Endokrynologia Polska
DOI: 10.5603/EP2015.0009
Tom/Volume 66; Numer/Number 1/2015
ISSN 0423–104X

Comparison of chromogranin A (CgA) levels in serum and plasma ($EDTA_2K$) and the respective reference ranges in healthy males

Porównanie stężeń chromograniny A (CgA) w surowicy i w osoczu ($EDTA_2K$) oraz odnośnych zakresów referencyjnych u zdrowych mężczyzn

Piotr Glinicki, Wojciech Jeske, Renata Kapuścińska, Wojciech Zgliczyński

Department of Endocrinology, The Centre of Postgraduate Medical Education, Warsaw, Poland

Abstract

Introduction: Chromogranin A (CgA) is a major, nonspecific marker of neuroendocrine tumours (NET). There are a few routinely used assays for the measurement of CgA concentration in serum or plasma. These assays differ in analytical techniques (radioimmunoassay, ELISA, CLIA, TRACE), have different calibrators, and use different antibodies which recognise different epitopes of CgA molecule.
Our study was designed to confirm the noted earlier differences in CgA levels measured in serum and plasma, and to establish respective reference ranges in a group of healthy males.
Material and methods: In 145 male blood donors (age 19–61 years, mean = 35.7), blood was collected into two tubes: one with $EDTA_2K$ (plasma) and one with clot activator (serum). Chromogranin A was measured by immunoradiometric kit (CIS bio, France).
Results: In blood donors, the median (and the range) of CgA concentration were as follows for serum samples — 42.0 ng/mL (16–108 ng/mL) and for plasma ($EDTA_2K$) samples — 58.0 ng/mL (23–153 ng/mL). The differences between serum and plasma ranged 15–75% (median 26%). Plasma CgA levels were significantly higher in relation to serum CgA levels ($p < 0.0001$). Correlation of CgA in serum and plasma was $r = 0.8493$; $p < 0.01$. The reference ranges for CgA measured in serum and plasma in males, expressed as 2.5 to 97.5 percentiles, were: 21.0–108.0 ng/mL and 31.0–153.0 ng/mL respectively.
Conclusions:

1. Significant differences in the concentrations of CgA measured in plasma and in serum demand the application of separate reference ranges adjusted to the type of investigated material.
2. Each laboratory should recommend only one sort of sample material for CgA assay.

(Endokrynol Pol 2015; 66 (1): 53–56)

Key words: *chromogranin A; CgA; neuroendocrine tumour; NET*

Streszczenie

Wstęp: Chromogranina A (CgA) jest głównym, niespecyficznym markerem guzów neuroendokrynnych (NET). Istnieje kilka rutynowych testów służących do oznaczania stężenia CgA w surowicy i w osoczu. Testy te różnią się techniką analityczną (izotopowe, ELISA, CLIA, TRACE), są różnie kalibrowane, użyto w nich różne przeciwciała rozpoznające odmienne epitopy na powierzchni cząsteczki CgA.
Badanie miało na celu potwierdzenie zaobserwowanych wcześniej różnic w mierzonym stężeniu CgA w surowicy i w osoczu oraz ustalenie odpowiednich zakresów referencyjnych w grupie zdrowych mężczyzn.
Materiał i metody: U 145 krwiodawców płci męskiej w wieku 19–61 lat (średnia wieku = 35,7) pobierano krew do 2 probówek: jednej z $EDTA_2K$ (osocze) i jednej z aktywatorem wykrzepiania (surowica). Stężenie CgA było oznaczane metodą immunoradiometryczną (CIS bio, Francja).
Wyniki: U dawców krwi, mediana oraz zakres stężeń CgA były następujące: dla surowicy — 42,0 ng/ml (16–108 ng/ml), a dla osocza ($EDTA_2K$) — 58,0 ng/ml (23–153 ng/ml). Różnice między surowicą a osoczem wyniosły 15–75% (mediana 26%). Stężenie CgA było istotnie statystycznie wyższe w osoczu niż w surowicy ($p < 0,0001$). Korelacja stężenia CgA w surowicy i w osoczu wyniosła $r = 0,8493$; $p < 0,01$. Zakresy referencyjne CgA oznaczanej w surowicy i w osoczu u mężczyzn wyrażone jako 2,5–97,5 percentyla wyniosły odpowiednio: 21,0–108,0 ng/ml oraz 31,0–153,0 ng/ml.
Wnioski:

1. Istnieją istotne różnice w stężeniu CgA mierzonej w surowicy i w osoczu, zatem uzyskiwane wyniki powinny być odnoszone do osobnych zakresów referencyjnych odpowiadających rodzajowi użytego materiału do badania.
2. Każde laboratorium powinno zalecać stosowanie tego samego rodzaju materiału do oznaczenia stężenia CgA.

