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Significance of plasma chromogranin A determination in neuroendocrine tumour (NET) diagnosis

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Abstract: The secretory nature of NETs implies the determination of the CgA concentration as a standard marker. The concentration of CgA in plasma correlates with the degree of histopathological differentiation, tumor stage, and is an essential prerequisite for therapy. A retrospective analysis of the results of the plasma CgA concentrations in relation to histopathological and clinical findings (type of NET according to the WHO classification, severity of disease based on the presence of metastases and clinical symptoms) as well as somatostatin receptor scintigraphy was performed in 41 patients with NET. The patients were treated in The Regional Oncology of Lublin from February 2005 to May 2008. Data from the literature and results of this study suggest the use of CgA in the diagnosis and prognosis of NET. Plasma CgA concentration analysed together with histopathological assessment of tumor and the clinical picture is a useful marker in the diagnosis of neuroendocrine tumours. High plasma CgA concentrations may indicate the presence of highly-differentiated NET (WDNEC), and also may indicate the presence of tumor metastasis. The highest CgA concentrations were observed in patients with neuroendocrine tumors associated with carcinoid symptoms and the presence of metastases to the liver.

Key words: chromogranin A (CgA), neuroendocrine tumour (NET)

Introduction

The Chromogranin A (CgA) is an acidic glycoprotein member of the granin family, which was discovered and described in 1960 [1-5]. This physiological protein of MW 48 kDa [6-8] is produced and stored together with biogenic amines and other peptide hormones in the secretory granules called large dense-core vesicles of the neuroendocrine cells [2,4-6,9,10], which are widespread in the whole organism and form the diffused endocrine system (DES) [11-13]. The neuroendocine cells can be found in: adrenal medulla, sympathetic nerves endings, cerebral cortex, pituitary gland, gastrointestinal tract, thyroid, parathyroid glands, pancreatic islets and lungs [2,4,8,10]. The synthesis and secretion of CgA may be intensified in the neuroendocrine tumour (NET) cells [4,9,14,15].

CgA is secreted to the extracellular space, so it's easily detectable in the blood [2,4,16]. Recent studies confirmed that the measurement of CgA levels in blood can also be used to diagnose and to monitor neuroendocrine tumours during treatment [6,10,14,16-18].

Neuroendocrine tumours are a rare and heterogeneous group of neoplasms characterized by embryological, biological and histopathological differences. Additional differences are related to either the presence or the absence of symptoms caused by endocrine activity (biologically active or inactive NETs) or the presence or absence of a somatostatin receptors expressed on neuroendocrine tumour cells found in scintigraphy [12,19-22]. Approximately 70% of all cancers are neuroendocrine tumors of the gastrointestinal tract called gastroenteropancreatic neuroendocrine tumours (GEP-NET) [19,23]. In every case of diagnosed NET determination of CgA concentration has a significant impact on the prognosis and the choice of treatment and clinical outcome. Prognosis for the patients with NET secreting CgA is better, having a longer survival time and a prolonged time to progression [5,10,18,22,24,25].

The present study was performed in order to assess the relationship between the concentration of CgA in plasma and patients' selected clinical data (type of NET according to the WHO classification, severity of disease based on the presence of metastases and clinical symp-

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

Parameter	Groups						P level
	Study group n=41			Control group 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

