Serum Chromogranin A and Neuron-Specific Enolase in diagnosis of biologically inactive neuroendocrine tumours

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Background. Human Chromogranin A (CgA) is largely distributed in secretory granules of endocrine and neuroendocrine cells. Serum levels of CgA are significantly elevated in neuroendocrine tumours and reflect both secretory activity and tumour burden.
Because intense proteolyisis CgA is processed and degraded in tissues and sera: so, total CgA detection is crucial to improve diagnostic accuracy of the marker. Recently a two-site immunoradiometric method has been developed by selecting two monoclonal antibodies against a median, relatively unprocessed, sequence of the molecule.
Methods. We selected 32 patients with histologically proved neuroendocrine tumours (NET) without clinical and biological signs of hormonal secretion and evaluated the diagnostic performance of serum CgA and NSE and the relationship between marker's expression in tissues and in sera.
Results. Overall diagnostic accuracy of CgA was better than NSE one. CgA serum levels are related with the extension of the tumour ($p<0.01$) and to its expression in tissues ($p<0.001$). On the contrary, no relationship was found between serum NSE, extension of the tumour and immunostaining.
Conclusions. CgA was found to be more accurate than NSE in non-functioning NET evaluation. We suggest that CgA ought to be a general neuroendocrine marker routinely screened in patients with suspected NET, independently by hormone secretion.

Introduction

Human Chromogranin-A (CgA) is a 48-kDa protein, encompassing 439 amino acids. It belong to the granine family and it's largely distributed in secretory granules of endocrine and neuroendocrine cells [1]. CgA is an important marker of neuroendocrine differentiation: its circulating levels are significantly elevated in neuroendocrine tumours (NETs) reflecting the secretory activity of the tumour [2].
CgA assessment in NETs with eutopic secretory activity may be more convenient than urinary detection of 5-hydroxyindolacetic acid (5-HIAA), catecholamines and metabolites [3]. Additionally, CgA measurement may be useful also for the diagnosis and follow up of patients without demonstrated hormonal secretion [4].
Neurone-specific Enolase is an useful immunohistochemical marker of NETs. Nevertheless, its serum measurement has not a definitive position in NETs diagnosis, except for patients with small cell lung cancer and neuroblastoma, because of relatively low sensitivity and specificity of the marker [5].
CgA serum measurement is a sensitive and specific marker in NETs and correlates with the extension and the secretory activity of the tumour [6,7]. Recently a positive relationship among CgA serum expression, immunohistochemical and ultrastructural findings was found[8].
Degradation and proteolysis of CgA may be responsible for the wide variability of the fragments found in normal and pathological tissues, blood and urine [9]. Apart from its differential expression in normal and neoplastic tissues, many studies have demonstrated the diagnostic value of circulating CgA detection [10, 11,12].
Because of CgA molecule exposition to intensive proteolytic activity, the specificity of the antibodies used for CgA detection is crucial. Recently a two-site sandwich immunoradiometric assay (IRMA) has been developed by selecting two monoclonal antibodies against the median, relatively unprocessed, molecular domain [13].
This IRMA method has been employed in our study in order to (1) evaluate the diagnostic performance of serum CgA in a group of patients affected by bio-

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logically inactive NET, (2) compare the sensitivity of CgA and NSE, (3) investigate the relationship between immunohistochemical expression of both CgA and NSE and their serum concentration.

Patients and methods

Thirty-two patients referred to our institution with documented neuroendocrine disease were enrolled. Eleven had pancreatic neuroendocrine tumour and 17 carcinoids (4 stomach, 1 duodenum, 2 bronchus, 3 jejunum, 4 ileum, 2 appendix and 1 rectum). Three patients had neuroendocrine breast carcinoma and 1 had a paraganglioma.
Diagnosis were histologically confirmed and clinical evaluation of the disease extension was performed before any therapy. Conventional imaging methods (X-ray, ultrasonography and helical-CT) and ^{111}In-pentetreotide somatostatin-receptors scintigraphy were used to stage the disease. Tumour was considered to be limited when only primary lesion was detected and to be extensive when any other localisation was demonstrated.
Immunohistochemical investigation using NSE and CgA antibodies was performed in all patients. The degree of immunohistochemical expression of the markers was expressed as percentage of immunoreactive cells [8]. The ultra structural evaluation of large-core dense granules by electron microscopy was performed in 6 patients by a previously described method[14].
No patients presented clinical signs neither symptoms of biologically functioning neuroendocrine tumours.
24-h urinary 5-hydroxyndolacetic excretion was assessed by HPLC in patients affected by carcinoid tumours. Gastrin (Gask-PR IRMA, Cis Bio International, France) insulin (Insulin RIA, DPC, US), glucagon (Glucagon RIA, DPC, US), calcitonin (CT-RIA, Dia Sorin, Italy) and ACTH (ACTH-IRMA Dia Sorin, Italy) were measured in patients with insular pancreatic tumours.
CgA and NSE were assayed in all patients and in 100 healthy subjects as controls. Serum CgA was tested using a novel two-site immunoradiometric assay (IRMA) based on monoclonal antibodies that bind two distinct epitopes within the 145-245 region of CgA (CGA RIA CT. Cis Bio International, France). The cut off value was fixed at 100 ng/mL.
NSE levels were detected by a two-site IRMA (Prolifigen-NSE, Sangtec AB, Sweden) and a cut-off level of 12.5 μg/L (microg/L) was employed to obtain a specificity of 95% in controls.