(Endokrynol Pol 2015; 66 (1): 53–56)

Słowa kluczowe: *chromogranina A; CgA; guzy neuroendokrynne; NET*

This work was supported by The Centre of Postgraduate Medical Education grant n° 506-1-08-01-13

Piotr Glinicki M.Sc., Ph.D., Department of Endocrinology, The Centre of Postgraduate Medical Education, Bielański Hospital, Cegłowska 80, 01–809 Warsaw, Poland, tel.: +48 22 569 02 93, fax: +48 22 834 31 31, e-mail: glinicki@cmkp.edu.pl

Table I. *Comparison of the CgA median (and the range) and mean ± SD in serum and plasma ($EDTA_2K$) in healthy males*
Tabela I. *Porównanie median (oraz zakresów) i średnich ± SD w surowicy i w osoczu ($EDTA_2K$) u zdrowych mężczyzn*

Serum [ng/mL] (n = 145)				Plasma ($EDTA_2K$) [ng/mL] (n = 145)			
Median	**Range**	**Mean**	**SD**	**Median**	**Range**	**Mean**	**SD**
42.0	16–108	45.7	15.8	58.0	23–153	61.9	21.3

Difference 15–79% (median 26%)
$p < 0.0001$

Introduction

Chromogranin A (CgA) is an acid hydrophilic protein that belongs to the chromogranin/secretogranin family. Human CgA is a 439 amino acids protein (48kDa) localised in the dense core secretory granules of many normal and neoplastic neuroendocrine cells of the diffuse neuroendocrine system (DES), and endocrine cells of the endocrine glands (e.g. pancreatic islets). CgA plays an important role in intracellular and extracellular function of endocrine and neuroendocrine cells [1, 2]. CgA has become a main, nonspecific marker of neuroendocrine tumours (NET) because it is secreted by most NETs, particularly GEP-NET (gastroenteropancreatic neuroendocrine tumours), midgut carcinoids, pancreatic tumours and pheochromocytoma [3, 4].

There are few routinely used assays for the measurement of CgA concentration in serum or plasma. These assays have different analytical techniques (radioimmunoassay, ELISA, CLIA, TRACE), have different calibrators, and use different antibodies which recognise different epitopes of CgA molecule [5].

Our study was designed to confirm the noted earlier differences in CgA levels measured in serum and plasma, and to establish the respective references ranges in a group of healthy males.

Material and methods

145 male blood donors (age mean ± SD 35.7 ± 9.4; range 19–61 years) were investigated. The following exclusion criteria in our study were settled: treatment with proton pump inhibitors, histamine H_2-receptors blockers, corticosteroids, presence of some chronic diseases such as impaired renal or hepatic function, inflammatory bowel diseases, and prostate cancer. At each collection, blood was withdrawn into two tubes: one with $EDTA_2K$ (plasma) and one with clot activator (serum). After blood collection, venous blood was centrifuged (10 minutes, 3,500 rpm) and plasma ($EDTA_2K$) and serum samples were frozen at –30°C and stored until assayed.

Chromogranin A was measured by immunoradiometric kit (IRMA) (CGA-RIA CT, CIS bio International, Gif-sur-Ivette cedex, France). Analytical sensitivity was 1.5 ng/mL. According to the kit producer, the reference value for serum sample was 19.4–98.1 ng/mL, with the median at 41.6 ng/mL. Intra-assay coefficients of variation (CV) were: 6% for 30 ng/mL, 3.8% for 144 ng/mL, and 2.2% for 996 ng/mL. Inter-assay CV values were: 8.5% for 29 ng/mL, 5.7% for 144 ng/mL and 5.3% for 996 ng/mL.