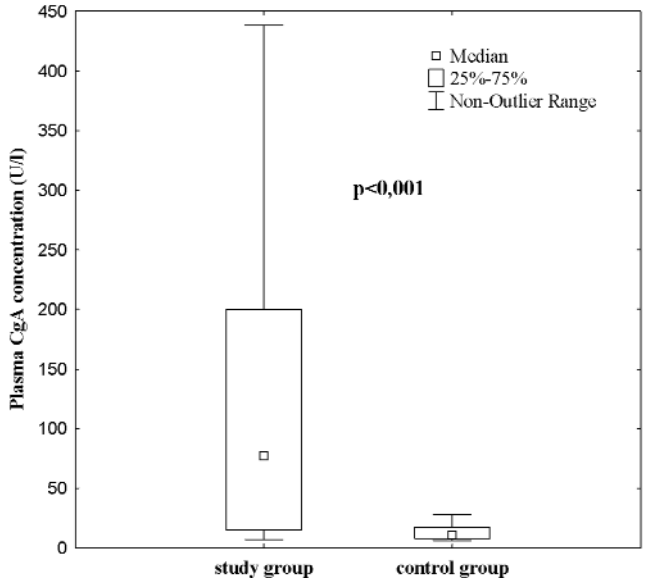

Fig. 1. Plasma levels of CgA [U/l] in the study and control group.

toms) and outcome of somatostatin receptor scintigraphy (SRS). Moreover we evaluated the diagnostic power of CgA determinations for the diagnosis of NET.

Materials and methods

A retrospective analysis of the results of the plasma CgA concentrations in relation to histopathological and clinical findings as well as somatostatin receptor scintigraphy was performed in 41 patients with NET. In most cases primary localisation was identified in the gastrointestinal tract (73%) and lungs (15%). In individual cases were ovarian cancers of unknown primary origin.

NET histological type was determined based on the WHO classification from 2000y. [26,27]:

1. WDNET well-differentiated neuroendocrine tumor (benign or low grade malignant)
2. WDNEC well-differentiated neuroendocrine carcinoma (low grade malignant)
3. PDNEC poorly-differentiated neuroendocrine carcinoma (high grade malignant)

The patients were treated in The Oncological Centre of Lublin Reguion from February 2005 to May 2008. The patients ages ranged from 38 to 76 years (mean 58±10 years). The group included 27 women (65,8%) and 14 men (34,2%), W/M index=1,9.

The control group was composed of healthy volunteers (n=15) with 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, W/M index=2.

CgA determinations. Peripheral venous blood samples were collected using standard veinpuncture technique. Volumes of 7 ml for CgA determination were drawn into sterile tubes with EDTA as anticoagulant and centrifuged within 1hr at 1000 rpm for 10 min. All plasma samples were separated and aliquoted into eppendorfs and stored at -20°C pending analysis.

The CgA plasma determinations were performed 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. Analytical sensitivity of the test was 2.0 U/l and imprecision expressed as CV was 8.6%.

Statistical analysis. Plasma CgA concentrations in the studied groups were reported with the use of descriptive statistic elements (median Me, range or percentile (25-75%), minimum Min, maximum Max) as appropriate and the results are shown in the tables and on the graphs. During statistical analysis the comparisons of CgA concentrations between patients and control groups were performed, as well as within the patients' group depending on the clinical presentation of the disease. For statistical analysis of obtained results, Statistica 7.0 StatSoft was used.

Distribution was tested for normality using Shapiro-Wilk W test. Analysed parameters were found skew-distributed and therefore non parametric tests U Mann-Whitney and Kruskal-Wallis were applied.

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 equations. 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 using Analyse-it Microsoft Excel program. A p value ≤0.05 was considered as statistically significant in all analyses.

Results

In the plasma of NET patients with tumors CgA levels were statistically significantly higher (p<0.001) compared to the control group. The summary of CgA plasma concentrations in the two groups are presented below (Table 1 and Fig. 1).

Patients were subdivided into two groups, depending on the concentrations of plasma Cg A.

Table 2 and 3 presents the results of plasma CgA and evaluation of CgA in immunohistopathological preparates in 41 patients with respect to clinical data (type of tumor NET according to the WHO classifica-

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Table 2. NET patients with normal concentrations of CgA