Statistics

Since tumour markers were not normally distributed, results were expressed as median and distribution range and non-parametric analysis were employed. Mann-Whitney U test was used to assess differences between two independent groups.
The relationship between two variables was assessed by linear regression analysis.
A p value <0.05 was considered statistically significant.

Results

Serum concentration of CgA was significantly higher in patients affected by NETs compared to controls (Mann Whitney U test, $p<0.001$) while no difference was found for NSE concentration [Figure 1].
Serum CgA concentration was elevated in 18/32 (56%) patients while NSE was positive in 7/32 (21%). Both serum markers were elevated in 6/32 (18%) patients. High CgA concentration associated with normal NSE were found in 12/32 (37%) patients. Raised NSE concentration associated with normal CgA concentration were found only in 1 patient and 11/32 (34%) patients were negative for both markers [Table I].
Elevated serum CgA concentration was found to be significantly related to disease extension: 7/15 (46%) patients with limited disease and 11/17 (64%) patients with extensive disease showed raised CgA concentration. Serum CgA concentration in limited disease group (median 82 ng/mL, range 5-315) was significantly lower than in extensive disease group (median 312 ng/mL, range 56-1300 ng/mL) [Mann-Whitney U test, $p<0.001$].
Patients with metastatic disease showed a significant relationship between serum concentration of CgA and the number of ^{111}In-pentetreotide uptake areas (linear regression, r^2 0.773, $p<0.01$) [Figure 2].
Elevated NSE serum concentration was found in 3/15 (20%) patients with limited disease and in 4/17 (23%) patients with extensive disease. No relationship was found between extension of the disease and serum NSE concentration.
Immunohistochemical expression of CgA in tissue showed a positive relationship with CgA serum levels (linear regression, r^2 0.855, $p<0.001$) [Figure 3].NSE immunostaining was positive in all patients but no relationship was demonstrated with serum concentration of this marker.
Electron microscopy evidence a poor granular expression in CgA-negative extensive NETs while 2 CgA-positive limited NETs showed intense granular expression.

Discussion

Immunohistochemical detection of NSE and CgA is a very useful tool for diagnosis of NETs and both molecules may be considered as general markers of neuroendocrine derivation. NSE and CgA reflect metabolic and secretory activity of the tumour respectively and their elevated serum concentration may have different meanings.

Figure 1: Cg-A and NSE distribution in patients with neuroendocrine tumours (NET) and controls.

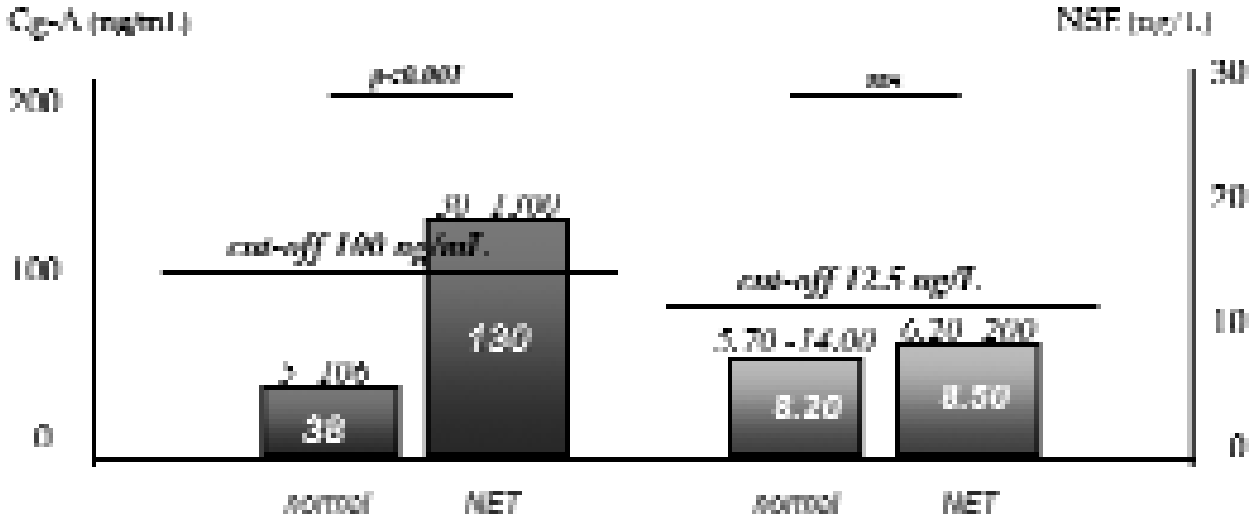


Figure 2. Relationship between serum chromogranin-A and number of 111In-pentetreotide uptake areas in extensive disease (n=17)

