ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIV, N 4, 17 SECTIO DDD 2011

[1]Departament of Biochemistry Diagnostics, Medical University in Lublin
[2]Saint John of Dukla Oncological Centre of Lublin Region

HELENA DONICA[1], MACIEJ KORPYSZ[1], ELŻBIETA STAROSŁAWSKA[2], ARLETA MALECHA-JĘDRASZEK[1], AGATA BURSKA[1], KAMIL KUĆ[2], BEATA WOJTYSIAK-DUMA[1], TOMASZ KUBIATOWSKI[2]

Comparison of diagnostics power of chromogranin A elisa test in general population of patients with neuroendocrine tumors and with metastases

Porównanie mocy diagnostycznej testu elisa do oznaczania chromograniny A w ogólnej populacji chorych z guzami neuroendokrynnymi i u chorych z przerzutami

INTRODUCTION

Neuroendocrine tumors (NET) are relatively infrequent; however include a broad group of tumors which arise from the endocrine cells of various organs [5]. Approximately 70% of all neoplasms are tumors of neuroendocrine gastrointestinal tract, known as gastroenteropancreatic neuroendocrine tumors (GEP-NET) [5,7,14].

The NET tumors are characterized by slow growth, often small size and presence of late occurring metastases [26]. They may be asymptomatic for many years or may manifest with non-specific symptoms. Most of NET are malicious, but in comparison with other malignancies allow patients long-term survival [15,26].

Due to rarity, biological distinctiveness and diverse clinical course of NET makes them a serious diagnostic problem. Because of their uncharacteristic symptoms NETs are often diagnosed too late, usually in advanced stage of the disease, when the metastases are already present [9,14]. Therefore, the progress in the diagnostic methods development is of high importance to allow tumor biomarkers discovery proper for early diagnosis, as well as assessment of their severity and choice of therapy.

In clinical practice the usefulness of tumor markers concentration determination is limited by diagnostic sensitivity, specificity and negative and positive predictive value [16,21]. The ideal tumor marker should be characterized by high sensitivity (i.e. be detected in patients affected with a disease regardless of clinical status) and high diagnostic specificity (i.e. to be undetectable in healthy individuals) [1,4,8,10,16,21]. Its concentration should be proportional to the size of the tumor and closely reflect disease severity and response to treatment. Biomarker organ specificity should be

an important feature [4,16,21]. In principle, the results of the marker determinations should also be characterized by a high positive and negative predictive value, i.e. the probability of disease exclusion at a low concentration of tumor marker and the probability of coexistence of elevated serum tumor marker with the presence of tumor [1,8,16].

In recent years, recognition of neuroendocrine tumors was clearly improved by the introduction into the diagnostic profile tests for somatostatin receptors detection and serum chromogranin A (CgA) concentrations as standard tumor markers. The CgA is a sensitive but non-specific biomarker in the diagnosis of GEP-NET, as it is released from different types of neuroendocrine tumors [6,12,24].

The purpose of this study was to evaluate the diagnostic usefulness of CgA Elisa test in the general population patients with NET and in selected groups of patients with metastases.

MATERIAL AND METHODS

The patients were treated at Saint John of Dukla Oncology Center of Lublin Region in Lublin from February 2005 to May 2011. The patients' age ranged from 20 to 80 years (mean age 60 ± 15 years).The group included 42 women (60%) with mean age 57 ± 10 years and 28 men (40%) with mean age 64 ± 10 years, female/male ratio =1.5. The control group was composed of healthy volunteers (n=33) with age range from 25 to 55 years (mean age 40 ± 10 years), gender distribution was the same as in the study group (female/male ratio = 1.5).

C g A d e t e r m i n a t i o n. Peripheral venous blood samples were collected using standard vein puncture technique. The blood samples were taken at rest, fasting in a sitting position. Afterwards clotted samples were centrifuged for 10 min at 2000 rpm/min. From the obtained samples serum was separated and aliquoted into eppendorfs and stored at –20°C pending analysis.