No	Sex/ age	CgA		Tumour type (WHO)	Primary origin	Presence of metastases	Clinical symptoms	Results of SRS
		Plasma [U/l]	Imm.-hist-pat.					
1	M/59	10.0	present (+)	I	ileum	no	no	n/a
2	K/68	8.8	present (+)	I	jejunum	no	no	n/a
3	K/53	11.2	present (+)	I	appendix	no	no	n/a
4	K/59	15.0	present (+)	I	appendix	no	no	neg
5	K/57	9.4	present (+)	I	rectum	no	no	pos
6	M/54	9.3	present (+)	I	rectum	no	no	neg
7	M/69	14.8	present (+)	I	rectum	no	no	n/a
8	K/56	11.1	present (+)	II	cecum	LN	no	neg
9	K/61	7.5	present (+)	II	cecum	LN	no	n/a
10	K/59	6.9	present (+)	II	pancreas (body)	L	no	n/a
11	M/38	10.8	present (+)	III	pancreas (head)	LN	no	neg
12	M/76	18.1	present (weak +)	III	liver	LN	no	pos
13	K/50	16.6	present (+)	III	lungs	LN, L	no	n/a

Imm-hist-pat – immunohistopathological test; SRS – somatostasin receptors scintigraphy; LN – lymphatic nodules metastases; L – liver metastases; B – bone metastases; P – lung metastases; C – CNS metastases; n/a – not available.

tion, severity of disease based on the presence of metastases and clinical symptoms) and SRS results. Elevated CgA in plasma was observed in 28 (68%) patients (Table 1), whereas in 13 (32%) patients CgA levels fell in reference ranges (Table 2). Immunocytochemical test for the presence of GgA in the material collected from the tumor site was positive in 97% of patients.

Table 4 and Fig. 2 summarises the plasma CgA concentration results in patients depending on the degree of neuroendocrine differentiation of NET.

Table 5 and Fig. 3 presents the codependence of plasma CgA levels in the patients with the presence of distant metastases.

Table 6 and Fig. 4 presents the patients plasma CgA concentration results depending on the presence of clinical symptoms. The predominant clinical symptoms of carcinoid were: flush, diarrhea, stomach contraction pain.

Compatibility between patients plasma CgA concentration and the results of SRS, were shown in Table 7. The compatibility between SRS and CgA determinations in 27 patients who underwent somatostatin receptor scintigraphy was 85%. 19 patients showed the presence of somatostatin receptors and had elevated plasma CgA, while 4 patients had negative SRS and the normal concentration of CgA.

Assessment of CgA diagnostic power in neuroendocrine tumors (NET)

Diagnostic sensitivity and specificity of CgA in NET and positive and negative predictive values for cut-off value 18 U/l are 71%, 87%, 93%, 52% respectively. Fig. 5 shows the ROC curve for CgA.

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

The secretory nature of NETs implies the determination of the CgA concentration as a standard marker. The concentration of CgA in plasma correlates with the degree of histopathological differentiation, tumor stage, and is an essential prerequisite for therapy [5-7, 10,17,18,25,28]. Relating this study results of CgA concentrations which were obtained over 3 years, to histopathological and clinical parameters has led to observations which have contributed to knowledge in the field of NET diagnosis.

We demonstrated that in NET patients the median value of CgA was significantly higher compared to healthy people, which is consistent with reports of other authors [10,14,20]. A comparison of plasma CgA results with the results of CgA immunohistochemical staining in the same patients (compiled in Table 2 and 3) clearly indicates that low concentrations of CgA do not exclude the presence of neuroendocrine tumor. The assessed relationships between serum CgA in plasma and histopathological tumor type showed that the concentration of plasma CgA was significantly higher in patients with well differentiated NET compared with low grade differentiation tumors. However in well differentiated tumors low median value of CgA was observed.

