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Evaluation of diagnostic power of CgA determinations in neuroendocrine tumours (net$_s$)

Ocena mocy diagnostycznej oznaczania CgA w guzach neuroendokrynnych (net$_s$)

INTRODUCTION

Chromogranin A (CgA) is a 48 kDa hydrophilic acidic glycoprotein member of the granin family produced and stored together with biogenic amines and other peptide hormones in the secretory granules of the neuroendocrine cells, which are widespread in the whole organism and form the diffused endocrine system (DES) [2, 4, 6, 10, 12]. CgA is found in adrenal medulla, pancreatic islet cells, endocrine cells of the gastrointestinal tract and in sympathetic nervous system. Immunocytochemical detection of CgA is currently performed to assess the endocrine character of tumoural cells. CgA is physiologically released by exocytosis and may be detected in blood. In particular, when a tumor develops in an endocrine tissue, it becomes the main source of circulating CgA [2, 4, 5].

Elevated circulating CgA levels have been demonstrated in serum or plasma of patients with various neuroendocrine tumors (NETs). NETs constitute a heterogeneous group of neoplasms, characterized by the presence a wide variety of functional or nonfunctional endocrine syndromes and biological and histopathological differences [2, 4, 5, 11].

Recent studies confirm that CgA is the useful marker for the diagnosis and follow-up of neuroendocrine tumors. However, these reports have shown various diagnostic sensitivity and specificity for circulating CgA ranging from 27% to 81% [1, 3, 6–8]. Therefore, the aim of this study was to evaluate the diagnostic power of CgA determinations for the diagnosis of NETs.

MATERIAL AND METHODS

Analysis of the results of the plasma CgA concentrations was performed in 41 patients with NETs. The patients were treated at the Oncological Centre of Lublin Region from February 2005

to May 2008. In most cases, primary localisation was identified in the gastrointestinal tract (73%) and lungs (15%). In individual cases ovarian cancers were of unknown primary origin. Among the enrolled subjects there were 27 women (65.8%) and 14 men (34.2%). The age range was from 38 to 76 years (mean 58±10 years).

The control group was composed of healthy subjects (n=15) with the age range from 23 to 53 years (mean age: 38±8 years). This group included 10 women (60%) with the mean age of 41 years ±9 and 5 men (40%) with the mean age 37±9 years.

CgA determinations. The material for the study was the peripheral blood obtained from the ulnar vein. Blood samples were drawn between 8:00 and 10:00 a.m. into tubes with K_3EDT**A** **a**s **a**ntico**a**gul**a**nt in volumes of 5 ml. Plasma was separated from the collected blood samples by centrifugation for 10 min at 1000 rpm, aliquoted and stored frozen at -20˚C until analysis.

CgA plasma determinations were measured with the use of ELISA immunoenzymatic assay of commercially available kit Chromogranin A (DakoCytomation, Denmark). Analyses were done according to the manufacturer's instructions. During the whole course of the study the kits from the same company were used.

Statistical analysis. For statistical analysis of the obtained results, Statistica 7.0 StatSoft was used. Plasma CgA concentrations in the study and control group were reported with the use of descriptive statistic elements (median Me, range or percentile (25–75%), minimum Min, maximum Max). Distribution was tested for normality using Shapiro-Wilk test and non parametric test U Mann-Whitney was applied. A p value ≤ 0.05 was considered as statistically significant in the analysis.

The cut-off value of 18 U/l was used according to the manufacturer's declaration. Sensitivity, specificity, positive and negative predictive values were calculated using the standard formulae. Sensitivity = true positive / true positive + false negative and specificity = true negative/true negative + false positive. Positive predictive value (PPV) = true positive/true positive + false positive and negative predictive value (NPV) = true negative/false negative + true negative.

In order to investigate the diagnostic value of plasma CgA we plotted ROC (Receiver Operating Characteristic) curve and the area under the curve (AUC) was calculated to describe the capability of the marker to discriminate between patients and controls using Analyse-it Microsoft Excel program.

RESULTS

CgA plasma levels were statistically significantly higher ($p<0.001$) in patients with NETs compared to the control group. Table 1 shows the results of determinations of CgA plasma concentrations in the patients with NETs and healthy participants.

