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Evaluation of a new immunoassay for chromogranin A measurement on the Kryptor system

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ABSTRACT

Background: Chromogranin A (CgA) is a biomarker for neuroendocrine tumors (NETs). The aims of this study were to evaluate differences in measurement between the ThermoFisher Brahms CgA Kryptor assay and the CisBio assay and to investigate the influence of patient covariates. Temperature stability of CgA using both assays was determined.

Design and Methods: 406 patients were analyzed for serum CgA using both assays. We performed a comparison study to determine whether several patient covariates (gender, use of protein pump inhibitors, impaired kidney function, referral department and tumor location) influenced the results. For the stability study, pooled serum samples were aliquoted and stored at different storage temperatures (room temperature, 4 °C and −20 °C) until assayed. In addition, 15 individual samples were evaluated after storage at 4 °C using the Kryptor assay.

Results: Differences in measured concentrations between the assays were statistically significant. Passing & Bablok fit showed $\ln Y(\text{Kryptor}) = 1.05 \ln X(\text{CisBio}) - 0.20$ with a bias of 1.0% after logarithmic transformation. Patient covariates were not associated. Patients' sera showed variable stability for CgA in the Kryptor assay at room temperature and 4 °C, whereas the recovery in the CisBio assay was stable at both temperatures.

Conclusion: Differences in measured CgA concentration between the assays could not be explained by the investigated patient covariates. Serum should be stored at –20 °C prior to determination using the Kryptor assay.

1. Introduction

Chromogranin A (CgA) is a member of the granin family and is an acidic glycoprotein consisting of 439 amino-acids with an approximate molecular mass of 48 kDa. CgA is produced and stored, together with the other members of the granin family (e.g. chromogranin B and C and other secretogranins), in large dense-core vesicles of neuroendocrine cell types. These cells can be found in the adrenal medulla, sympathetic nerve endings, cerebral cortex, pituitary gland, gastrointestinal tract, thyroid, parathyroid glands, pancreatic islets and lungs. Different neuroendocrine cells can process CgA differently in the cell. CgA acts as a pro-hormone which produces biologically active peptides for a wide range of biological activity by proteolytic cleavage [1,2].

The serum concentration of CgA is a sensitive but nonspecific marker for neuroendocrine tumors (NETs). NETs are rare neoplasms characterized by the ability to synthesize, store and secrete different peptides and neuroamines. Less than 10% of NETs are functional, that is, functional (hormone-secreting) with specific hypersecretory symptoms/syndromes. Most are non-functional. Classification of NETs can be done by localization and the embryological origin of the involved organ. Foregut carcinoid tumors are found in the thymus, bronchus, stomach, first portion of the duodenum, pancreas and ovaries. Midgut carcinoid tumors occur in the second part of the duodenum, small bowel and ascending colon, while hindgut tumors occur in the transverse colon, descending colon and rectum. Typically, in

Abbreviations: CgA, chromogranin A; NET, neuroendocrine tumor; TRACE, time-resolved amplified cryptate emission; ELISA, enzyme-linked immuno sorbent assay; ENETS, European Neuroendocrine Tumor Society; PPI, proton pump inhibitor; H2RA, H2-receptor antagonist; MDRD, modification of diet in renal diseases; LD, lactate dehydrogenase; GEP-NET, gastroentropancreatic NET

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well-differentiated NETs, the CgA elevation is more pronounced and often correlated with clinical symptoms, tumor mass, differentiation and presence of metastases [1–3]. Different tumors can release different molecular forms of CgA after post-translational processing with difference in circulating epitopes [1,4,5]. Also, different analytical methods with possible variable antibody–epitope binding characteristics can give different results [5–8]. Reliable quantification of CgA is important since it is used as a marker for NETs and for long-term follow up and treatment of the tumor.

Clinical interpretation of CgA results from different laboratories is hampered by considerable heterogeneity between available CgA assays. In our department of Clinical Chemistry the Kryptor CgA assay was introduced to replace the CisBio ELISA because the Kryptor assay facilitates a faster turn-around-time.