The CgA serum determinations were performed with the use of ELISA immunoenzymatic assay of commercially available kit Chromogranin A (DakoCtomation, 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%.

S t a t i s t i c a l a n a l y s i s. Serum CgA concentrations in the studied groups were reported with the use of descriptive statistic elements (median (Me), range or percentile (25-75%), minimum-maximum (min- max)) as appropriate and the results are shown in the tables. 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 metastases presence and localisation. For statistical analysis of obtained results, Statistica 7.0 StatSoft was used. Distribution was tested for normality using Shapiro-Wilk W test. The analysed parameters were found skew-distributed and therefore for analysis of differences non parametric tests U Mann-Whitney and Kruskal-Wallis were applied. The cut-off value of 19 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 usefulness of serum CgA we plotted ROC curves (Receiver Operating Characteristic - the dependence of the sensitivity and specificity) and the area under the curve (AUC) was calculated. To carry out these calculations we used MedCalc Version 11.6 program. A P value ≤ 0.05 was considered as statistically significant for all analyses.

RESULTS

In the serum of patients with NET we found statistically significantly higher CgA levels (P <0.001) in comparison to the control group. Table 1 shows the concentration of CgA in the studied groups.

Table 1. CgA [U/l] concentration in the group of patients diagnosed with NET and controls

Parameter	Group					
	NET (n=70)			Control (n=33)		
CgA	Me	25-75%	min – max	Me	25-75%	min – max
	31.1 *	13-133.3	6.9-770.7	11.9	8.6- 16.4	2.1 – 39.3

* p<0,001

We calculated diagnostic sensitivity, specificity of the CgA concentrations in NET and positive and negative predictive value of the test. Obtained results were respectively: 67%, 85%, 89% and 55%. The most optimal cut-off value was set as 19 U/l. The AUC of 0.8 with p <0.001 indicated a good diagnostic usefulness of the serum CgA determinations in the diagnosis of patients with NET. Figure 1 shows the ROC curve for the CgA results.

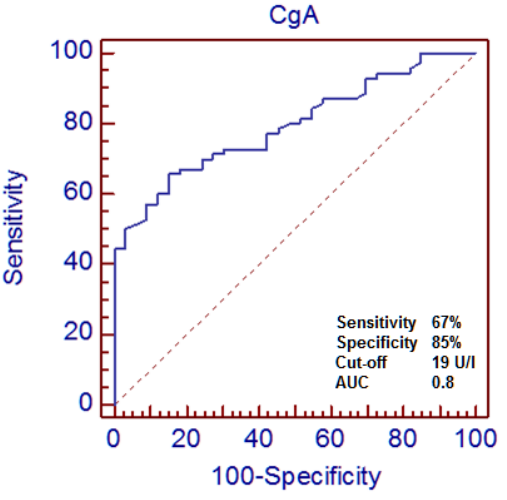

Figure 1. The Reciever Operating Curve for the CgA.

In this study we also evaluated serum CgA concentration in respect to the presence of metastases and their location, moreover the CgA diagnostic sensitivity was calculated for patients with the specific location of metastases.

The imaging tests results, clinical signs and in some cases the surgery procedure, have revealed metastases to the lymph nodes, liver, bones, lungs, brain in 52 patients (74%) while in 18 patients (26%) no metastases were detected. The Table 2 shows the CgA levels changes depending on the presence of metastases and their location.

For 28 patients (54%) most metastases have been detected in the liver and/or (bone/lung/brain/lymph nodes), for 16 patients (31%) only lymph nodes were affected and for 8 patients (15%) metastases were found in organs like only lungs, bones, brain.

