Midgut carcinoids; surgical aspects, biogenic amines and vascular effects

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Chapter 4

DIAGNOSTIC VALUE OF SERUM CHROMOGRANIN A AND PLATELET AND URINARY INDOLES IN CARCINOID AND ISLET CELL TUMOR PATIENTS

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Abstract

Aim of this study was to compare the diagnostic value of the neuroendocrine marker serum chromogranin A (CgA) with platelet serotonin and serotonin and 5-HIAA in urine in metastatic carcinoid patients, patients with islet cell tumors and controls.

Serotonin in platelets, urine serotonin, urine 5-HIAA and CgA were measured in carcinoid patients and patients with pancreatic islet cell tumors. Serum samples of sex- and age-matched controls were obtained from healthy volunteers. Samples from 60 carcinoid patients and 12 patients with a pancreatic islet cell tumor were available for CgA analysis. Serum CgA concentrations did not differ between the two patient groups. Both groups had higher CgA compared to controls. Carcinoid patients had higher platelet serotonin, urine serotonin and urine 5-HIAA when compared to patients with an islet cell tumor. The diagnostic sensitivity of serum CgA proved to be at least equal to platelet serotonin for carcinoid diagnosis (88.3% [76.8-94.8] and 98.3% [89.9-99.9] respectively), but CgA appeared superior in diagnosing islet cell tumors: 91.7% [59.8-99.6] versus serotonin in platelets: 30.0% [8.1-64.6], $p<0.01$ urine serotonin 37.5%[10.2-74.1], $p<0.03$ and urine 5-HIAA 37.5% [10.2-74.1], $p<0.03$. The diagnostic sensitivity of CgA proved to be better than urine 5-HIAA and urine serotonin.

Introduction

Neuroendocrine tumor cells are characterized by a common phenotype consisting of the simultaneous expression of general protein markers of and hormonal products specific to each cell type.[1,2] Products are stored in secretory vesicles and may cause specific clinical symptoms such as carcinoid- (serotonin), Zollinger–Ellison- (gastrin) and hypoglycemic syndrome (insulin). Neuroendocrine tumors may arise in nearly every organ, but primary sites in the gastrointestinal- and bronchopulmonary tract are most frequent (57% and 25% respectively).[3] Carcinoid tumors are neuroendocrine tumors and are usually classified on the basis of their site of origin into fore-, mid- and hindgut tumors. They are often slowly growing malignancies with an annual incidence of approximately 2-4 per 100,000.[3] They are part of the group of neuroendocrine tumors, a collection of malignancies that originate from the diffuse neuroendocrine system which is spread throughout the body.[4-7] Their endocrine manifestation is diverse, dependent on tumor-product, related to the site of origin and the presence of metastases. Carcinoid tumors can produce a wide variety of peptide hormones and amines, such as serotonin, neurotensin, substance P, and catecholamines.[8,9]

For the biochemical diagnosis of carcinoid tumors several markers have been advocated. Their diagnostic performance is dependent on the distribution between for- mid- and hindgut in the groups studied. Serotonin overproduction, a hallmark of carcinoid tumors, can be established by measuring platelet serotonin and urinary 5HIAA and serotonin. Specific products such as ACTH and catecholamines can be used to explain tumor related endocrine symptoms.

Chromogranins are the major components of the secretory dense core granules of most neuroendocrine cells.[10,11] Within the secretory pathway, chromogranins are involved in granulogenesis, and in sorting and processing of secretory protein cargo prior to secretion. The chromogranin family includes chromogranins A (CgA) and B (CgB) and secretogranin II (SgII, formerly called chromogranin C). CgA, a 439 amino acid protein with a molecular weight of 49 kD, is water-soluble glycoprotein distributed in the secretory granules of endocrine and neuroendocrine cells. These glycoproteins are co-released with amines. Because of this co-release, elevated serum levels of CgA can be found in a variety of neuroendocrine tumors.[12] CgA levels can be particularly helpful in so-called "non-functioning" endocrine tumors. Once elevated levels have been detected, alterations in CgA levels can serve as a marker for tumor volume.[13] Detection of chromogranins depends on the use of antibodies, therefore different analytical properties of the commercial available kits can display different results.[14] Moreover, CgA compared to secretory hormones (e.g. serotonin in carcinoids) is more heterogeneous because of an extensive