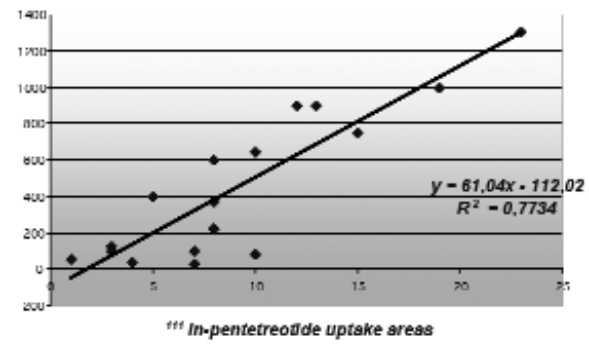
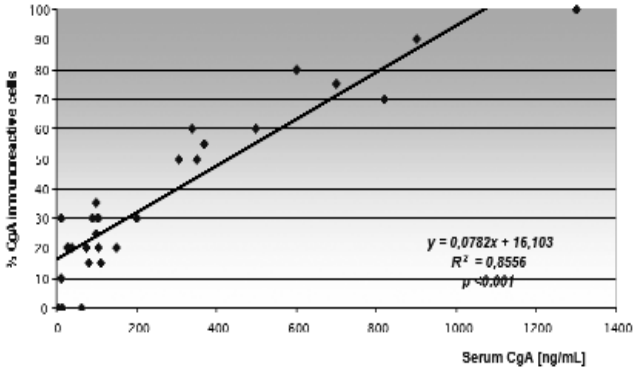


Figure 3. Relationship between serum CgA and immunohistochemical expression



The present paper evaluated the diagnostic performance of both markers in a group of clinically and biologically non–functioning NETs.

CgA showed a better diagnostic sensitivity than NSE and its serum concentration was related to disease extension.

Baudin and co-workers obtained similar results in a group of patients with both functioning and non-functioning NET. In the Authors' opinion the secretory activity more than tumour burden may be the

Table I: Positive-rate of CgA and Nse in limited and extensive NETs.

Neuroendocrine tumours	Limited disease (n=15)		Extensive disease (n=17)	
	Cg-A	**NSE**	**Cg-A**	**NSE**
Pancreatic NET (n=11)	2/6	1/6	4/5	2/5
Carcinoids (n=17)				
Stomach (n=4)	1/1	1/1	3/3	1/3
Duodenum (n=1)	-	-	0/1	1/1
Bronchus (n=2)	0/2	0/2	-	-
Jejunum (n=3)	2/2	1/2	1/1	0/1
Ileum (n=4)	1/1	0/1	2/3	0/3
Appendix (n=2)	0/2	0/2	-	-
Rectum (n=1)	-	-	1/1	0/1
Breast NE carcinomas (n=3)	-	-	0/3	0/3
Paraganglioma (n=1)	1/1	0/1	-	-
	7/15	3/15	11/17	4/17

"key-element" and CgA detection may have low value in patients with limited disease or in patients without peptide hormone secretion. On the other hand, CgA serum concentration above the cut-off was found in 28% of the patients having no secretion and mainly extensive disease and 89% of selected patients … undergone … treatments … potentially decreasing the sensitivity of the marker[15].

Some Authors previously demonstrated a poor correlation between serum levels of CgA and specific secretory products of the tumours. In facts, NETs that are not able either to secrete hormone peptide or amines and NETs that release undetectable hormonal products, frequently retained the ability to secrete significant amounts of CgA. These so-called chromograninomas were first described by Sobol et al. [16].

Our data confirmed that non-functioning NETs enhance serum CgA expression related with tumour burden. Immunohistochemical stains demonstrate that CgA levels are related to CgA expression in the tissue. Moreover electron microscopy showed that 4 CgA-negative extensive NET express a limited number of large-core dense granules and 2 CgA-positive small NET demonstrate a rich granular expression.

Serum CgA levels can be considered as the result of a complex function of large-core granules expression in neoplastic tissues and tumour burden. Increased serum CgA levels in non-functioning NET probably reflects undetectable hormonal secretion or alternative pathways of CgA secretion, uncoupled with hormone release.

Diagnostic performance of serum NSE was poor but NSE immunostaining was positive in all patients. Previous studies claimed that NSE may reflect cell necrosis rather than tumour burden: so, only in the presence of cellular lysis the marker is released in the circulation [17]. Because large population of necrotic cell is frequently encountered in poorly differentiated neuroendocrine carcinoma, NSE should be considered as marker of "aggressive" NETs [18].

In conclusion CgA was found to be more sensitive than NSE in diagnosis of non-functioning NETs. We suggest that CgA ought to be routinely measured in patients with suspected NETs, independently by hormone peptides secretion.

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