistics and presented as a median and interquartile range. Since the majority of parameters did not follow normal distribution we used nonparametric tests as follows: independent continuous variables were compared with Mann-Whitney U test and categorical variables were compared using Fisher's exact test. Receiver operating characteristic (ROC) analysis was used to analyze the diagnostic accuracy of CgA at each point during AOST. The results of (ROC) analysis were presented with area under the curve (AUC), 95% confidence interval, sensitivity and specificity for each CgA cut-off. Two-tailed P values <0.05 were considered significant. Statistical analyses were performed by using SPSS Version 20.0 and MedCalc Version 14.8.1.

Results

There were no differences in demographic characteristics and serum CgA levels between nonNEN and NEN patients. Age and gender were not associated with plasma CgA levels or with the magnitude of change in plasma CgA during the AOST. NonNEN patients had more pronounced decrease in serum CgA (Table 1, Figure 1). Characteristics of patients with NENs are presented in table 2. A total of 7 patients were taking PPIs prior to AOST (5 patients admitted via emergency department and 2 patients with suspicion of NEN (the presence of NEN was excluded after work-up). Among patients taking PPIs, 5 had increased baseline serum CgA and 2 patients had normal CgA. NonNEN patients had the following factors and comorbidities that might be associated with serum CgA: PPI use in 5(17.2%), acute infection in 5(17.2%), autoimmune diseases in 7(24.1%), other chronic noninfectious diseases in 8 (27.6%) patients and other malignant disease in 4 (13.8%) patients.

Patients with increased baseline serum CgA had more pronounced decrease in serum CgA during AOST although it did not reach statistical significance [-74.4% (-129.73 - (-7.81) vs. -27.8% (-59.18 – (-15.09), P=0.083]. However, due to substantial difference in recruitment of patients with increased baseline serum CgA and normal baseline serum CgA, these two groups were analyzed separately. Normal baseline serum CgA consisted of 17 patients (6 NEN patients), while increased baseline serum CgA consisted of 28 patients (10 NEN patients). The proportion of NEN patients was similar in both groups. The proportion of patients with G1 NENs was higher [4 (66.7%) vs. 1 (12.5%)] in the normal baseline serum CgA group, although it did not reach statistical significance. The proportion of patients with localized disease was similar in both groups (1 (20.0%) vs 1 (11.1%)).

In current study population, single measurement of CgA did not have any diagnostic accuracy in detecting NENs (AUC 0.477, 95% CI 0.296 - 0.659, P = 0.803).

In patients with increased baseline serum CgA, the decrease of serum CgA at 6th hour of AOST by <51.3% had a 90.0% sensitivity and 88.9% specificity in detecting patients with NEN (AUC 0.906, 95% CI 0.790 -1.000). There was only one falsely negative patient with localized G3 rectal NEC and serum NSE of 110.3 ng/ml (normal range <15 ng/ml). There were two false positive patients, a 65 year-old male patient admitted due to diabetic ketoacidosis and sepsis and a 24 year-old female who was admitted due to increased serum CgA and nonspecific dermatitis. All 5 patients who were taking PPIs and had increased baseline CgA, had the decrease in serum CgA >51.3% [median 94.5% (78.8 – 143.4)].

In patients with normal baseline serum CgA the decrease of serum CgA at 3rd hour of AOST by <17.6% had a 83.3% sensitivity and 81.8% specificity in detecting patients with NENs (AUC 0.788, 95% CI 0.544 - 1.000). There was only one false negative patient with metastatic G2 rectal NEN (Ki67 8%) who had features of G3 NEN with large necrotic liver metastases and a serum NSE of 28.5 ng/ml. There were two false positive patients admitted via emergency department due to pulmonary embolism and metastatic gastric adenocarcinoma (Figure 2b). Two patients with normal baseline CgA while taking PPIs, had the decrease in serum CgA by 50.7% and 33.8%, respectively.

The diagnostic performance of AOST is presented in table 3. Overall, the diagnostic accuracy of AOST in the entire study population was 86.7%. In a subgroup analysis, there was a trend of higher diagnostic accuracy in patients with pancreatic NENs, as well as in patients with G1 and G2 NENs and patients with metastatic disease, but these differences were not statistically significant due to small sample size (Table 4).