Table 2. Serum CgA concentration in the study group depending on the presence and location of metastases

Distant metastases	**n**	**CgA concentration (U/l)**			**n of results > 19 U/l**
		Me	**25-75%**	**Min – Max**	
Absent	18 (26%)	19.2*	10-47.2	7.4-97.2	9 (50%)
Present	52 (74%)	38.2	16.1-168.1	6.9-770.7	38 (73%)
Lymp nodes	16 (31%)	13.5†	11.1-29.9	6.9-611.7	7 (43%)
Liver and/or other organs	28 (54%)	122.6‡	28.4-209.3	6.9-770.7	25 (89%)
Other organs	8 (15%)	57.8**	31.8-120.8	9.8-438.4	6 (75%)

*$p<0.05$ patients without metastases vs. with metastases; **$p<0.05$ metastases in other organs vs. without metastases; †$p<0.01$ metastases in lymph nodes vs. liver and/or other organs; ‡$p<0.001$ liver and/or other organs vs. without metastases

The serum levels of CgA concentrations were significantly elevated in patients with metastases comparing to patients without metastases ($p<0.05$). Moreover the CgA levels were also significantly elevated ($p<0.05$) in patients with metastases to distant organs (brain, lungs, bones) compared to patients without metastases. Statistically significantly lower ($p<0.01$) CgA values were found among patients with lymph node metastases compared with patients diagnosed with metastases in the liver (and/or bone, lung, brain, lymph nodes) and statistically significantly higher ($p<0.001$) CgA concentrations were found in patients with liver metastases and/or other organs, compared with patients in whom metastases did not occur.

Among patients with NET increased CgA concentration was found in 73% of cases with metastases and in 50% of cases without metastases. In most cases metastases were located in the liver and/or lymph nodes, bones, brain and lungs and in these patients CgA levels were the highest.

The ROC (Fig. 2) curve confirms successful application of CgA determinations in liver metastases and/or (lymph nodes, bones, brain and lungs) diagnosis.

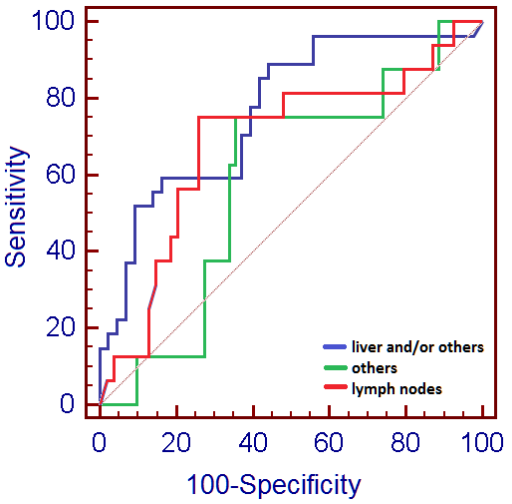

Figure. 2. Comparison of the diagnostics sensitivity and specificity of the CgA results in patients with the specific metastases localisation

For the CgA levels in patients with liver metastases and/or lymph nodes, bones, brain and lungs the sensitivity was 89% and AUC 0.76, while for the patents with the lymph nodes and distant organs metastases the sensitivity was 75% and AUC 0.68 and 0.59 respectively.

DISCUSION

The increasing incidence and diagnosis of NET is thought to be largely a result of the introduction to biochemical diagnostics profile of the new biomarker - serum CgA. This non-specific biomarker is now a well-recognized indicator of a neuroendocrine cells secretory activity and has become the most important circulating marker for NET at present.

The serum CgA results obtained in our study has become a source of interesting observations, which potentially extend the diagnostic expertise in the area of neuroendocrine tumors.

We have found significantly higher median values of CgA in patients with NET in comparison to healthy people, which is consistent with reports by others (2,17].

In our study we evaluated the diagnostic power of ELISA test for the CgA determination in serum of patients diagnosed with NET. Diagnostic sensitivity and specificity for the used ELISA were 67% and 85% respectively at the accepted cut-off value of 19 U/l, which was calculated from the ROC curve.

From the other authors' observations the CgA sensitivity as a marker of the gastrointestinal tract NET varies between 10-100% with specificity of 68-100% [3,14]. The available values of the CgA diagnostic sensitivity and specificity in the literature vary and it mostly depends on the method used for CgA determination, the accepted cut-off values [3,6,17,27], histopathological differentiation, tumor stage, size and secretory activity [11,20].