In preliminary work using randomly selected patients' samples with a request for CgA, we found a satisfactory comparison for CgA concentrations up to 300 μg/L (range: 19–300 μg/L) with a slope of 0.99 and an intercept of –1.15 ($n=91$). The difference plot of the values obtained provided a mean difference of 0.44 μg/L (2.7%). Therefore the reference value of CgA (<98 μg/L) provided by the CisBio manufacturer was not altered when the Kryptor assay was introduced. However, a larger bias was found at higher concentrations in NET patients and we therefore started a large comparison study in our clinic.

Since patients' samples are often stored for some time before determination of CgA, we determined the influence of different storage temperatures on the recovery of the CgA in the two assays.

In the present large comparison study of patients with known or suspected neuroendocrine tumors, we compare the analytical performance of the Kryptor assay with the CisBio assay. We hypothesized that the different NET locations in patients may result in different forms of circulating molecular CgA, which could lead to different results when measured with two assays raised against different epitopes. Also other factors (e.g. gender, use of proton pump inhibitors) might affect the result. To our knowledge, this is the largest published comparison study to investigate these factors.

2. Methods

2.1. Study population

In the Erasmus MC Rotterdam (ENETS centre) 406 consecutive patients were included over a nine-month period (March 2012–January 2013) from the Department of Internal Medicine (section of Endocrinology and Nuclear Medicine). In contrast to the Department of Endocrinology, almost all patients referred from the Department of Nuclear Medicine have metastatic disease. The referral department is therefore used as a marker for advanced disease in this study.

During this period all patient CgA concentrations were determined simultaneously with the Kryptor and CisBio assays. Patients either had a known NET for which they were being treated, or were into follow-up or were new cases with a suspected NET submitted for investigation.

2.2. Assays

The 29-min Kryptor assay is a sandwich immunoassay using Time-Resolved Amplified Cryptate Emission (TRACE) technology (ThermoFisher Brahms, Hennigsdorf, Germany). TRACE technology is based on a non-radiative energy transfer between a donor (europium cryptate) and an acceptor (XL665). The Kryptor assay facilitates automatic dilution of the sample inboard in case the default upper range of assay linearity is reached (measuring range, including automatic dilution 7–1,000,000 μg/L). The assay uses two mouse monoclonal antibodies. The epitope of the LK2H10 antibody has been identified as part of the amino acid sequence 250–301 [9,10]. The epitope of the monoclonal antibody PHE5 is not described in the literature. The inter-assay coefficient of variation (CV) for the Kryptor assay was 12.6% and 3.1% at 9.1 μg/L and 417.1 μg/L, respectively and the intra-assay CV of the Kryptor assay in our lab was 2.1% and 0.70% at 48.6 μg/L and 395.2 μg/L, respectively.

The CisBio assay is a solid-phase ELISA (CisBio Bioassays, Codolet, France). Sample dilution for the CisBio assay needs to be done manually (in the next run) after a result in the upper range is found. Performance of the CisBio assay takes 18 hours and it must be performed in batch mode. The assay uses two monoclonal antibodies against the central domain of CgA (amino acids 145–245). For the CisBio assay the inter-assay CV was 12% and 7% at 40 μg/L and 170 μg/L, respectively and the intra-assay CV was 4.2% and 3.7% at 100 μg/L and 400 μg/L, respectively.

2.3. Sample collection and comparison of CgA methods

Blood samples were obtained using venepuncture technique in 7 mL vacuum collection serum-separating tubes and centrifuged within 6 h at 1000–1200*g* for 10 min. Serum, the recommended matrix by the manufacturer, was frozen in aliquots and kept at −20 °C before analysis.

2.4. Stability conditions of the assays

We made two pools by mixing sera from 5–10 patients with approximately the same level of CgA. Concentrations of the pools were 80–109 μg/L and 390–529 μg/L. They were aliquoted and stored for 0, 2, 4 or 8 days at room temperature, 4 °C or −20 °C. Subsequently, samples were stored 0–8 days at −80 °C until assayed in singlicate by either the CisBio or Kryptor assay. Because the recovery was found to be very low (20%) for the Kryptor assay in pooled samples after 24 h at 4 °C, we duplicated the recovery data using a new serum pool at 4 °C. Additionally, 15 individual samples with a concentration range of 48–1169 μg/L were measured in singlicate at 4 °C in the Kryptor assay.