proteolysis which leads to a large heterogeneity of circulating fragments of CgA which may result in lower sensitivity and specificity.[15,16]
The last decade several neuroendocrine markers with respect to detection and diagnosis have been reported. Serengi et al found CgA to be superior to 5-HIAA in the diagnosis of neuroendocrine tumors, sensitivity 68% *vs* 35% and specificity 86% *vs* 100% respectively.[17] In the follow up the concordance of tumor evolution and biomarkers for CgA was 81% and for serotonin 54%.[18] To date CgA is therefore supposed to be a superior marker in neuroendocrine tumors. No studies were performed including CgA and platelet serotonin as a tumor marker although several studies report platelet serotonin to be a superior marker in diagnosing carcinoids, especially those producing only small amounts of serotonin.[19,20] Aim of the present study was to compare the diagnostic performance of the neuroendocrine markers platelet serotonin, serotonin in urine and 5-HIAA in urine with serum CgA in metastatic carcinoid patients, patients with islet cell tumors and controls.

Patients and Methods

Subjects

All metastatic carcinoid patients and patients with pancreatic islet cell tumors diagnosed at the University Medical Center Groningen, in the period 1989-2001 were identified. Included were patients of whom archival samples were available for CgA analysis. The initial results of platelet serotonin, urine serotonin and urine 5-HIAA measurements were used for comparison. A maximum interval of 3 months was allowed between the samples collected for indoles and CgA analysis, provided that no therapeutic interventions were made within this period. Serum samples of sex- and age-matched controls derived from samples of apparently healthy volunteers who participated in 1999 in a previous study on reference values.[21] Samples were coded, meaning that the participants of the original study were not traceable.
All serum and urine samples were obtained during standard treatment and follow-up. For the present study, all relevant data were retrieved from a large computerized database into a separate anonymous database. In this separate database, patient identity was protected by study-specific, unique patient codes, which were known to two data managers, who also have responsibility for the larger database. In case of uncertainties with respect to the data, the larger databases could only be checked through the data managers, thereby ascertaining the protection of patient's identity. The volunteers gave their informed consent for storage of coded samples in the original study. Volunteers and patients were not informed about the research outcome according to rulings of the ethics committee.

Analytical methods

Sampling procedures of the blood samples are described in detail elsewhere.[20,22] Indoles were determined within 2 weeks after sample collection and storage at -20 ºC. Platelet-rich plasma and urine serotonin concentrations were determined by cation exchange chromatography clean-up combined with HPLC and fluorometric detection as previously described.[23] From 2000 onwards, serotonin concentrations in platelet-rich plasma were analyzed by automated in line solid phase extraction combined with HPLC with fluorometric detection.[22] Both methods of the measurement of platelet-rich plasma serotonin are highly correlated (r= .99). Platelet concentrations were measured in platelet-rich plasma with a Coulter STKS (Coulter Corporation). Urinary 5-HIAA concentrations were determined in ether extracts by HPLC with fluorometric detection, essentially as described by Rosano et al.[24] Creatinine levels were measured by a picric acid method on a Mega autoanalyzer (Merck, Darmstadt, Germany). Serum CgA of patients was analyzed immediately or after storage for a maximum period of 11 years at -20ºC. Serum samples of controls were analyzed for CgA in 2004, i.e. after 4.5 years of storage at -20 ºC. It has previously been reported that storage has no influence on serum CgA concentrations.[25] Serum CgA was determined using a radioimmunoassay (Bionovo BV, Purmerend, The Netherlands).

Data evaluation and statistics

Pearson's Chi-square tests were used to test differences between groups in gender-distributions and percentage subjects above reference limits. Differences between groups in ages, platelet serotonin contents, urine serotonin and 5-HIAA concentrations, and serum CgA concentrations were evaluated using the non-parametric Mann-Whitney U test. In all calculations a p-level <0.05 indicated a significant difference. Bonferroni adjustments were made to correct for type 1 errors in multiple comparisons. Spearman correlation tests were performed to investigate age-dependency, and correlations between indoles and serum CgA. Serum CgA reference values were calculated as 95% confidence intervals[26], using the data of the sex- and age-matched healthy volunteers. The upper-limit of the new CgA reference values and the previously established local reference values of platelet serotonin (2.8-5.4 nmol/10^9 platelets), urine serotonin (<66 µmol/mol creatinine) and urine 5-HIAA (0.8-3.8 mmol/mol creatinine) were used as cut-off level. Serum CgA and indole concentration differences between the patient subgroups (foregut, midgut, islet cell tumors) were investigated to gain insight in the specificity of these tumor markers.