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Table 3. NET patients with elevated CgA concentration

No	sex/ age	CgA		Tumour type (WHO)	Primary origin	Presence of metastases	Clinical symptoms	Results of SRS
		plasma [U/l]	Imm.-hist.-pat					
1	K/71	207.2	present (+)	II	ileum	LN, L	yes	neg
2	M/62	37.8	present (+)	II	cecum	L, P	no	n/a
3	K/70	83.7	present (+)	II	ileum	LN, L, P	no	pos
4	M/69	135.0	present (+)	II	jejunum	LN, L	yes	pos
5	K/38	635.0	present (++)	II	ileum	LN, L	yes	pos
6	K/66	135.0	present (+++)	II	cecum	LN, L	yes	n/a
7	M/57	111.8	present (+)	II	ileum	LN, L	yes	n/a
8	M/66	165.2	present (+)	II	appendix	LN	no	pos
9	M/76	133.4	present (+)	II	ileum	LN, L	no	pos
10	K/58	252.0	present (+)	II	ileum	LN, L	yes	pos
11	K/58	555.0	present (++)	II	gallbladder	L	no	pos
12	K/40	171.0	present (+)	II	stomach	LN, L	no	pos
13	K/61	139.0	present (+)	II	unknown	LN, L	yes	pos
14	K/55	211.4	present (+++)	II	unknown	LN, L, B, C	yes	pos
15	K/49	707.2	present (+)	II	unknown	LN, L	yes	pos
16	K/49	32.0	present (+)	II	pancreas (tail)	L, P	no	pos
17	M/42	338.0	present (+)	II	lungs	LN, L	yes	pos
18	M/61	19.5	absent (-)	II	rectum	LN	no	n/a
19	K/59	22.7	present (+)	II	pancreas (head)	LN, L	no	pos
20	M/61	97.2	present (+)	I	stomach (body)	no	no	neg
21	K/70	51.2	present (+)	I	rectum	no	no	pos
22	K/58	30.9	present (+)	I	pancreas	no	no	n/a
23	K/41	76.9	present (+)	I	lungs	no	no	n/a
24	K/46	438.4	present (+)	III	ovary	LN, P	no	pos
25	M/72	770.7	present (+)	II	unknown	L	yes	pos
26	K/57	199.6	present (+)	II	lungs	LN, L	no	pos
27	K/55	255.0	present (+)	III	lungs	LN	no	n/a
28	K/61	30.8	present (weak+)	III	lung	L	no	pos

Imm-hist-pat – immunohistopathological test; SRS – somatostasin receptors scintigraphy; LN – lymphatic nodules metastases; L – liver metastases; B – bone metastases; P – lung metastases; C – CNS metastases; n/a – not available.

Table 4. The concentration of plasma CgA of patients depending on the histopathological type of tumor (WHO classification)

Tumour type (WHO)	n patients	plasma CgA [U/l]			n results > 18 U/l	P level
		Me	25%-75%	Min-Max		
WDNET	11(27%)	14.8	9.4-51.2	8.8-97.2	4(36%)	*0.002 **0.159
WDNEC	24(58%)	137.0	34.9-231.7	6.9-770.7	21(87%)	**0.306
PDNEC	6(15%)	24.45	16.6-255.0	10.8-438.4	3(50%)	-

p – level of statistical significance ($p<0.05$); * p level in comparison with WDNEC; ** p level in comparison with PDNEC

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Table 5. The concentration of plasma CgA in the patients' blood, depending on the presence or absence of distant metastases

Distant mestastases	n patients	plasma CgA [U/l]			n results > 18 U/l	P level
		Me	25%-75%	Min-Max		
absent	18 (44%)	14.9	10.0-51.2	7.5-255.0	7 (38%)	<0.001
present	23 (56%)	139.0	37.8-338.0	6.9-770.7	21(95%)	

p – level of statistical significance (p<0,05)

Table 6. The concentration of CgA in the blood plasma of patients, depending on the presence of clinical symptoms of carcinoid syndrome

Distant mestastases	n patients	plasma CgA [U/l]			n results > 18 U/l	P level
		Me	25%-75%	Min-Max		
absent	18 (44%)	14.9	10.0-51.2	7.5-255.0	7 (38%)	<0.001
present	23 (56%)	139.0	37.8-338.0	6.9-770.7	21(95%)	

p – level of statistical significance

Table 7. Compatibility between patients plasma CgA and the results of SRS

SRS Pos/ CgA Pos	SRS Pos/ CgA Neg	SRS Neg/ CgA Pos	SRS Neg/ CgA Neg	n	compatibility
19	2	2	4	27	85%