Discussion

To the best of our knowledge, this is the first study which analyzed the diagnostic performance of AOST in detecting patients with NENs. We have shown that AOST has excellent diagnostic performance in patients with increased baseline plasma CgA. In these patients, the decrease in serum CgA 6 hours after octreotide administration for less than 51.3%, had 91% diagnostic accuracy in detecting patients with NEN. Surprisingly, our results suggest that AOST may have important role in patients with normal baseline serum CgA, since the decrease of serum CgA for <17.6% 3 hours after octreotide administration had 79% diagnostic accuracy in detecting patients with NEN. This means that AOST has excellent performance in detecting patients with falsely increased serum CgA, but might have important role in detecting patients with NENs and normal baseline serum CgA. The difference in response to AOST between NEN patients with normal and increased baseline serum CgA was observed by Kos-Kudla et al[16]. Their study included 32 patients with gastroenteropancreatic and lung NETs and serum CgA was measured 30, 60, 90 and 120 minutes after 0.1 mg octreotide injection. Patients with increased baseline serum CgA had significant decrease in serum CgA. On the other hand, serum CgA did not change in patients with baseline normal serum CgA. Interestingly, in patients with increased baseline serum CgA, 25% of patients had a decrease in serum CgA >60% at the end of the test. A study by Massironi et al. included 38 patients with gastroenteropancreatic NENs and plasma CgA was measured 3 and 6 hours after 0.2 mg octreotide injection, approximately 50% of all patients had the decrease in plasma CgA for >50%[17]. Findings from both of these studies greatly differ from our results, suggesting that our AOST would have too many false negative results. However, several methodological differences between these studies make such conclusions impossible. For instance, we used different laboratory technique for serum CgA measurement and therefore any comparisons in serum CgA during AOST and cut-off values with other studies are difficult[19,20].

Our AOST protocol was similar to one reported by Massironi et al[17]. However, participants in our study were required to fast during the test and not to take proton pump inhibitors prior to test, both of which has not been specified in the aforementioned study. This may be important since both feeding and proton pump inhibitors may influence serum CgA levels[21,11]. Moreover, patients with poorly differentiated tumors, large tumor burden and poor functional status were excluded from their study, which might be important when interpreting AOST results.

Only two patients with NEN had false negative result of AOST (the decrease in serum CgA >51% and both had rectal NENs. One patient had localized G3 NEC (Ki67 90%) and profoundly increased serum NSE levels. We can hypothesize that mildly increased baseline serum CgA was not caused by autonomous tumor secretion, but rather with systemic response to highly malignant disease[22,23]. In this setting, one would expect to see pronounced decrease in serum CgA during AOST. The other patient had metastatic G2 rectal NET with tumor burden of >75%. Her baseline serum CgA was normal but serum NSE was also increased. Similar to previous case, we can speculate that serum CgA was not associated with autonomous tumor secretion, and hence, associated with profound decrease during AOST. Thus, we suggest that AOST may not be accurate in patients with G3 NEC and patients with increased NSE that suggest aggressive biology. However, further studies are needed to elucidate this issue.

On the other hand, it is a bit difficult to explain false positive results of AOST. MRI of the abdomen and thorax and 99mTc-Tektrotyd scintigraphy failed to identify NEN in 24-year old female patient with paradoxical rise in serum CgA during AOST. We repeated AOST in this patient 6 months after the initial testing. During the last AOST her dermatitis-associated symptoms were still present and her baseline serum CgA increased to 748 ng/ml. However, it

decreased to 202 ng/ml at the end of AOST, which suggests the absence of NEN (this data have not been added to current analysis).

Several points need to be addressed when discussing the potential clinical utility of AOST. First of all, we must highlight that this is a proof-of-concept study and that our results need to be validated in a different cohort prior to its use in clinical practice. We would like to propose a few suggestions when planning future studies with AOST. Ideally, future studies should be multicentric and should include only patients with well-differentiated NENs. Patients with poorly differentiated morphology and increased serum NSE or other tumor markers should be excluded, because our data suggests that these patients do not exhibit autonomous CgA secretion associated with NEN, leading to increased rate of false negative results. Moreover, AOST should be repeated in each patient with different octreotide dose (0.1 mg and 0.5 mg), in order to find the dose which provides the highest sensitivity and specificity of AOST. We suggest to measure serum CgA at both third and sixth hour of the test, due to significant difference in diagnostic performance at each time point in regard to baseline serum CgA concentration. Finally, AOST should be performed with different assays for CgA measurement, before it's wider use in everyday clinical practice.

In conclusion, our pilot study suggests that AOST is a safe and accurate method which may substantially improve the role of CgA as a screening tool for detecting NENs. In patients with increased baseline serum CgA, the decrease of serum CgA at 6^{th} hour of AOST by <51.3% had 91% diagnostic accuracy, while the decrease of serum CgA at 3^{rd} hour of AOST by <17.6% had 79% diagnostic accuracy in patients with normal baseline serum CgA. Hence, we can conclude that patients with NEN have less pronounced decrease in serum CgA during the AOST. Further validation studies are needed in order to implement this test in everyday clinical practice.

Conflict of interest: None.

Funding: None.

Previous presentations of this work: This work has been presented in a form of poster presentation at 15th Annual ENETS Conference, March 2018, Barcelona (Spain) and awarded with travel grant.