2.5. Covariates

Covariates investigated were gender, location of the tumor, use of proton pump inhibitor/ H2-receptor antagonist (PPI/H2RA), impaired kidney function (estimated GFR by MDRD equation, mL/min/1.73 m^2) and referral department (Internal Medicine and Nuclear Medicine). Categorical data were partly obtained by research nurses in the outpatient clinic and from patient file research. The location of the NET was classified according to anatomic location and functionality. PPI use and renal failure were classified as dichotomous variables. Estimated GFR (MDRD) < 60 mL/min/1.73 m^2 was used as the cut-off value to define impaired kidney function.

2.6. Statistical analyses

After natural logarithm-transformation of the measurements, a normal distribution was seen in the 406 patients. A paired *t*-test was performed on these data and the concordance between the assays was measured by the correlation coefficient. Passing & Bablok curve fitting and a difference plot was performed. Linear regression analysis of covariates was made. For analysis of the covariate location, we used deviation coding in which the mean difference is used as reference. The CgA stability over time and at different temperatures was calculated as a recovery percentage of the CgA concentration at time 0. Recoveries ranging from 90% to 110% were deemed to be acceptable.

All analyses were performed with the R statistical package (version 3.1.0, 2014; www.r-project.org). Passing–Bablok and difference plot analysis were done in Analyse-it v2.30 (Analyse-It Software Ltd, www.analyse-it.com). Two-tailed $P < 0.05$ was considered statistically significant.

3. Results

3.1. Performance of assays

Of the 406 patients, 186 (45.8%) were female and 220 (54.2%) were male. 122 (30%) used PPI or H2RB. Measured CgA levels of the Kryptor assay were on average higher compared to the CisBio assay ($P=0.012$), Pearson's correlation 0.97. The distribution of the CgA concentration in the patients' samples is summarized in Table 1.

When classifying patients for the covariate location, 59 patients (14.5%) were diagnosed with a NET with unknown primary. Another 22 patients (5.4%) were diagnosed with a high probability of a certain location and were classified for this specific location. Seven patients were expected to have normal CgA concentrations since no tumor was diagnosed. One patient of these seven had an increased CgA concentration of 4040 μg/L with the CisBio, and 3571 μg/L with Kryptor. The increased CgA level could be explained since he was using a PPI and had impaired renal function (estimated GFR 59 mL/min/1.73 m^2). This patient has known diabetes mellitus type II and is being followed up at the endocrinology outpatient clinic. The other 6 patients with no diagnosed tumor neither used PPI nor had altered renal function. One of these had a slightly increased CgA concentration (109 μg/L) using the Kryptor assay. All other concentrations were not increased.

Five patients were diagnosed with a tumor other than NET. Of the 394 remaining patients with a diagnosed NET, 138 patients (35.0%) were found to have non-increased CgA levels in the CisBio assay. For Kryptor this figure was 135 (34.3%).

Eighty-one patients with foregut tumors ($n=105$) had tumors of pancreatic origin. The other tumors were located in lung ($n=11$), stomach ($n=4$), duodenum ($n=4$), thymus ($n=3$) or ovary ($n=2$). Midgut tumors ($n=158$) were located in the small bowel or ascending colon. Hindgut tumors ($n=16$) were located in the transverse or descending colon or the rectum. Of the functional NETs ($n=31$), most were diagnosed with gastrinoma ($n=11$). Other functional NETs were diagnosed as insulinoma ($n=10$), glucagonoma ($n=4$), VIPoma ($n=3$) or a combination of these ($n=3$). Thirteen (3.2%) patients were diagnosed with paraganglioma. Other patients had pheochromocytoma ($n=6$), medullary carcinoma of the thyroid ($n=4$), carcinoid of the adrenal medulla ($n=1$) and NET of the cranial clivus ($n=1$).

Since in clinical practice CgA measurement is performed both in patients with and without NETs, all 406 patients were included in the comparison between the CisBio and Kryptor assays. The Passing & Bablok fit of the values showed a slope of 1.05 with 95% CI of 1.03–1.07 and an intercept of −0.20 with 95% CI of −0.28 to −0.12 (Fig. 1A). The difference plot ($N=406$) showed an overall positive bias of 1.0% with a 95% CI of 0.0–1.9% (Fig. 1B). We found that, in the concentration range of the natural logarithm means 5–6 (corresponding to 150–400 μg/L, $n=95$), a bias towards higher concentrations with the Kryptor assay is seen compared to the CisBio assay. No significant covariate associations were found in this subpopulation. Sixteen patients with much lower Kryptor values compared to those determined by the CisBio method also showed similar characteristics to the overall study population.