Pos- positive result
Neg- negative result

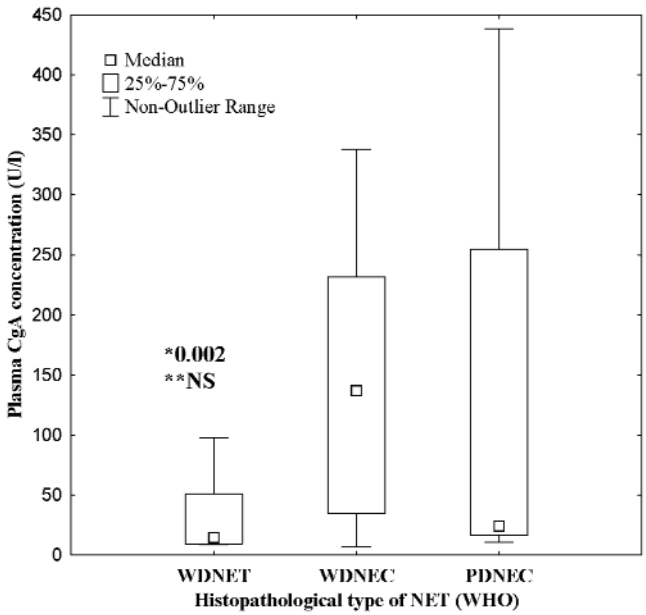

Fig. 2. The concentration of plasma CgA in the patients depending on the degree of NET differentiation.
* p level in comparison with WDNEC
** p level in comparison with PDNEC

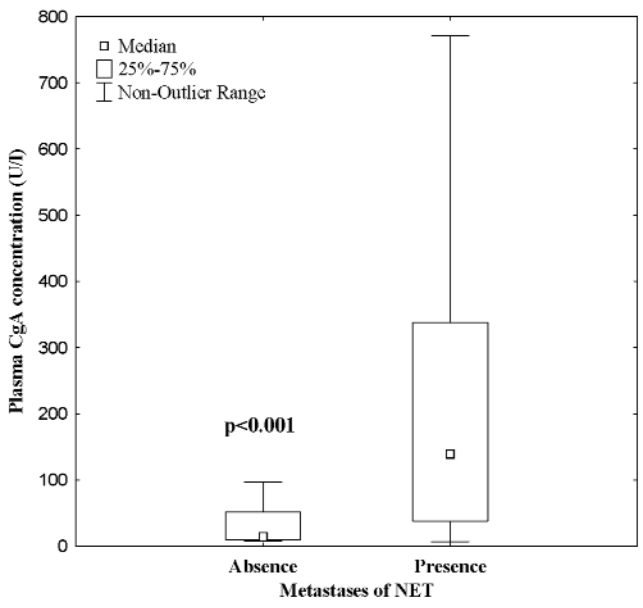

Fig. 3. The concentration of plasma CgA of patients, depending on the presence of distant metastases.
p – level of statistical significance

According to the Seregni *et al* [28] and Stivanello *et al* [29] the CgA plasma concentration in NET patients reflects the differentiation grade of neuroendocrine tumor. Furthermore, Giovanella *et al* [7] results indicate that a very high CgA concentration is a good prognostic marker and may indicate well-differentiated neuroendocrine carcinomas. The author has shown a correlation between the secretory granules