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Table 1. The difference in demographic characteristics and serum chromogranin A levels during the acute octreotide suppression test between controls (nonNEN) and patients with neuroendocrine neoplasms

	nonNEN N=29	NEN N=16	P
Age (years)	64.0 (56.0 – 74.5)	54.5 (47.0 – 69.0)	0.090
Male (%)	14 (48.3)	11 (68.8)	0.224
Baseline CgA increased (%)	18 (62.1)	10 (62.5)	1.000
Baseline CgA (ng/ml)	169 (61 – 385)	126 (75 – 330)	0.803
CgA 3rd hour (ng/ml)	132 (45 – 265)	103 (64 – 267)	0.713
CgA 6th hour (ng/ml)	96 (36 – 201)	119 (55 – 233)	0.361
Change (CgA 6h – CgA 0) %	-83.3 (-127.4 – (-41.0))	-13.8 (-43.6 – 0.0)	<0.001
Change (CgA 3h – CgA 0) %	-33.3 (-66.7 – (-19.6))	-9.2 (-27.3 – 6.2)	0.010
Change (CgA 6h – CgA 3h) %	-25.8 (-44.3 – (-14.6))	-6.4 (-23.1 – 3.0)	0.011

Table 2. Characteristics of patients with neuroendocrine neoplasms

Characteristic	n (%)
Primary tumor site	
Midgut and hindgut carcinoids	9 (56.3)
Pancreatic NENs	4 (25.0)
Lung	1 (6.3)
Pheochromocytoma	2 (12.5)
WHO tumor grade	
1	7 (43.8)
2	7 (43.8)
3	2 (12.5)
Tumor spread	
Localized disease	4 (25.0)
Locoregional metastases	1 (6.3)
Distant metastases	1 (6.3)
Locoregional + distant	10 (62.5)
Functional NEN	8 (50.0)

Table 3. Diagnostic performance of change in serum CgA during acute octreotide suppression test.

	AUC	95% CI for AUC		P	Cut-off (%)	Sensitivity (%)	Specificity (%)
Increased baseline CgA							
Change (CgA 6h – CgA 0)	.906	.790	1.000	.000	-51.3	90.0	.111
Change (CgA 3h – CgA 0)	.733	.543	.924	.044	-31.7	80.0	.278
Change (CgA 6h – CgA 3h)	.917	.812	1.000	.000	-11.6	80.0	.056
Normal baseline CgA							
Change (CgA 6h – CgA 0)	.697	.436	.958	.191	-34.4	66.7	.455
Change (CgA 3h – CgA 0)	.788	.544	1.000	.056	-17.6	83.3	.182
Change (CgA 6h – CgA 3h)	.470	.114	.826	.841	-6.4	50.0	.364
Entire study population							
Change (CgA 6h – CgA 0)	.832	.712	.952	.000	-51.5	93.8	.276
Change (CgA 3h – CgA 0)	.735	.587	.883	.010	-31.7	81.3	.379
Change (CgA 6h – CgA 3h)	.731	.565	.896	.011	-12.3	68.8	.241

Table 4. Subgroup analysis showing diagnostic performance of AOST in different cohorts of patients with NEN (AOST was defined positive if patients with normal baseline serum CgA had the decrease of serum CgA at 3rd hour by >17.6% and if patients with increased baseline serum CgA had the decrease at 6th hour by >51.3%)

	AOST negative	AOST positive	P
Grade n(%)			0.192
1	0 (0.0)	5 (100.0)	
2	1 (11.1)	8 (88.9)	
3	1 (50.0)	1 (50.0)	
Primary tumor n(%)			0.383
Pancreas	0 (0.0)	4 (100.0)	
Other	2 (16.7)	10 (83.3)	
Functionality n(%)			1.000
Nonfunctional	1 (12.5)	7 (87.5)	
Functional	1 (12.5)	7 (87.5)	
Stage n(%)			
Localized	1 (25.0)	3 (75.0)	0.383
Metastatic	1 (8.3)	11 (91.7)	