Results

Subjects

Samples from 60 carcinoid patients and 12 patients with an islet cell tumor were available for CgA analysis. Their characteristics, together with those of the selected controls, are presented in the Table 1. Patients groups and the control group had similar ages and gender distributions. Serum CgA concentrations did not differ between the patient groups. Both patient groups had however higher serum CgA when compared with controls. Carcinoid patients had higher platelet serotonin, urine serotonin and urine 5-HIAA when compared with patients with an islet cell tumor. The percentage subjects with indole levels above the reference limits was also higher in the carcinoid patients group, as was expected. For details see Table 1.

Serum Chromogranin A reference values

CgA reference values were determined after log-transformation of the original data. CgA proved independent of age and gender. Retransformation of the mean ± 1.96 standard deviation of the transformed data gave rise to an age- and gender-independent reference interval of 21.5 - 75.1 mg/l.

Sensitivity and specificity

Figure 1 shows the serum CgA concentrations plotted against the indole contents and concentrations of both patient groups, together with the upper-limits of the reference levels of the corresponding biomarkers. Levels of CgA were significantly related to platelet serotonin ($r= 0.386$, $p< 0.0005$), urine serotonin ($r= 0.658$, $p< 0.0005$) and urine 5-HIAA ($r= 0.641$, $p< 0.0005$).

Carcinoid patients

Fifty-three of 60 patients (88.3% [76.8-94.8]) had elevated serum CgA (figure panel A,B,C and table 1). All of them had also elevated platelet serotonin (98.3% [89.9-99.9], panel A and table 1). Six of 60 (4 midgut, 2 unknown) had only elevated platelet serotonin. One of 60 (foregut) had normal values for both markers. Of the patients with elevated CgA, 10 had a normal urine serotonin excretion (1 foregut, 3 midgut, 6 unknown, panel B). Three of 60 patients (1 midgut, 2 unknown) had isolated positive urine serotonin. Four of 60 (1 foregut, 3 midgut) were negative for both markers. Two of the 53 CgA positive patients had no elevated 5-HIAA excretion (1 foregut, 1 unknown, panel C). Four of 60 patients (3 midgut, 1 unknown) had isolated positive 5-HIAA. Three of 60 (1 foregut, 1 midgut, 1 unknown) were negative for both markers.

Pancreatic islet cell tumor patients

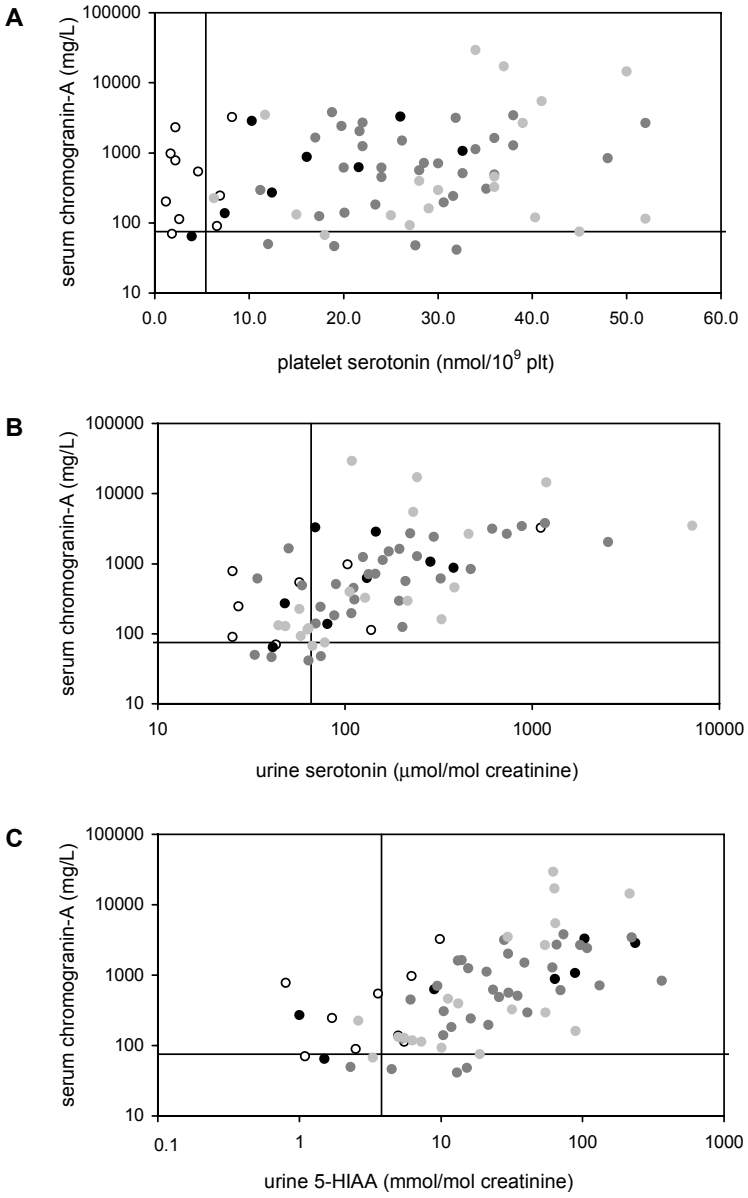
Nine of 10 patients (90% [59.8-99.6]) had an elevated serum CgA, of whom 3 (30% [8.1-64.6]) had only mildly elevated platelet serotonin levels (figure panel A and Table 1). Three of the CgA positive patients had an elevated urine serotonin and urine 5-HIAA excretion (panel B,C). The patients with a normal CgA had also no increased platelet serotonin, urine serotonin and urine 5-HIAA levels (panel A,B,C).