Campana et al. [6] evaluated the diagnostic power of CgA in 238 patients with endocrine tumors located in the gastrointestinal tract and lungs, and 48 healthy subjects. Using the same cut-off point as in our study (19 U/l), they received a greater diagnostic sensitivity (85%) and specificity (96%) of the CgA for NET. The reason of better diagnostic power is probably due to the larger size of patient cohort enrolled in their study in comparison to our group.

In other publications, authors used CgA cut-off values from 17 to 34 U/l and obtained different diagnostic sensitivity ranges from 79 to 92% and specificity ranges from 83 to 91% [20,23].

Interesting observations were made by Zatelli et al. [27] who compared the diagnostic sensitivity and specificity of two methods for CgA concentration measurement: IRMA and ELISA in patients with NET. The authors demonstrated higher diagnostic sensitivity and specificity of ELISA (84 and 85% respectively) compared with the IRMA (71.3 and 77.8% respectively) in the diagnosis of GEP-NET. Similar results were obtained by Stridsberg et al. [23].

In the present study we also examined how the CgA diagnostic value may be used to detect patients with metastases from the studied cohort. Significantly elevated levels of CgA in patients with metastases were confirmed by a range of authors in their publications [3,17-19,27], but there is no literature evaluating the diagnostic value of the CgA test for the determination of in patients with NET with metastases available.

In our study the metastases were found in 74% of patients, in 73% of them elevated CgA levels were detected. The highest CgA concentrations were observed in patients with liver metastases and/or bone, brain, lymph nodes and lungs. The chromogranin A diagnostic sensitivity in patients with liver metastases and/or lymph nodes, bones, brain and lungs was 89%, while in patients with lymph node and distant organs metastases, the sensitivity was 75%.

Our results are consistent with these of Nehar et al. [17] who in 124 patients with NET showed significant correlation between CgA levels and disease severity ($p < 0.001$). Elevated levels of this marker were found in 73% of patients with metastases and in 26% of patients without metastases. These authors found that the percentage of elevated CgA results differed significantly ($p < 0.001$) between the groups of patients with regional lymph nodes metastases (38%), liver metastases (69%) and very advanced disease with metastases to the liver, lungs, bones, spleen (100%).

Similar results were obtained by Nobels et al. [18] and Peracchi et al. [19]. Furthermore, Seregni et al. [20] and Sivanello et al. [22] demonstrated that the plasma CgA concentrations are elevated in patients with NET and strongly correlate with the tumor burden.

Baudin et al. [3] and Tomassetti et al. [25] suggested that plasma CgA levels reflects spread of the tumor with the highest concentrations in the presence metastases in the liver, this was also confirmed by our results. Janson et al. [13] found a correlation between increasing concentration of plasma CgA and the number of metastatic foci in the liver in patients with carcinoid. According to Campana et al. [6] the CgA concentration of 282 U/l is the best cut-off value for diagnosis of patients with advanced tumors (sensitivity 71%, specificity 79%).

CONCLUSION

To conclude we would like to stress that CgA is an important tumour marker for all neuroendocrine tumours. However, different analytical methods give different results, which must be taken into consideration when comparing results from different clinical studies. The ELISA test from DacoCytomation for the determination of chromogranin A in serum used in this study with accepted cutoff value of 19 U/L has a good diagnostic power in detecting neuroendocrine tumors.