This study was approved by the Bioethics Committee of The Centre of Postgraduate Medical Education.

Data is presented as median (and the range) and mean ± SD. Normality of the distribution was studied by the Shapiro-Wilk test. Distribution of results differs from the theoretical normal distribution. The Wilcoxon's signed-rank test was performed to estimate differences between groups. The relationship between the compared results was expressed using Spearman's rank correlation analysis. The reference ranges were expressed as 2.5 to 97.5 percentiles. A p-value of < 0.05 was considered to be significant and $p < 0.01$ highly significant. All statistical analyses were performed using statistical software (PQStat ver. 1.4.2.324).

Results

In blood donors, the median (and the range) of CgA concentration determined for serum samples was 42.0 ng/mL (16-108 ng/mL) and for plasma samples was 58.0 ng/mL (23-153 ng/mL). The differences between serum and plasma ranged from 15% to 79% (median 26%) (Table I). Plasma CgA levels were significantly higher in relation to serum CgA levels ($p < 0.0001$) (Fig. 1). Correlation of CgA in serum and plasma was $r = 0.8493$; $p < 0.01$ (Fig 2). The determined reference ranges for CgA measured in serum and plasma in males expressed as 2.5 to 97.5 percentile were: 21.0–108.0 ng/mL and 31.0–153.0 ng/mL respectively (Table II).

Discussion

Chromogranin A (CgA) is known as the most useful nonspecific biomarker of various types of neuroendocrine tumours. Currently, several commercial methods allow determination of CgA concentrations in blood. These assays differ in analytical techniques, have different calibrators, and use different antibodies which recognise dif-

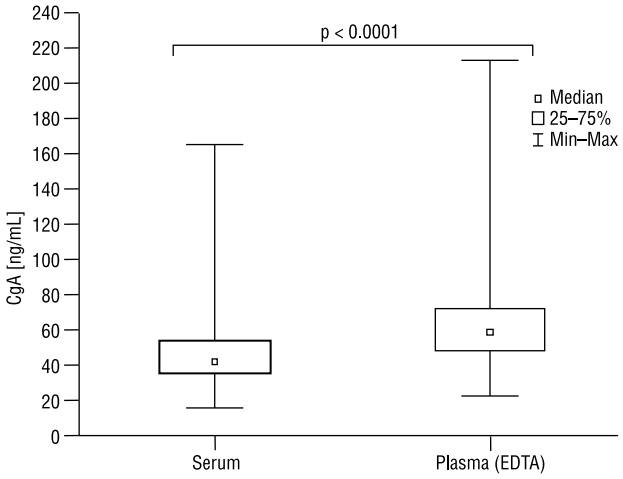

Figure 1. *The median and the ranges of CgA levels in serum and plasma (EDTA$_2$K)*
Rycina 1. *Mediana oraz zakresy stężenia CgA w surowicy i w osoczu (EDTA$_2$K)*

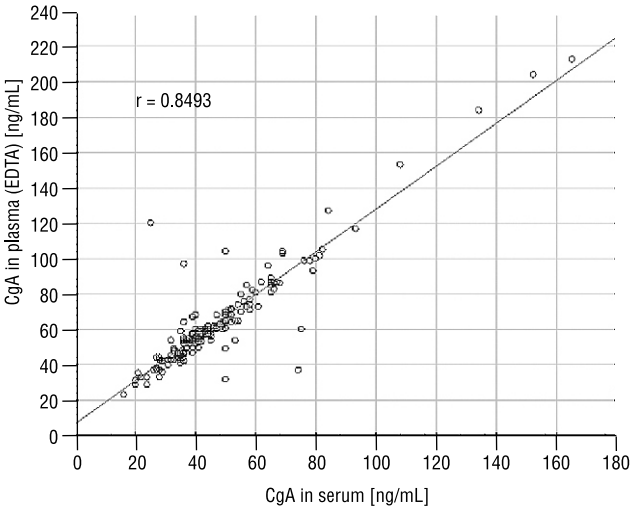

Figure 2. *Correlation of CgA in serum and plasma (EDTA$_2$K)*
Rycina 2. *Korelacja CgA w surowicy i w osoczu (EDTA$_2$K)*