There were no differences in CgA concentrations in-between the carcinoid subtypes as well as the islet cell tumors. However, clear differences in indole concentrations were found between the different carcinoid subtypes. Notably platelet serotonin differed between carcinoid patients and islet cell tumor patients (98% above the upper reference limit (URL) versus 30% respectively, $p<0.01$)

Carcinoid patients with a midgut or unknown primary tumor localization had higher platelet serotonin contents compared with foregut carcinoid patients ($p<0.01$). No other differences in biomarkers existed between the various carcinoid patients. Carcinoid patients with midgut or unknown primary tumor location had higher urine 5-HIAA concentrations when compared with those with an islet cell tumor ($p<0.01$).

		Carcinoid	Islet cell tumor	Control
Number		60	12	27
Age (years)		62 ± 10	61 ± 12	59 ± 10
Male / female		25 / 35	5 / 7	13 / 14
Primary carcinoid localization	Foregut	8		
	Midgut	33		
	Unknown origin	19		
Liver metastasis	No	2		
	Yes	55	7	
	Unknown	3	5	
Lymfnode metastasis	No	2		
	Yes	28		
	Unknown	30	12	
Serum chromogranin A (mg/l)	median	537	394.5	36.9
	range	(47.2-15865.0) [2]	(75.5-12351.5) [2]	(24.3-64.4)
	> 75.1 (=URL), (95%CI)	88.3% (76.8-94.8)	91.7% (59.8-99.6)	
Platelet serotonin (nmol/10^9 platelets)	median	27.8	2.4	
	range	(6.8-51.1)	(1.3-7.9) [1]	
	> 5.4 (=URL), (95%CI]	98.3% (89.9-99.9)	30.0% (8.1-64.6)[1]	
Urine serotonin (µmol/mol creatinine)	median	129.5	49.9	
	range	(37.2-1906.0)	(25.0-943.2) [1]	
	> 66 (=URL), (95%CI)	76.7% (63.7-86.2)	37.5% (10.2-74.1)[1]	
Urine 5-HIAA	median	22.5	3.1	
	range	(1.9-229.2)	(0.9-9.2) [1]	
	> 3.8 (=URL), (95%CI)	91.7% (80.9-96.9)	37.5 %(10.2-74.1)[1]	

Table 1: Patient and control characteristics
1: versus carcinoid patients, p<0.03. 2: versus controls, p<0.0005.



Legend to figures

Serum Chromogranin A versus platelet serotonin (A), urine serotonin (B) and urine 5-HIAA (C) for 60 carcinoid patients and 10 patients with an islet cell tumor.

Lines indicate the upper-limits of the reference values of the corresponding parameters. The closed dots represent the data of the carcinoid patients (primary tumor localization: foregut (●); midgut (●); unknown origin (●)); open dots indicate the patients with pancreatic islet cell tumors.