Figure 1. Flowchart of the study population

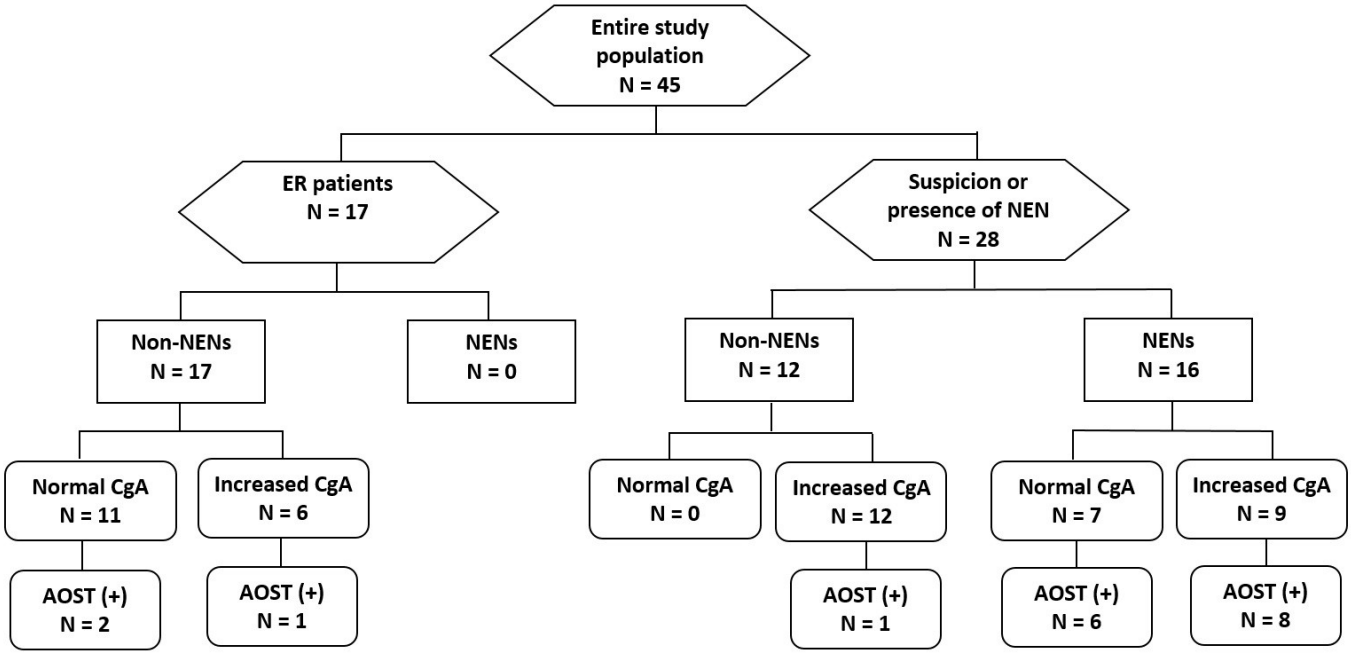