Table II. ***Percentile distribution of CgA reference range in serum and plasma (EDTA$_2$K)***
Tabela II. ***Rozkład percentyli zakresu referencyjnego CgA w surowicy i w osoczu (EDTA$_2$K)***

CgA	Percentiles								
	2.5th	5th	10th	25th	50th	75th	90th	95th	97.5th
Serum	21	25	28	36	42.5	54	69	81	108
Plasma (EDTA$_2$K)	31	33	39	48	58.5	72	98	105	153

ferent epitopes of CgA molecule. As yet, an international standard for CgA assay is unavailable. Altogether, CgA measurements performed with the use of these assays are barely comparable and some of them are expressed in different units (a comparison of the selected methods used for CgA determination is presented in Table III).

Some differences of CgA results might partly be caused also by the matrix effect related to the type of investigated biological material [6].

Some regions of the CgA molecule are homologues with calcium-binding protein - calmodulin. Therefore, CgA has a high capacity for calcium binding. In the presence of free Ca^{2+} CgA aggregate rapidly (the extent and rate of aggregation are highly dependent on Ca^{2+} concentration). Anticoagulation occurs through the binding of Ca^{2+} ions and the inhibition of thrombin action, whereas in venous blood samples drawn into tubes containing clot activator, the calcium ions remain free [7–10]. Partial aggregation of CgA in serum samples might explain the measured lower CgA concentration in serum than in plasma samples.

Such a possible explanation was considered by us after completing our previous study [11] in which, using two routinely used methods (IRMA and ELISA), we compared the results of CgA measurements performed in serum and plasma (EDTA$_2$K) samples collected concomitantly from patients with various neuroendocrine tumours.

In other studies, this problem of matrix effect in relation to CgA was not recognised. The reported upper reference values for the serum CgA concentration determined by IRMA method (manufactured by CIS-bio) were as follows: Leon — 87 ng/mL [12], Ferrari— 70 ng/mL [13], Stridsberg — 99 ng/mL [14]. The cited authors did not declare that those values were valid only for the serum samples, while for instance, Stridsberg et al. measured CgA in heparinised plasma samples.

Bernini et al. [15] followed the manufacturer's reference values established for serum samples and accepted this CgA value rounded up to 100 ng/mL for plasma samples. Ramachandran et al. [16] established the upper reference value at 94 ng/mL, but they calculated this value as the 85th percentile of 185 plasma (EDTA$_2$K) samples of healthy patients and patients with non-NETs diseases.

We calculated due references ranges separately for the serum and plasma samples of 145 healthy males, expressed as 2.5 to 97.5 percentiles. Such a calculation seems to be more accurate because it covers a larger range of population.

Table III. *Comparison of the currently available methods for determination of CgA concentration*
Tabela III. *Porównanie obecnie stosowanych metod oznaczania stężenia CgA*

Method	ImmunoradiometricELISA (IRMA)		ELISA	Radioimmunoassay (RIA)	ELISA	Automated immunometric assay (Kryptor)
Kit producer	CISbio	CISbio	DAKO	EuroDiagnostica	ALPCO	B.R.A.H.M.S.
Antibody	2 monoclonal	2 monoclonal	2 polyclonal	1 polyclonal	2 polyclonal	2 monoclonal
Standard	rh CgA	rh CgA	23 kDa C-terminal fragment of CgA	CgA fraction purified from urine (patients with carcinoid tumours)	rh CgA	rh CgA
Unit	ng/mL	ng/mL	U/L	nmol/L	ng/mL	μg/L
Sort of biological material (according to the kit producer)	Serum Plasma	Serum Plasma	Plasma (EDTA, heparin)	Serum Plasma (EDTA, heparin)	Plasma (EDTA)	Serum
Cut-off (according to the kit producer)	Serum 98 ng/mL Plasma ?	Serum 98 ng/mL Plasma ?	Serum? Plasma 2–18 U/L	Serum and plasma ≤ 3 nmol/L	Serum ? Plasma 100 ng/mL	Serum Male: 84.7 μg/L Female: 43.2 μg/L Plasma ?

rh CgA — human recombinant CgA

Using the IRMA (Cis bio) method, we obtained a very good correlation between CgA determination in serum and plasma. In one other study, the authors reported a low correlation (r = 0.61) between serum and plasma CgA concentration, which was probably because they correlated two different assays: the ELISA (DAKO) for plasma samples and IRMA (CIS bio) for serum samples [13].