Gender was not associated with the difference between CisBio and Kryptor measurements ($P=0.18$), neither was PPI use ($P=0.60$), impaired renal function ($P=0.66$) or referral department ($P=0.21$). Also, tumor location was not associated.

Table 1
Descriptive statistics in 406 (suspected) NET patients.

	CisBio assay CgA (μg/L)	Kryptor assay CgA (μg/L)
Mean	1571	1862
Median	148.5	160.5
Range	5–137,000	6–143,100

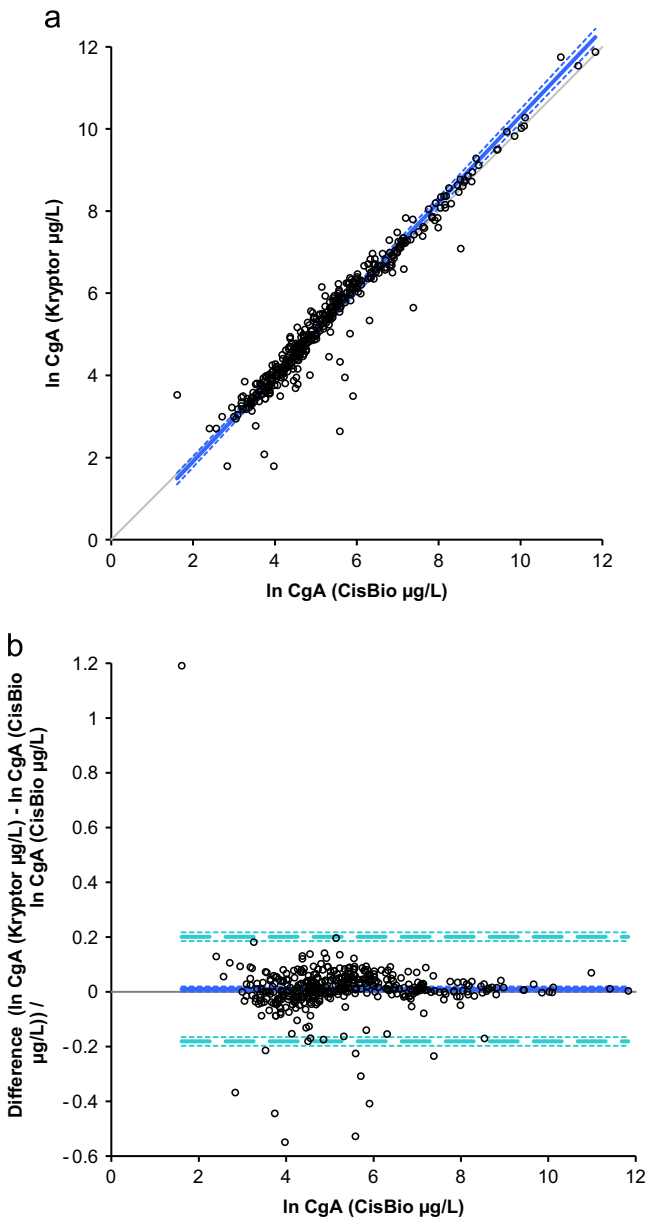

Fig. 1. (A) Comparison between CisBio and Kryptor CgA assay (Passing & Bablok curve fit, all samples). $y = 1.05–0.20x$. (B) Difference plot of Kryptor and CisBio CgA measurements (all samples). Bias of +1.0% (95% CI=0.0–1.9).

3.2. *Stability of the assays*

Storage at –80°C showed good recoveries of 99.9–104.5% after 24 h. Figs. 2 and 3 show results of the stability of CgA over 8 days using pooled samples.

The Kryptor assay shows a recovery of 94–97% after 8 h at 4 °C. After 24 h, however, recovery at 4 °C decreased to 80–81% and at day 8 had fallen to 13–20%. At room temperature, the recoveries were 98.0–98.2% after 24 h, decreasing to 62.6–76.3% at day 8. At −20 °C, a good recovery of 95.2–95.4% was found at day 8 (Fig. 2).

The CisBio showed a recovery of 91.3–100.8% at day 8 at both 4 °C and −20 °C. At room temperature this was 79.2–116.0% (Fig. 3).