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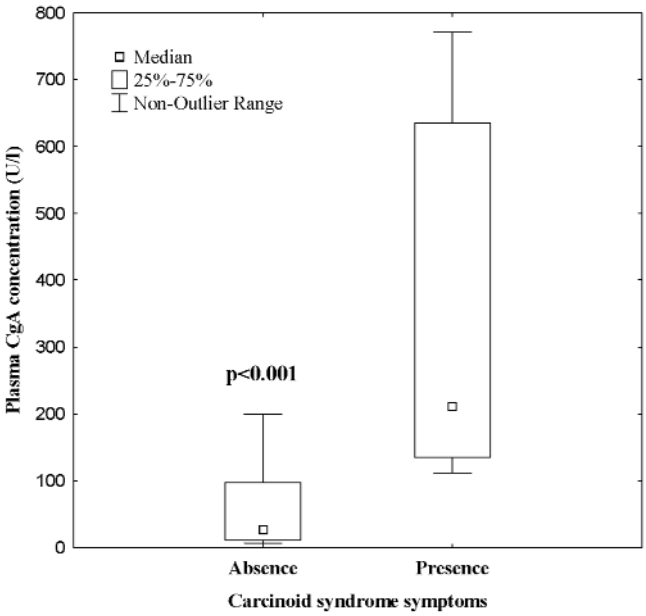

Fig. 4. The concentration of CgA in the blood plasma of patients, depending on the presence of clinical symptoms of carcinoid syndrome.

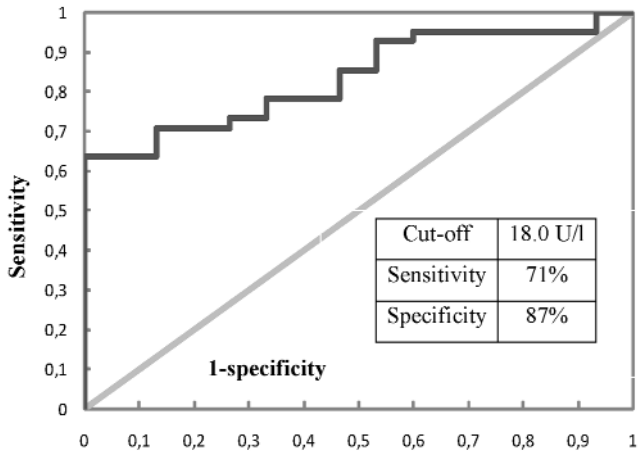

Fig. 5. ROC curve for CgA

density in tumor cells and plasma CgA. Results of their study showed that well-differentiated carcinomas are characterized by numerous secretory vesicles and relatively high plasma CgA, while low-differentiated endocrine carcinomas contain fewer of secretory granules in the cells, and therefore are characterized by relatively low CgA levels in plasma. Also, Rindi and Klöppel [27] showed that CgA is usually absent or only focally expressed in poorly differentiated neuroendocrine tumors because of the lost capacity of these tumors to produce CgA. Our findings are in agreement with above mentioned already published data.

In conducted retrospective analysis we demonstrated the relationship between serum CgA levels and the presence of metastases. Elevated CgA was found in 95% of cases with distant metastases and only 38% of cases without metastases. The highest concentrations of CgA were observed in NET with liver metastases.

These relationships found confirmation in the results obtained by Nehar *et al* [10]. In 124 GEP-NET patients and 34 with MEN-1 (multiple endocrine neoplasma type 1) they demonstrated that the concentration of CgA correlates with the severity of disease ($p<0.001$). Elevated levels of this marker were observed in 73% of patients with metastases and in 26% of patients without metastases. These authors noted that the percentage of elevated CgA results differed significantly ($p<0.001$) between patients with metastases to regional lymph nodes (38%), liver metastases (69%) and very advanced disease, with metastases to the liver, lungs, bone and spleen (100%).

Similar results were obtained by Nobles *et al* [18] and Peracchi *et al* [30] in their studies. Seregni *et al* [28] and Sivanello *et al* [29] also demonstrated that the CgA plasma concentration is elevated in patients with tumors NET and strongly correlates with the severity of the neoplastic process.

Baudin *et al* [17] and Tomassetti *et al* [22] suggest that the CgA concentration in NET patients reflects the spread of the tumor, with the highest values in the presence of metastases in the liver, which is confirmed by our results. Janson *et al* [24] found a correlation between increasing levels of plasma CgA in patients with carcinoid and the number of metastatic foci in the liver. According to Campana *et al* [6] the concentration of CgA of 282 U/l was the best cut-off value for diagnosis of patients with advanced neoplasm (sensitivity 71%, specificity 79%).