Table 1. Plasma levels of CgA (U/l) in the study and control group

Parameter	Group						p level
	Study n=41			Control n=15			
	Me	25%–75%	Min-Max	Me	25%–75%	Min-Max	
CgA (U/l)	76.9	15.0–199.6	6.9–770.7	10.5	7.9–17.2	5.9–27.7	<0.001

p – level of statistical significance ($p<0.05$)

Diagnostic sensitivity and specificity of CgA in NETs and positive and negative predictive values for cut-off value 18 U/l are 71%, 87%, 93%, 52% respectively. Fig. 1 shows the ROC curve for CgA.

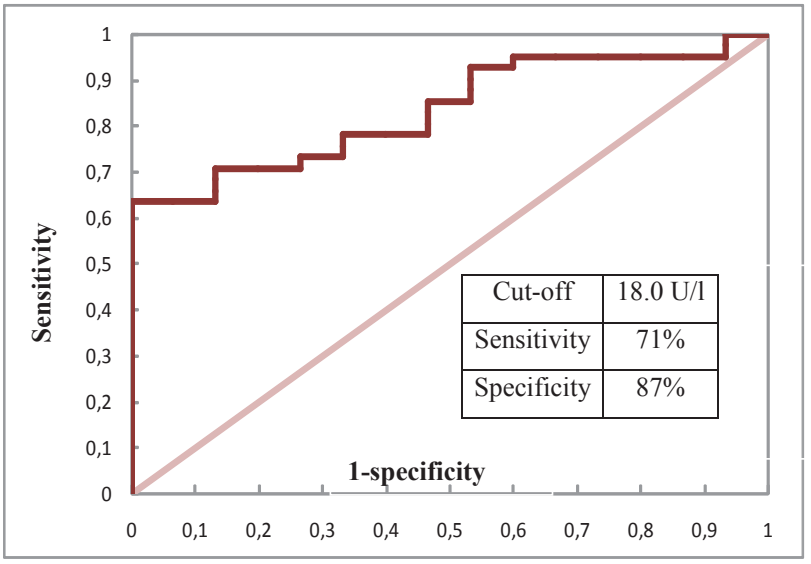

Fig. 1. Receiver-operating characteristics curve for CgA obtained with 41 NET patients and 15 healthy subjects. The area under the curve was 0.84 ($p<0.0001$)

The area under the curve equal to 0.84 ($p<0.0001$) indicates a good diagnostic usefulness of CgA in the detection of NET.

DISCUSSION

We demonstrated that the median value of CgA was significantly higher compared to healthy participants, which is consistent with reports of other authors [3–5].

Reports in the literature have shown that serum levels of CgA may be a sensitive and specific marker of NETs [1, 5–9]. In this study, we focused on assessing the value of CgA as a biochemical marker for detection of NET tumors, therefore the evaluation of the diagnostic power of CgA ELISA determination in NETs was the subject of the present study.

We calculated diagnostic sensitivity and specificity of CgA for the cut-off value 18 U/l proposed by the manufacturer obtaining values of 71% and 87%, respectively. In other author's observations the sensitivity of CgA as a marker of neuroendocrine tumors of the gastrointestinal tract varies between 10–100% with specificity of 68–100% [1,3]. Recent studies have shown different ranges of diagnostic sensitivity and specificity for circulating CgA, according to secretory activity, histological characteristics of the tumour and to metastatic disease spread. These parameters have been

demonstrated to depend also on the method used to determine the serum or plasma concentration of CgA and on the cut-off value considered as pathologic [1, 2, 4, 5, 9, 11, 12].

Campana et al. [2] evaluated the diagnostic power of CgA in the group of 238 NET patients localized in the gastrointestinal tract and lungs, and 48 healthy subjects. Using the same cut-off point (18 U/l) and ELISA kit as in our study, they received higher diagnostic sensitivity (85%) and specificity (96%) for the diagnosis of NETs. These findings are in agreement with others reported in the literature, where a variable cutoff value from 17 to 34 U/l was identified, with a variable values of the diagnostic sensitivity of CgA from 79 to 92% (6,9) and specificity from 83 to 91% [6, 9, 11].

In conclusion, data from the literature and results of this study suggest the use of CgA in the diagnosis of NETs. The DacoCytomation ELISA test for the determination of chromogranin A in plasma with the accepted cut-off value of 18 U/l that was used in this study has a good diagnostic power in detecting neuroendocrine tumors.