Since a very low CgA recovery was found in pooled sera with the Kryptor assay after storage at 4 °C, additional measurements in individual sera were performed. Fig. 4 shows recoveries of 15 unpooled sera with baseline concentrations of 48–1169 µg/L, stored for seven days at 4 °C and measured by the Kryptor assay. Here, variations in recovery ranging from 36.8% to 102.8% at 24 h and < 8.6–97.4% after seven days were found.

4. Discussion

In this study, we performed a large comparison study in NET patients using the Kryptor CgA assay and CisBio assay.

Overall, Kryptor measured significantly higher levels of CgA compared to CisBio. To our knowledge, this is the largest population in which the covariates tumor location, gender, use of PPI, impaired kidney function and referral department (endocrinology or nuclear

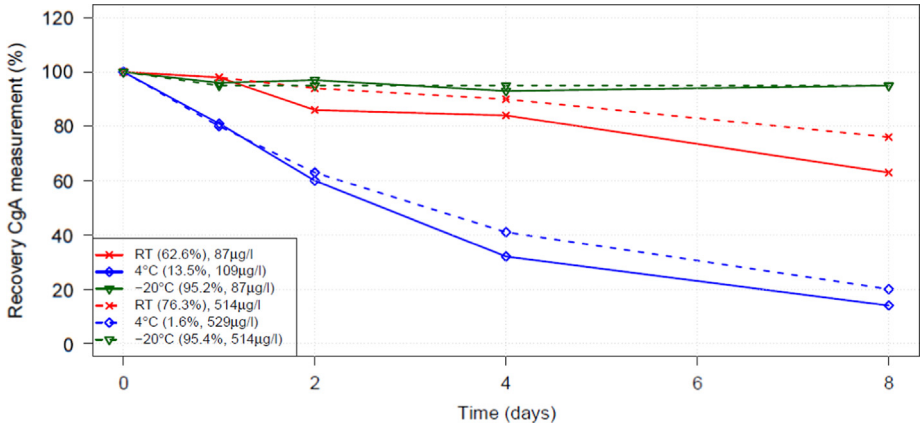

Fig. 2. Recoveries of CgA with the Kryptor assay at different temperature conditions in pooled samples. Percentages shown in the legend are recoveries on the last day of storage. Initial sample concentrations are also shown.

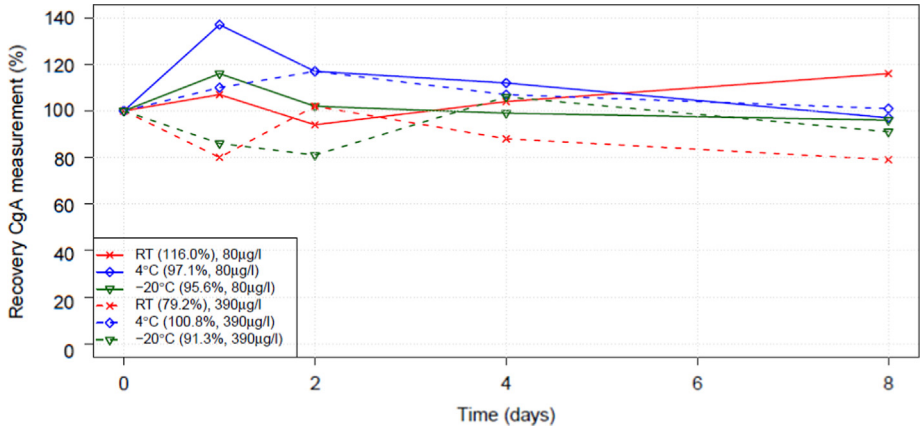

Fig. 3. Recoveries of CgA with the CisBio assay at different temperature conditions in pooled samples. Percentages shown in the legend are recoveries on the last day of storage. Initial sample concentrations are also shown.