In previous studies [17], no differences were found in CgA levels between men and women. In the latest study of Braga et al. [18], however, the authors showed that serum CgA concentrations were significantly higher in women than in men, but all values were below the cut-off value.

A weak point of our work is a failure to collect a similarly large group of healthy females, which can be explained by the fact that most blood donors are males.

Conclusions

1. Significant differences in the concentrations of CgA measured in plasma and in serum demand the application of separate reference ranges adjusted to the type of investigated material.
2. Each laboratory should recommend only one sort of sample material for CgA assay.

References

1. Louthan O. Chromogranin A in physiology and oncology. Folia Biologica (Praha) 2011; 57: 173–181.
2. Peng Loh Y, Cheng Y, Mahata SK et al. Chromogranin A and derived peptides in health and disease. J Mol Neurosci 2012; 48: 347–356.
3. Kanakis G, Kaltsas G. Biochemical markers for gastroenteropancreatic neuroendocrine tumours (GEP-NETs). Best Pract Res Clin Gastroenterol 2012; 26: 791–802.
4. Singh S, Law C. Chromogranin A: a sensitive biomarker for the detection and post-treatment monitoring of gastroenteropancreatic neuroendocrine tumours. Expert Rev Gastroenterol Hepatol 2012; 6: 313–334.
5. Lawrence B, Gustafsson BI, Kidd M et al. The clinical relevance of chromogranin A as a biomarker for gastroenteropancreatic neuroendocrine tumours. Endocrinol Metab Clin N Am 2011; 40: 111–134.
6. Glinicki P, Kapuścińska R, Jeske W. Improved diagnostics accuracy for neuroendocrine neoplasms using two chromogranin A assays: the importance of protein matrix effects. Clin Endocrinol (Oxf) 2013; 79: 295–296.
7. Jain RK, Changã WT, Geetha Ch et al. In vitro aggregation of the regulated secretory protein chromogranin A. Biochem J 2002; 368: 605–610.
8. Koshimizu H, Kim T, Cawley NX et al. Chromogranin A: a new proposal for trafficking, processing and induction of granule biogenesis. Regul Pept 2010; 160: 153–159.
9. Yoo SH, Albanesi JP. Ca^{2+} — induced conformational change and aggregation of chromogranin A. J Biol Chem 1990; 265: 14414–14421.
10. Glinicki P, Jeske W. Chromogranin A (CgA) — the influence of various factors in vivo and in vitro, and existing disorders on it's concentration in blood. Endokrynol Pol 2010; 61: 384–387.
11. Glinicki P, Kapuścińska R, Jeske W. The differences in chromogranin A (CgA) concentrations measured in serum and in plasma by IRMA and ELISA methods. Endokrynol Pol 2010; 61: 346–350.
12. Leon A, Torta M, Dittadi R et al. Comparison between two methods in the determination of circulating chromogranin A in neuroendocrine tumors (NETs): results of a prospective multicenter observational study. Int J Biol Markers 2005; 20: 156–168.
13. Ferrari L, Seregni E, Lucignani G et al. Accuracy and clinical correlates of two different methods for chromogranin A assay in neuroendocrine tumors. Int J Biol Markers 2004; 19: 295–304.
14. Stridsberg M, Eriksson B, Öberg K et al. A comparison between three commercial kits for chromogranin A measurements. J Endocrinol 2003; 177: 337–341.
15. Bernini GP, Moretti A, Ferdeghini M et al. A new human chromogranin A immunoradiometric assay for the diagnosis of neuroendocrine tumours. Br J Cancer 2001; 84: 636–642.
16. Ramachandran R, Bech P, Murphy KG et al. Improved diagnostic accuracy for neuroendocrie neoplasms using two chromogranin A assays. Clin Endocrinol 2012; 76: 831–836.
17. Dittadi R, Meo S, Gion M. Biological variation of plasma chromogranin A. Clin Chem Lab Med 2004; 42: 109–110.
18. Braga F, Ferraro S, Mozzi R et al. Biological variation of neuroendocrine tumor markers chromogranin A and neuron-specific enolase. Clin Biochem 2013; 46: 148–151.