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SUMMARY

Chromogranin A (CgA) is a 48 kDa hydrophilic acidic glycoprotein member of the granin family produced and stored together with biogenic amines and other peptide hormones in the secretory granules of the neuroendocrine cells, which are widespread in the whole organism and form the diffused endocrine system (DES). CgA is found in adrenal medulla, pancreatic islet cells, endocrine cells of the gastrointestinal tract and in sympathetic nervous system. CgA is physiologically released by exocytosis and may be detected in blood. Elevated circulating CgA levels have been demonstrated in serum or plasma of patients with various neuroendocrine tumors (NETs). Recent studies confirm that CgA is a useful marker for the diagnosis and follow-up of NETs. The aim of this study was to evaluate the diagnostic power of CgA determinations for the diagnosis of NETs. Analysis of the results of plasma CgA concentrations was performed in 41 patients with NETs. The control group was composed of healthy subjects (n=15). CgA plasma determinations were measured with the use of ELISA immunoenzymatic assay of commercially available kit Chromogranin A (DakoCytomation, Denmark). In order to investigate the diagnostic value of plasma CgA we plotted ROC (Receiver Operating Characteristic) curve and the area under the curve (AUC) was calculated. In our study, CgA plasma levels were statistically significantly higher ($p<0.001$) in patients with NETs compared to the control group. We calculated diagnostic sensitivity and specificity of CgA in NETs and positive and negative predictive values using the standard equations for the cut-off 18 U/l proposed by the manufacturer obtaining values of 71%, 87%, 93%, 52%, respectively. In conclusion, the DacoCytomation ELISA test for the determination of chromogranin A in plasma with the accepted cut-off value of 18 U/l that was used in this study has a good diagnostic power in detecting neuroendocrine tumors.

Key words: chromogranin A, CgA, neuroendocrine tumors, NETs, diagnostic power

STRESZCZENIE

Chromogranina A (CgA) jest kwaśną hydrofilową glikoproteiną należącą do rodziny granin o masie cząsteczkowej 48 kDa, wytwarzaną i magazynowaną wspólnie z aminami biogennymi i innymi hormonami peptydowymi w ziarnistościach sekrecyjnych komórek neuroendokrynnych, które są rozproszone w całym organizmie człowieka i tworzą rozlany system endokrynny (DES). Jest obecna w rdzeniu nadnerczy, komórkach wysp trzustkowych i endokrynnych komórkach przewodu pokarmowego oraz w układzie nerwowym współczulnym. W warunkach fizjologicznych CgA jest uwalniana w procesie egzocytozy do przestrzeni pozakomórkowej i dzięki temu można wykryć jej obecność we krwi. Podwyższone stężenia CgA w osoczu lub surowicy stwierdza się u pacjentów z guzami neuroendokrynnymi (NETs). Badania ostatnich lat potwierdziły istotne znaczenie chromograniny A w diagnostyce i monitorowaniu leczenia guzów pochodzenia neuroendokrynnego. Celem pracy była ocena mocy diagnostycznej oznaczania CgA w guzach NET. Oceniano wyniki stężeń chromograniny A w osoczu u 41 chorych z guzami NET. Grupę kontrolną stanowili zdrowi ochotnicy (n=15). W celu ilościowego oznaczenia chromograniny A w osoczu krwi wykorzystano metodę immunoenzymatyczną ELISA. W badaniu zastosowano komercyjny

zestaw odczynnikowy Chromogranin A ELISA Kit, firmy DakoCytomation, Denmark. Do oceny przydatności diagnostycznej chromograniny A wykreślono krzywą ROC (Receiver Operating Curve) i obliczono pole pod krzywą. W osoczu krwi chorych z guzami NET stwierdzono statystycznie istotnie wyższe stężenie chromograniny A ($p<0.001$) w porównaniu z grupą kontrolną. Czułość i swoistość diagnostyczna CgA w guzach NET oraz wartości predykcyjne dodatnie i ujemne, obliczone według typowych wzorów dla punktu odcięcia 18 U/l przyjętego zgodnie z deklaracją producenta, wynosiły odpowiednio 71%, 87%, 93%, 52%. Zastosowany test ELISA firmy DacoCytomation do oznaczania chromograniny A w osoczu krwi z przyjętą wartością odcięcia 18 U/l ma dobrą moc diagnostyczną w wykrywaniu guzów neuroendokrynnych.

Key words: chromogranina A, CgA, guzy neuroendokrynne, NETs, wartość diagnostyczne