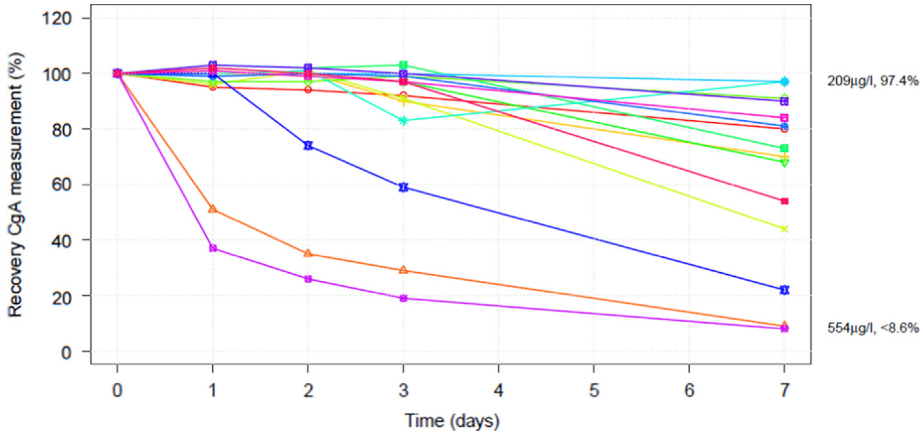

Fig. 4. Recoveries of CgA with the Kryptor assay in 15 individual (unspooled) samples stored at 4 °C. Percentages shown are the highest and lowest recovery in these samples. The initial CgA concentrations for these samples are also shown.

medicine) have been investigated. We hypothesized that patient dependent covariates could affect the molecular structure of excreted CgA molecules. In this way different assays could detect different levels of CgA. PPI usage has been shown to increase CgA levels in several commercial assays [11–13]. In one study, use of PPI did not give alterations in measured concentrations of the different region-specific assays [8]. Molina et al. [6] showed in a subgroup of patients without cancer that different assays can be influenced differently by renal failure. Stridsberg et al. [8] showed in a small study population with NETs that for an individual patient, impaired kidney function can influence immunoreactivity in a particular region on the CgA molecule (amino acids 17–38).

In our study we found no associations for the covariates studied. In healthy persons, Dittadi et al. [14] also found no association with gender using the CisBio assay. However, Braga et al. [15], using the Kryptor CgA assay, found higher values in women compared to men.

Different endocrine cell types process the CgA molecule differently [16]. In the intestine most enterochromaffin cells are positive for N-terminal and mid-portion antibodies, whereas C-terminal antibodies do not give rise to immunoreactivity. Gastrin cells display immunoreactivity to both N- and C-terminal antibodies [10]. Also in human endocrine pancreas, different cell types show different distribution patterns of region specific antibody binding [9]. CgA is an early marker of NETs in the foregut and the midgut [1]. Lower concentrations were found in hindgut tumors and other NETs. NETs of unknown primary site are diverse tumors with variable clinical behavior, predicted by tumor grade or differentiation. Their reported prevalence is 13% of all NETs, which is likely to be an underestimate due to reporting bias and assignment of tumors to suspected but unconfirmed sites [17]. In our study population 14.5% of the patients were found to have a NET of unknown primary. Another 5.4% had a NET of unknown primary, but had a high probability of a certain location mentioned in their patient file, mostly midgut tumors. We chose to classify these patients according to the most probable location mentioned. This could have introduced misclassification but this misclassification would be larger if this 5.4% was added to the NET unknown primary group. We hypothesized that tumor location could be associated with differences between the assays since different cell types process CgA differently. However, this was not found in our study with a large population of NET patients.

A recent study compared the Kryptor and CisBio CgA assay in 78 patients suspected of NETs [13]. The authors reported a mean difference of 10.1 μg/L, with CisBio giving higher concentrations compared to Kryptor. However, we found higher concentrations measured with the Kryptor assay as compared to the CisBio assay, mainly in the concentration range of the natural logarithm means 5–6 (corresponding to 150–400 μg/L). Patient covariates were not different in this range. Because our population included not only patients suspected of a NET, but also patients for follow-up or under treatment, CgA produced by the tumors after post-translational modifications could differ in our population compared to the population of 78 patients suspected of NETs in the study of Popovici et al [13]. Because the untransformed data was not normally distributed, only log transformed data should be used in the analysis. Consequently, we cannot compare directly the mean difference we found with the study of Popovici et al.

Interestingly, significant differences can occur in assays using the same antibodies, but with a different assay architecture. This was found in a study by Dittadi et al. [18], in which the CisBio ELISA was compared to the CisBio IRMA. The ELISA showed significant underestimation compared to IRMA in plasma EDTA samples.