REFERENCES

1. Akobeng A.K.: Understanding diagnostics tests 1: sensitivity, specificity and predictive values. Acta Paediatr. 96, 338, 2007.
2. Bajetta E., Ferrari L., Martinetti A. et al.: Chromogranin A, neuron-specific enolase, carcinoembryonic antigen and hydroxyindole acetic acid evaluation in patients with neuroendocrine tumors. Cancer, 86, 858, 1999.
3. Baudin E., Bidart J.M., Bachelot A. et al.: Impact of chromogranin A measurement in the work-up of neuroendocrine tumors. Ann. Oncol. 12, 79, 2001.
4. Będkowska G.E., Ławicki S., Szmitkowski M.: Markery nowotworowe przydatne w diagnostyce i monitorowaniu raka endometrium i szyjki macicy. Postepy Hig. Med. Dosw. 61, 122, 2007.
5. Bolanowski M.: Wybrane aspekty kliniczne guzów neuroendokrynnych. Fam. Med. Prim. Care Rev. 10, 788, 2008.
6. Campana D., Nori F., Piscitelli L. et al.: Chromogranin A: Is It a Useful Marker of neuroendocrine Tumors?. J. Clin. Oncol. 25, 1967, 2007.
7. Caplin M.E., Wiedenmann B.: The management of patients with neuroendocrine tumors. Endoc. Relat. Cancer, 10, 425, 2003.
8. Cotter K., Peipert J.F.: Can you handle the truth (and know it when you see it)? Understanding sensitivity, specificity and predictive values, and ROC curves. J. Minim. Invasive Gynecol. 12, 385, 2005.
9. Ćwikła J.B., Nasierowska-Guttmejer A., Jeziorski K.G. et al.: Diagnostic algorithm of neuroendocrine tumors of the digestive system (GEP-NET) and bronchi. Pol. J. Radiol. 70, 87, 2005.
10. Dziarkowska K., Wieczorek P.: Nowotwory tarczycy-klasyczne techniki diagnostyczne i markery nowotworowe. Kosmos, 55, 267, 2006.
11. Giovanella L.: Chromogranin A a circulating neuroendocrine marker. Cis. Bio. 1, 2003.
12. Glinicki P., Jeske W.: Chromogranina A (CgA) — charakterystyka dostępnych metod badawczych i uwarunkowań mogących mieć wpływ na uzyskane wyniki. Endokrynol. Pol. 60, 415, 2009.
13. Janson E.T., Holmberg L., Stridsberg M. et al.: Carcinoid tumors: analysis of prognostic factors and survival in 301 patients. Ann. Oncol. 8, 685, 1997.
14. Kaltas G.A., Besser G.M., Grossman A.B.: The diagnosis and medical management of advanced neuroendocrine tumors. Endocr. Rev. 25, 458, 2004.
15. Kos-Kudła B.: Guzy neuroendokrynne przewodu pokarmowego. Onkologia po Dyplomie, 5, 2005.
16. Kulpa J., Rychlik U.: Markery nowotworowe w diagnostyce laboratoryjnej cz. I. Bad. Diagn. 10, 9, 2004.

17. Nehar D., Lombard-Bohas C., Olivieri S. et al.: Interest of Chromogranin A for diagnosis and follow-up of endocrine tumours. Clin. Endocrinol. 60, 644, 2004.
18. Nobels F.R.E., Kwekkeboom D.J., Bouillon R. et al.: Chromogranin A: its clinical value as marker of neuroendocrine tumours. Eur. J. Clin. Invest. 24, 431, 1998.
19. Peracchi M., Conte D., Gebbia C. et al.: Plasma chromogranin A in patients with sporadic gastro-entero-pancreatic neuroendocrine tumours or multiple endocrine neoplasia type 1. Eur. J. Endocrinol. 148, 39, 2003.
20. Seregni E., Ferrari L., Bajetta E., et al: Clinical significance of blood chromogranin A measurement in neuroendocrine tumours. Ann. Oncol. 12, S69, 2001.
21. Soborczyk A., Deptała A.: Markery nowotworowe w praktyce klinicznej. Chor. Serca Naczyń, 4, 184, 2007.
22. Stivanello M., Berruti A., Torta M. et al.: Circulating chromogranin A in the assessment of patients with neuroendocrine tumours. A single institution experience. Ann. Oncol. 12, 573, 2001.
23. Stridsberg M., Eriksson B., Oberg K. et al.: A comparison between three commercial kits for chromogranin A measurements. J. Endocrinol. 177, 337, 2003.
24. Taupenot L., Harper K.L., O'Connor D.T.: The Chromogranin – Secretogranin Family. N. Engl. J. Med. 348, 1134, 2003.
25. Tomassetti P., Miglior M., Simoni P. et al.: Diagnostic value of plasma chromogranin A in neuroendocrine tumours. Eur. J. Gastroenterol. Hepatol. 13, 55, 2001.
26. Vinik A.: Carcinoid tumors. Diffuse Hormonal System. Endotext.com 2004.
27. Zatelli M.C., Torta M., Leon A. et al.: Chromogranin A as a marker of neuroendonecrine neoplasia: an Italaian Multicenter study. Endocr. Relat. Cancer, 14, 473, 2007.