The high concentration of CgA may be a good prognostic indicator for neuroendocrine cancer with a high degree of differentiation, may also indicate the presence of metastases as shown in our own observations and the outlined literature. Therefore, the results of CgA should be closely interpreted with histopathological data and clinical patient phenotype.

A further subject of our study was to find relationships between CgA plasma concentration and the presence of clinical symptoms of carcinoid. It has been shown that the CgA plasma concentration is significantly higher in patients with typical symptoms of carcinoid syndrome (flush, diarrhoea) compared with patients with tumours endocrinally nonactive. Elevated plasma CgA was observed in all patients (100%) with clinical symptoms and 56% of cases without any symptoms. Our results are consistent with reports of other authors [10,17,18,31].

According to Vinik A. [13] the plasma CgA concentrations in patients with GEP-NET may be dependent on its secretory activity. CgA levels are significantly elevated in the majority of GEP-NET, but the highest values are observed in the classic carcinoid

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syndrome with metastasis, in which the concentration of CgA may be increased from 100 to 1000 times.

Nehar *et al* [10] showed higher diagnostic sensitivity of CgA in hormonally active tumors than inactive (73 vs. 45%, $p<0.003$). The percentage of elevated CgA concentrations was greatest in patients with secreting GEP-NET with metastases (78%) and lowest (0%) with inactive tumors without metastases, as confirmed by our observations. Similar results were obtained by Bajetta *et al* [14] and Nobles *et al* [18].

Moreover in the present study we assessed the relationship between the plasma CgA concentration in the blood of NET patients and the presence of somatostatin receptors. We obtained accordance of 85% between the parameters. In a study conducted by Cimitan *et al* [31], this agreement was 75%.

The evaluation of the diagnostics power of CgA ELISA determination in NET was the next 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. From other authors observations the sensitivity of CgA as a marker of neuroendocrine tumors of the gastrointestinal tract varies between 10-100% with specificity of 68-100% [17,20]. Values of CgA diagnostic sensitivity and specificity available in the literature differ depending on the secretory activity and the degree of tumor and also depend on the method used to determine the concentration of CgA and accepted cut-off value [5,6,10,17,25,32].

Campana *et al* [6] 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) as in our study, they received greater diagnostic sensitivity (85%) and specificity (96%) for the diagnosis of NET. With cut-off values from 17 to 34 U/l the estimated value of the diagnostic sensitivity of CgA from 79 to 92% [30,33] and specificity from 83 to 91% [22,30,33] was obtained in various other studies.

It is noteworthy that the concentration of CgA may be distorted in patients treated with proton pump inhibitors as demonstrated in previous reports [10]. Moreover increase in the false positive CgA concentrations can be observed in patients with chronic renal failure, liver disease and gastric enterochromaffin-like cell hyperplasia associated with hypergastrinemy [6,10].

Data from the literature and results of this study suggest the use of CgA in the diagnosis and prognosis of NET.

Conclusions

Analysis of this research results allows us to formulate the following conclusions:

1. Plasma CgA concentration analysed together with histopathological assessment of tumor and the clinical picture is a useful marker in the diagnosis of neuroendocrine tumors.
2. Low plasma CgA concentrations do not exclude the presence of neuroendocrine tumor.
3. High plasma CgA concentrations may indicate the presence of highly-differentiated NET (WDNEC), and also may indicate the presence of tumor metastasis.
4. The highest CgA concentrations were observed in patients with neuroendocrine tumors associated with carcinoid symptoms and the presence of metastases to the liver.
5. The DacoCytomation ELISA test for the determination of chromogranin A in plasma with the accepted cutoff value of 18 U/l that was used in this study has a good diagnostic power in detecting neuroendocrine tumors

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