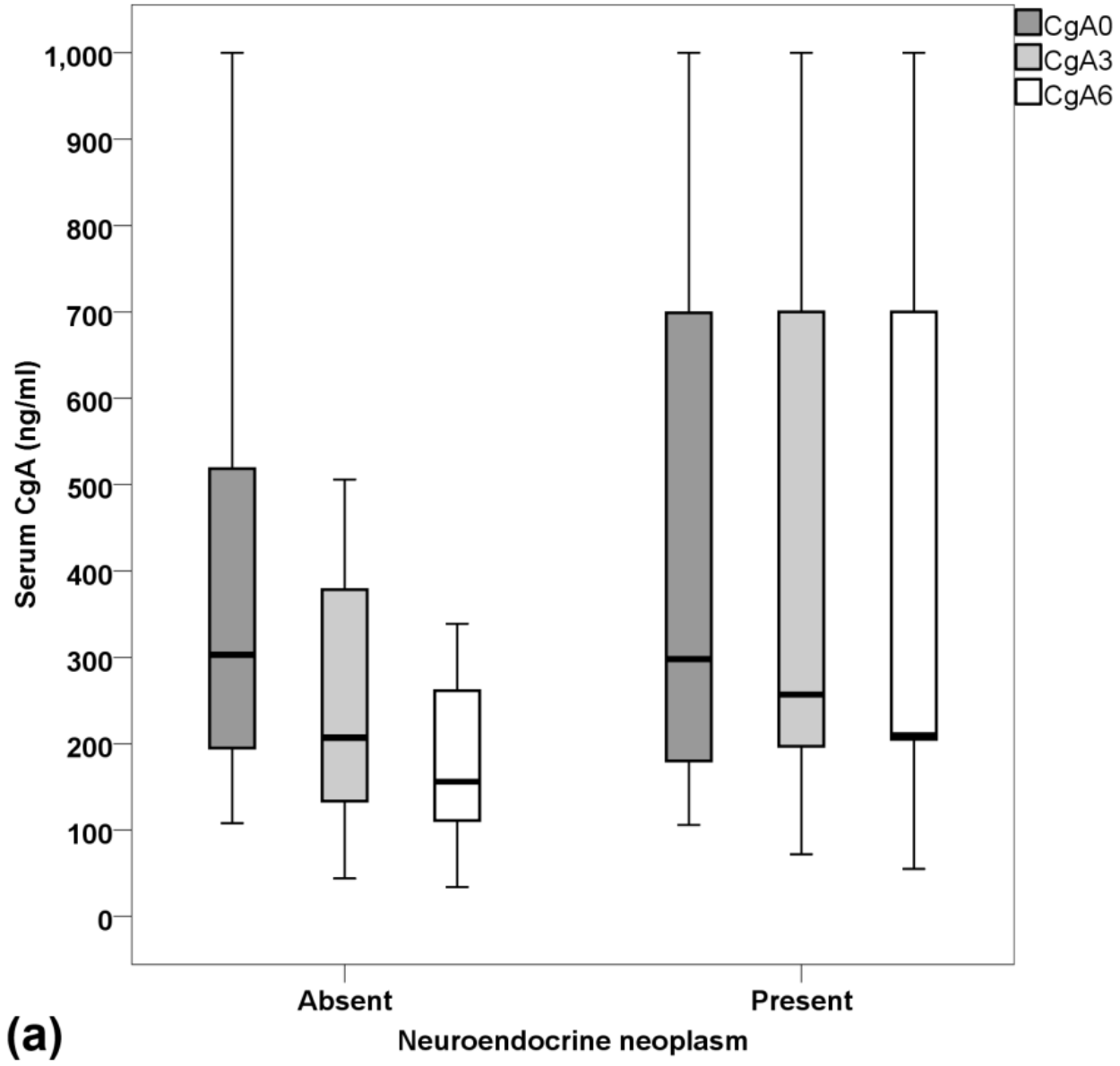