Discussion

We compared the value of serum CgA with that of platelet serotonin, urine serotonin and urine 5-HIAA for the diagnosis of carcinoids and pancreatic islet cell tumors. To our knowledge this is the first report using platelet serotonin in comparison. To date, serum CgA in carcinoid disease has usually been compared with 5-HIAA. Platelet serotonin proved to be superior to CgA in carcinoid diagnosis, but in diagnosing islet cell tumors CgA was superior to all of the investigated indoles. Urine serotonin and urine 5-HIAA did not have an added value. There is evidence that once the diagnosis carcinoid tumor is established, CgA can be useful in monitoring tumor volume.[17,27] As described earlier by Serengi et al, CgA sensitivity in diagnosing carcinoid disease depends on the stage of disease (50% for stage I and II, 60% for stage III and 100% for stage IV tumors).[17] In our series we included only metastatic (stage IV) carcinoid patients. The number of patients with islet cell tumor was relatively low, however all but one islet cell tumor patient had elevated CgA levels. This is in concordance with literature.[17,28] Panzuto found the diagnostic value to be augmented by combining CgA findings with pancreatic polypeptide.[29]

In our series the platelet serotonin test showed the smallest overlap between carcinoids and islet cell tumors and therefore appeared to be the most discriminative test. Raising URL of platelet serotonin to 10 nmol/10^9 platelets in our series would augment this discrimination. A serum CgA above the URL and a platelet serotonin below 10 nmol/10^9 platelets would point in the direction of a neuroendocrine non-carcinoid tumor. However elevated CgA levels are not always related to neuroendocrine tumors. They can also be found in patients with an adenocarcinoma, atrophic gastritis, patients treated with gastric secretion inhibitors and in patients with renal failure or without any obvious cause.[30]

We conclude that in our patient groups the diagnostic sensitivity of platelet serotonin proved to be at least equal to serum CgA in carcinoid diagnosis, but serum CgA appeared superior in diagnosing islet cell tumors. The diagnostic sensitivity of urine 5-HIAA and urine serotonin proved to be lower than CgA in all cases.

References

1. Lundqvist M, Arnberg H, Candell J, Malmgren M, Wilander E, Grimelius L, Oberg K. Silver stains for identification of neuroendocrine cells. A study of the chemical background. *Histochem J* 1990; 22:615-623.
2. Solcia E, Sessa F, Rindi G, Villani L, Riva C, Buffa R, Capella C. Classification and histogenesis of gastroenteropancreatic endocrine tumours. *Eur J Clin Invest* 1990; 20 Suppl 1:S72-S81.
3. Modlin IM, Lye KD, Kidd M. A 5-decade analysis of 13,715 carcinoid tumors. *Cancer* 2003; 97:934-959.
4. Ahlman H, Nilsson. The gut as the largest endocrine organ in the body. *Ann Oncol* 2001; 12 Suppl 2:S63-S68.
5. DeLellis RA. The neuroendocrine system and its tumors: an overview. *Am J Clin Pathol* 2001; 115 Suppl:S5-16.
6. Moertel CG. Karnofsky memorial lecture. An Odyssey in the land of small tumors. *J Clin Oncol* 1987; 5:1502-1522.
7. Modlin IM, Kidd M, Latich I, Zikusoka MN, Shapiro MD. Current status of gastrointestinal carcinoids. *Gastroenterology* 2005; 128:1717-1751.
8. Kema IP, de Vries EGE, Slooff MJ, Biesma B, Muskiet FA. Serotonin, catecholamines, histamine, and their metabolites in urine, platelets, and tumor tissue of patients with carcinoid tumors. *Clin Chem* 1994; 40:86-95.
9. Theodorsson Norheim E, Oberg K, Rosell S, Bostrom H. Neurotensinlike immunoreactivity in plasma and tumor tissue from patients with endocrine tumors of the pancreas and gut. *Gastroenterology* 1983; 85:881-889.
10. Blaschko H, Comline RS, Schneider FH, Silver M, Smith AD. Secretion of a chromaffin granule protein, chromogranin, from the adrenal gland after splanchnic stimulation. *Nature* 1967; 215:58-59.
11. Taupenot L, Harper KL, O'Connor DT. The chromogranin secretogranin family. *N Engl J Med* 2003; 348:1134-1149.
12. Nobels FR, Kwekkeboom DJ, Coopmans W, Schoenmakers CH, Lindemans J, de Herder WW, Krenning EP, Bouillon R, Lamberts SW. Chromogranin A as serum marker for neuroendocrine neoplasia: comparison with neuron-specific enolase and the alpha-subunit of glycoprotein hormones. *J Clin Endocrinol Metab* 1997; 82:2622-2628.
13. Nobels FR, Kwekkeboom DJ, Bouillon R, Lamberts SW. Chromogranin A: its clinical value as marker of neuroendocrine tumours. *Eur J Clin Invest* 1998; 28:431-440.
14. Stridsberg M, Eriksson B, Oberg K, Janson ET. A comparison between three commercial kits for chromogranin A measurements. *J Endocrinol* 2003; 177:337-341.
15. Degorce F, Goumon Y, Jacquemart L, Vidaud C, Bellanger L, Pons-Anicet D, Seguin P, Metz-Boutigue MH, Aunis D. A new human chromogranin A (CgA) immunoradiometric assay involving monoclonal antibodies raised against the unprocessed central domain (145-245). *Br J Cancer* 1999; 79:65-71.
16. Corti A, Gasparri A, Chen FX, Pelagi M, Brandazza A, Sidoli A, Siccardi AG. Characterisation of circulating chromogranin A in human cancer patients. *Br J Cancer* 1996; 73:924-932.
17. Seregni E, Ferrari L, Bajetta E, Martinetti A, Bombardieri E. Clinical significance of blood chromogranin A measurement in neuroendocrine tumours. *Ann Oncol* 2001; 12 Suppl 2:S69-S72.