SUMMARY

Neuroendocrine tumours (NET) are a rare and heterogeneous group of neoplasms derived from neuroendocrine cells scattered in the body. These cells mainly produce numerous peptides and biogenic amines i.e. chromogranin A. Despite many limitations CgA has been successfully used in the diagnosis of NET. It is a well-recognized marker for treatment monitoring and prognosis of disease course for patients diagnosed with NET. The purpose of this study was to evaluate the diagnostic usefulness of CgA Elisa test in the group of patients with NET and in selected groups of patients with metastases. The evaluation was performed in the group of 70 patients diagnosed with NET and 52 (74%) of them had confirmed metastases, 18 (26%) had no metastases detected. In order to investigate the diagnostic usefulness of serum CgA we plotted ROC curves (Receiver Operating Characteristic) and area under the curve (AUC) was calculated. Sensitivity, specificity, positive and negative predictive values were calculated using the standard equations for CgA cut-off set at 19 U/l obtaining values of 67%, 85%, 89%, 55% respectively. We also evaluated diagnostics power of CgA evaluation in selected groups of patients: with metastases to the liver and/or bone, lung, brain, lymph nodes. Diagnostics sensitivity of CgA in this group was 89%, however in the group of patients with metastases to the lymph nodes and distant organs reached the level of 75%. We conclude that the ELISA test from DacoCytomation for the determination of chromogranin A in serum used in this study has a good diagnostic power in detecting neuroendocrine tumors.

Keywords: diagnostic power of CgA Elisa test, neuroendocrine tumours

STRESZCZENIE

Guzy neuroendokrynne (NET) stanowią heterogenną grupę nowotworów, wywodzących się z komórek endokrynnych rozproszonych po całym organizmie człowieka. Komórki te zdolne są do produkcji licznych peptydów i/lub amin biogennych m.in. chromograniny A. CgA pomimo swoich ograniczeń znalazła zastosowanie w diagnostyce guzów NET. Szczególną rolę przypisuje się jej w monitorowaniu leczenia i prognozowaniu przebiegu choroby. Celem niniejszej pracy było porównanie mocy diagnostycznej testu ELISA do oznaczania CgA w całej badanej grupie pacjentów z guzami NET i grupach pacjentów z przerzutami. Ocena stężenia chromograniny A została przeprowadzona u 70 chorych z potwierdzonymi guzami neuroendokrynnymi (NET). W grupie tej było 52 (74%) chorych, u których stwierdzono obecność przerzutów, natomiast u 18 chorych (26%) wykazano ich brak. Oceny przydatności diagnostycznej dokonano w oparciu o wykreśloną krzywą ROC i obliczono pole pod krzywą. Czułość, swoistość diagnostyczną oraz dodatnią i ujemną wartość predykcyjną obliczono według odpowiednich wzorów dla punktu odcięcia 19 U/l, uzyskując odpowiednio następujące wyniki 67%, 85%, 89%, 55%. Przeprowadzono również ocenę mocy diagnostycznej CgA w wyodrębnionych grupach: z przerzutami do wątroby i/lub (węzłów chłonnych, kości, mózgu oraz płuc) czułość diagnostyczna CgA wynosiła 89%, natomiast w grupie pacjentów z przerzutami do węzłów chłonnych i narządów odległych czułość wynosiła odpowiednio 75%. Zastosowany w niniejszej pracy test ELISA do oznaczania chromograniny A ma dobrą moc diagnostyczną w wykrywaniu guzów neuroendokrynnych.

Słowa kluczowe: moc diagnostyczna testu Elisa CgA, guzy neuroendokrynne