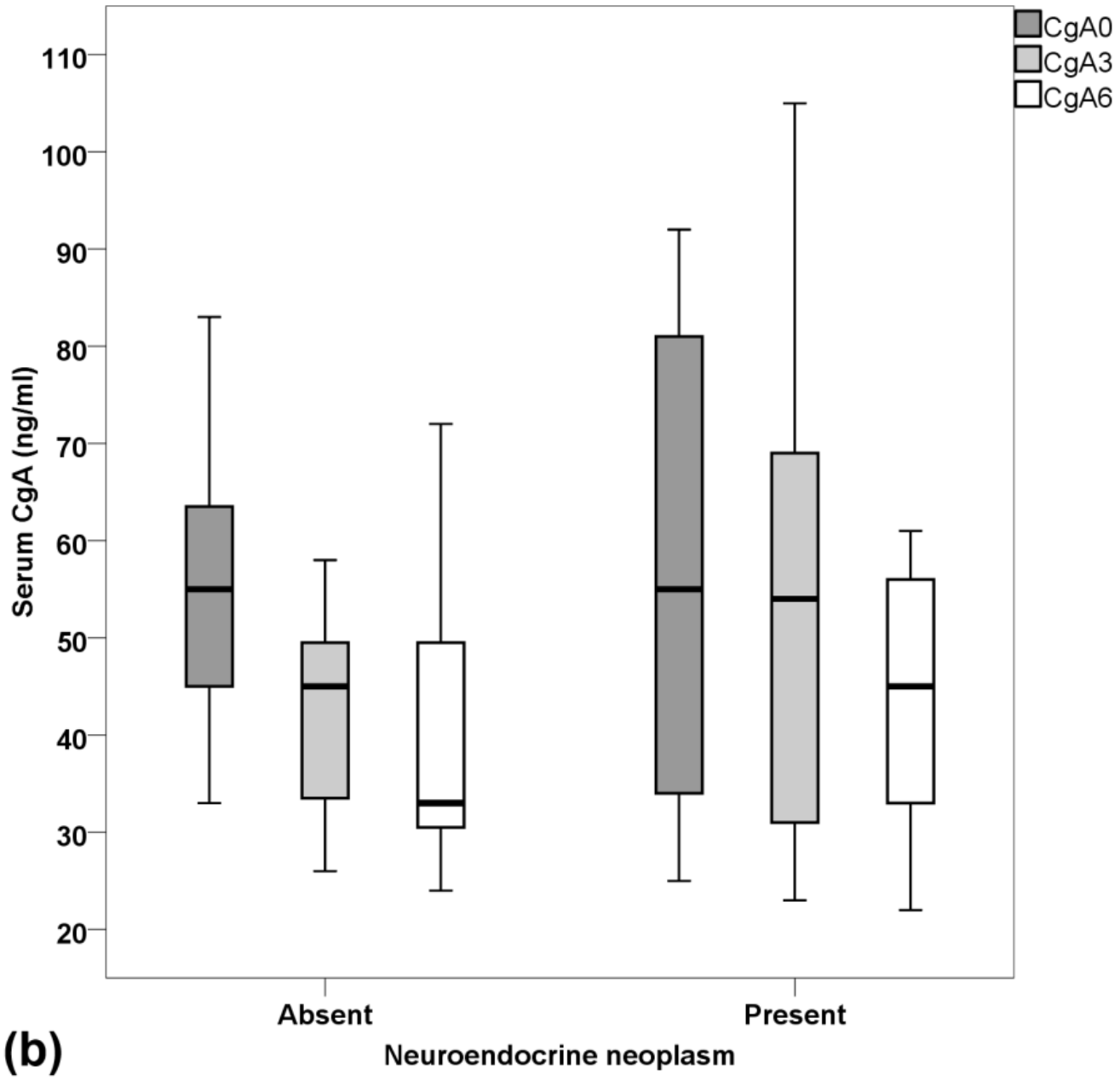
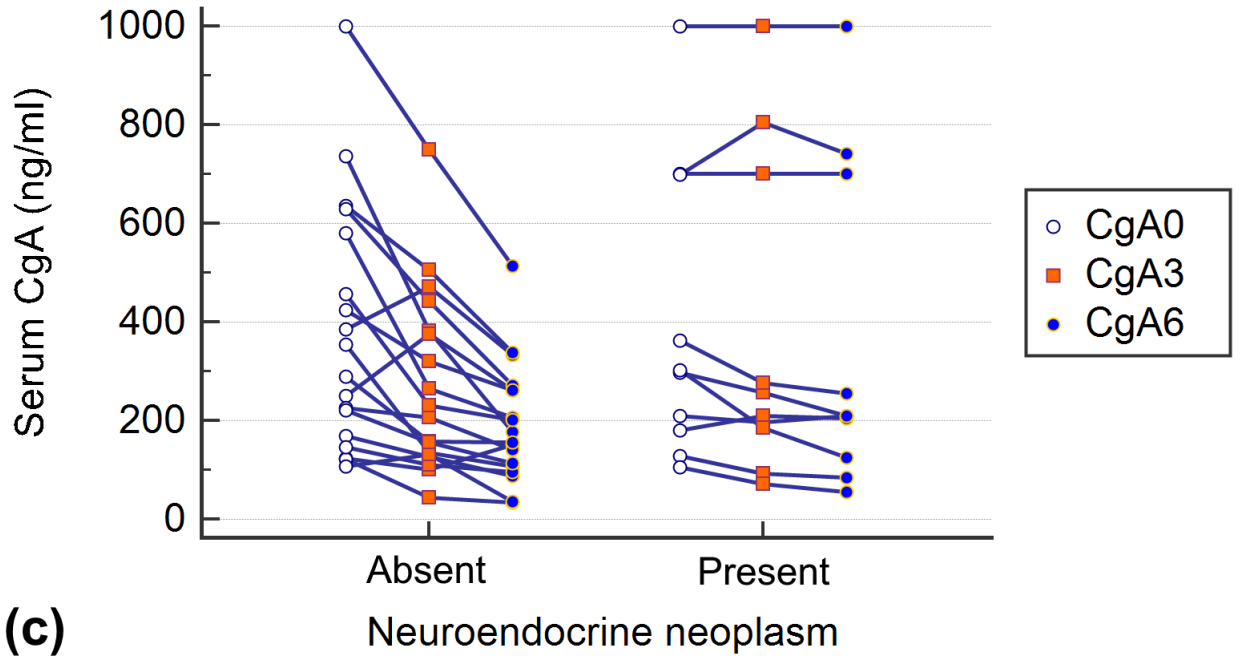