18. Nehar D, Lombard-Bohas C, Olivieri S, Claustrat B, Chayvialle JA, Penes MC, Sassolas G, Borson-Chazot F. Interest of Chromogranin A for diagnosis and follow-up of endocrine tumours. *Clin Endocrinol* 2004; 60:644-652.
19. Kema IP, de Vries EGE, Schellings AM, Postmus PE, Muskiet FA. Improved diagnosis of carcinoid tumors by measurement of platelet serotonin. *Clin Chem* 1992; 38:534-540.
20. Meijer WG, Kema IP, Volmer M, Willemse PHB, de Vries EGE. Discriminating capacity of indole markers in the diagnosis of carcinoid tumors. *Clin Chem* 2000; 46:1588-1596.
21. Fokkema MR, Weijer JM, Dijck-Brouwer DA, van Doormaal JJ, Muskiet FA. Influence of vitamin-optimized plasma homocysteine cutoff values on the prevalence of hyperhomocysteinemia in healthy adults. *Clin Chem* 2001; 47:1001-1007.
22. Kema IP, Meijer WG, Meiborg G, Ooms B, Willemse PHB, de Vries EGE. Profiling of tryptophan-related plasma indoles in patients with carcinoid tumors by automated, online, solid-phase extraction and HPLC with fluorescence detection. *Clin Chem* 2001; 47:1811-1820.
23. Kwarts E, Kwarts J, Rutgers H. A simple paired-ion liquid chromatography assay for serotonin in cerebrospinal fluid, platelet-rich plasma, serum and urine. *Ann Clin Biochem* 1984; 21:425-429.
24. Rosano TG, Meola JM, Swift TA. Liquid-chromatographic determination of urinary 5-hydroxy-3- indoleacetic acid, with fluorescence detection. *Clin Chem* 1982; 28:207-208.
25. O'Connor DT, Pandlan MR, Carlton E, Cervenka JH, Hslao RJ. Rapid radioimmunoassay of circulating chromogranin A: in vitro stability, exploration of the neuroendocrine character of neoplasia, and assessment of the effects of organ failure. *Clin Chem* 1989; 35:1631-1637.
26. International Federation of Clinical Chemistry. Approved recommendation on the theory of reference values. Part 5. Statistical treatment of collected reference values. *Clin Chim Acta* 1987; 170:S13-S32.
27. Kolby L, Bernhardt P, Sward C, Johanson V, Ahlman H, Forssell-Aronsson E, Stridsberg M, Wangberg B, Nilsson O. Chromogranin A as a determinant of midgut carcinoid tumour volume. *Regul Pept* 2004; 120:269-273.
28. Peracchi M, Conte D, Gebbia C, Penati C, Pizzinelli S, Arosio M, Corbetta S, Spada A. Plasma chromogranin A in patients with sporadic gastro-entero-pancreatic neuroendocrine tumors or multiple endocrine neoplasia type 1. *Eur J Endocrinol* 2003; 148:39-43.
29. Panzuto F, Severi C, Cannizzaro R, Falconi M, Angeletti S, Pasquali A, Corleto VD, Annibale B, Buonadonna A, Pederzoli P, Delle FG. Utility of combined use of plasma levels of chromogranin A and pancreatic polypeptide in the diagnosis of gastrointestinal and pancreatic endocrine tumors. *J Endocrinol Invest* 2004; 27:6-11.
30. Syversen U, Ramstad H, Gamme K, Qvigstad G, Falkmer S, Waldum HL. Clinical significance of elevated serum chromogranin A levels. *Scand J Gastroenterol* 2004; 39:969-973.