Figure 2. The difference in serum chromogranin A during acute octreotide suppression test between patients with neuroendocrine neoplasm and controls; in patients with increased baseline serum CgA (a) and normal baseline serum CgA (b) presented with box-plot graph; in patients with increased baseline serum CgA (c) and normal baseline serum CgA (d) presented with dot-line diagram for each patient.

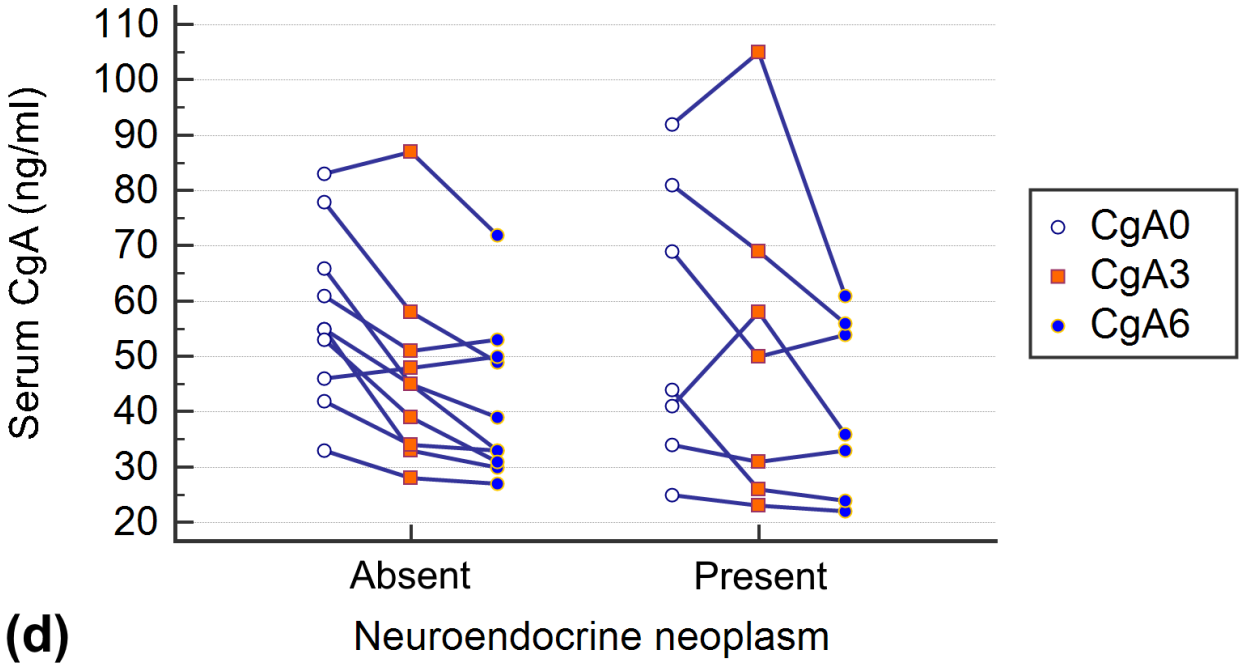



CgA0
CgA3
CgA6
110
100
90
80
70
60
50
40
30
20
Serum CgA (ng/ml)
Absent
Present
Neuroendocrine neoplasm

(b)

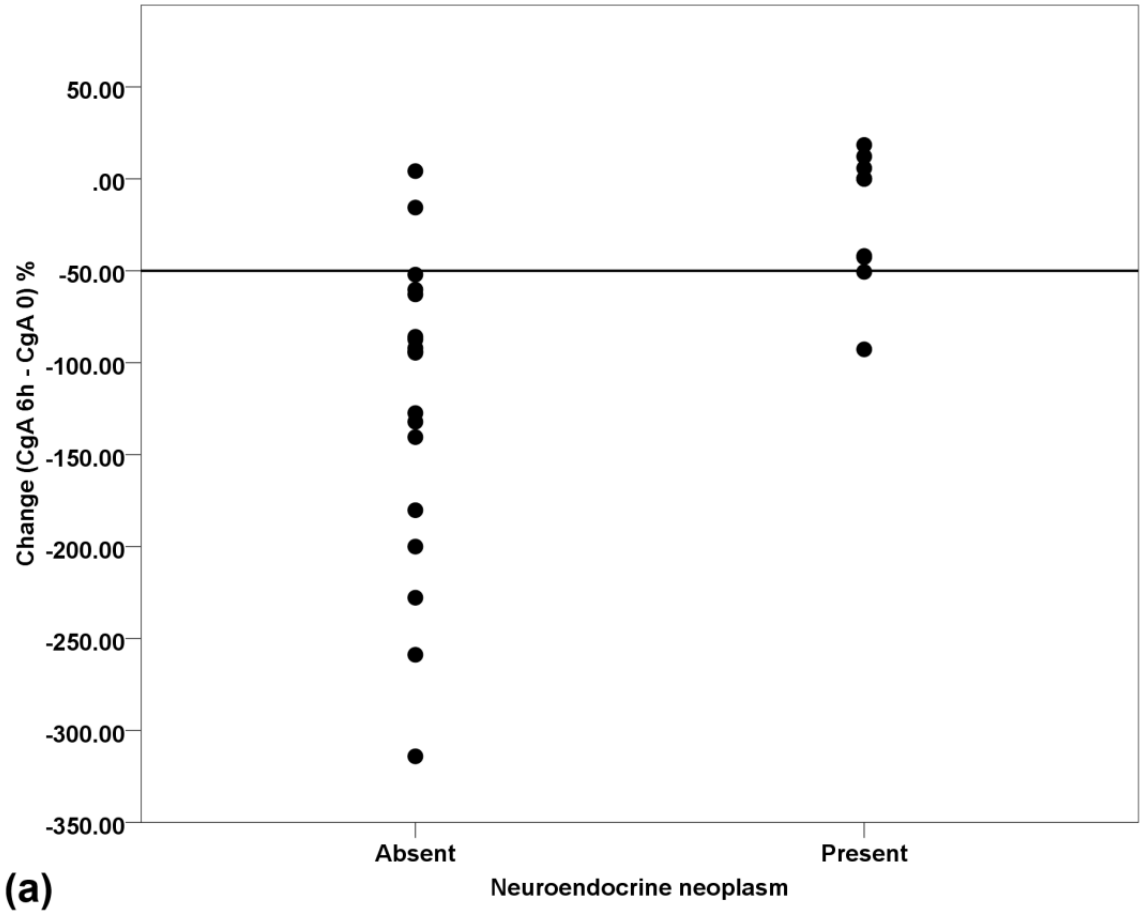

Serum CgA (ng/ml)
1000
800
600
400
200
0
Absent
Present
Neuroendocrine neoplasm
CgA0
CgA3
CgA6

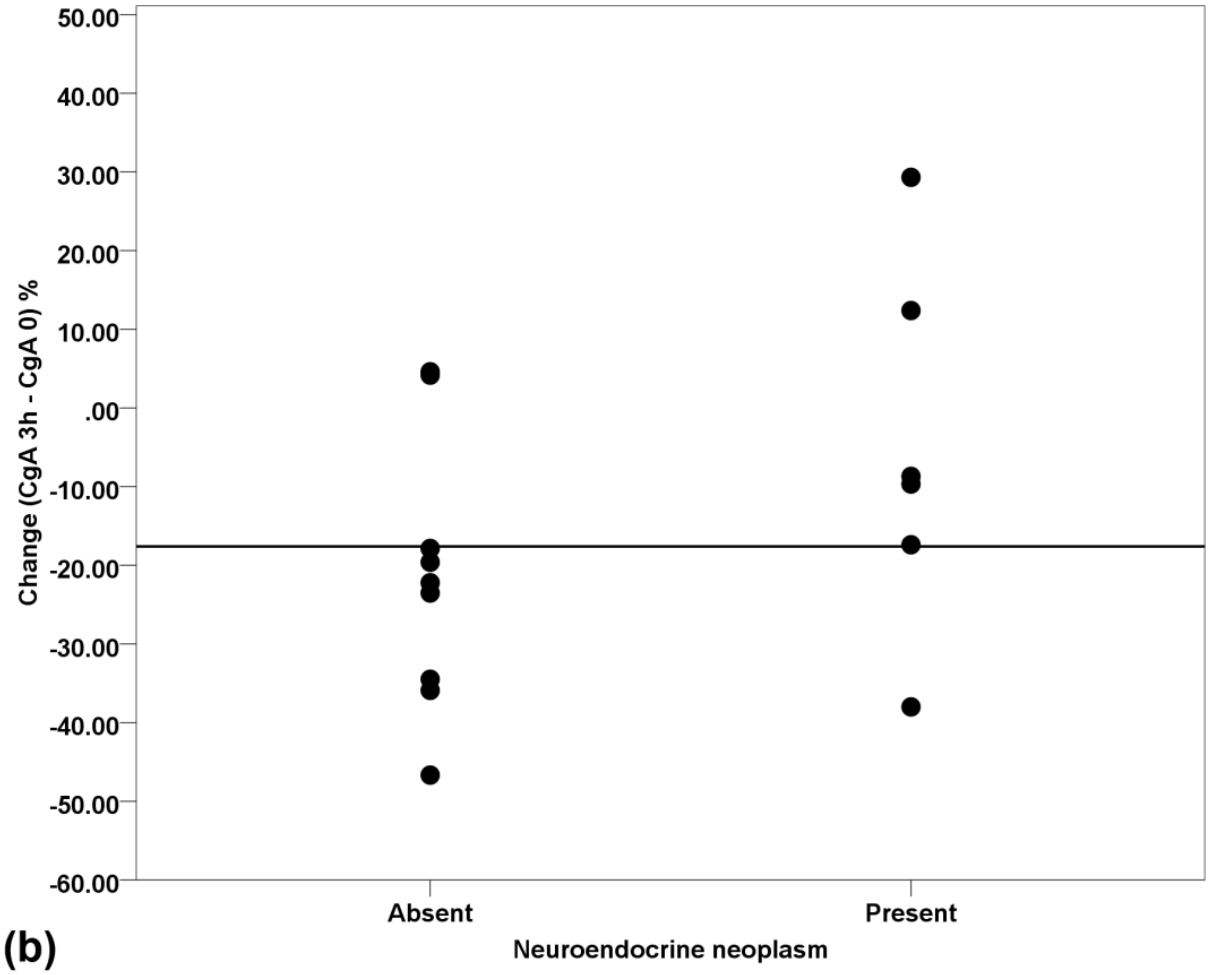
(c)


Serum CgA (ng/ml)
110
100
90
80
70
60
50
40
30
20
Absent
Present
Neuroendocrine neoplasm
CgA0
CgA3
CgA6

(d)

Figure 3. The difference in change of serum chromogranin A during acute octreotide suppression test between controls and patients with NEN; in patients with increased baseline serum chromogranin A (a) and normal baseline serum chromogranin A levels (b)




50.00
40.00
30.00
20.00
10.00
.00
-10.00
-20.00
-30.00
-40.00
-50.00
-60.00
Change (CgA 3h - CgA 0) %
Absent
Present
Neuroendocrine neoplasm

(b)