Optimal shipment and storage conditions are important for reliable CgA determinations. Using the CisBio assay, CgA in serum is stable at both 4 °C and −20 °C. Although the CgA is stable at −20 °C using the Kryptor assay, the assay showed a low recovery in pooled samples after storage at room temperature and even lower at 4 °C. This is probably due to variation in stability in individual patient samples, as shown in Fig. 4. Similar results were found for storage at 4 °C using a recently launched ELISA, EDI KT-820 assay (Epitope Diagnostic Inc. San Diego, USA) (de Rijke, unpublished results). Our data are in agreement with a recent study showing an average decrease of Kryptor CgA in individual samples of 15.6% and 44.0% after 24 and 48 h at 4 °C, respectively [19].

Hypothetically, epitope exposure is different at the temperatures studied because of CgA polymerization, folding or degradation by proteinases. The CgA molecule may start to fold at temperatures of 2–8 °C and the antibodies used in the assay may be prevented from binding to their epitope on the irreversible folded structure. Therefore the CgA molecule may not be detected by the assay. If this is a continuous process, this will result in a decreasing concentration over time (ThermoFisher, personal communication). Intracellular CgA can form dimers or tetramers with chromogranin B in secretion vesicles [20]. Hypothetically, this could also occur after blood sampling and result in decreased availability of the epitope. Less CgA degradation might be expected at lower concentrations of calcium ions since endoproteinase activity depends on Ca^{2+} [21]. Another explanation might be the formation of irreversible CgA–antibody complexes at lower temperatures, as has been described for lactate dehydrogenase (LD) which showed this property at 0–7 °C due to a cold-induced conformational change in the LD tetrameric molecule [22]. When stored at −75 °C or room temperature for 24 h, no decrease in LD activity was seen. Cold denaturation could be another explanation of our findings, but this is unlikely since, for most studied proteins, cold-induced denaturation temperature lies well below the freezing point of water [23,24].

5. Conclusion

Our study showed that the CisBio assay can conveniently be replaced by the Kryptor assay with the advantage of having a lower coefficient of variation, higher throughput and a better turn-around time. However, storage and shipping conditions can have a major influence on CgA concentrations. Serum should be stored at −20 °C using the Kryptor assay. Storage at room temperature and 4 °C should be avoided when using the Kryptor assay since rapid decrease in recovery can occur in individual serum samples.

Conflict of interest

None declared.

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References

[1] Modlin IM, Gustafsson BI, Moss SF, Pavel M, Tsolakis AV, Kidd M. Chromogranin A – biological function and clinical utility in neuro endocrine tumor disease. Ann Surg Oncol 2010;17:2427–43.
[2] Vinik AI, Silva MP, Woltering EA, Go VL, Warner R, Caplin M. Biochemical testing for neuroendocrine tumors. Pancreas 2009;38:876–89.
[3] Nobels FR, Kwekkeboom DJ, Coopmans W, Schoenmakers CH, Lindemans J, De Herder WW, et al. 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;82:2622–8.
[4] de Herder WW. Biochemistry of neuroendocrine tumours. Best Pract Res Clin Endocrinol Metab 2007;21:33–41.

[5] Ramachandran R, Bech P, Murphy KG, Dhillo WS, Meeran KM, Chapman RS, et al. Improved diagnostic accuracy for neuroendocrine neoplasms using two chromogranin A assays. Clin Endocrinol (Oxf) 2012;76:831–6.
[6] Molina R, Alvarez E, Aniel-Quiroga A, Borque M, Candas B, Leon A, et al. Evaluation of chromogranin A determined by three different procedures in patients with benign diseases, neuroendocrine tumors and other malignancies. Tumour Biol 2011;32:13–22.
[7] Stridsberg M, Eriksson B, Oberg K, Janson ET. A comparison between three commercial kits for chromogranin A measurements. J Endocrinol 2003;177:337–41.
[8] Stridsberg M, Eriksson B, Oberg K, Janson ET. A panel of 11 region-specific radioimmunoassays for measurements of human chromogranin A. Regul Pept 2004;117:219–27.
[9] Portela-Gomes GM, Stridsberg M. Selective processing of chromogranin A in the different islet cells in human pancreas. J Histochem Cytochem 2001;49:483–90.
[10] Portela-Gomes GM, Stridsberg M. Chromogranin A in the human gastrointestinal tract: an immunocytochemical study with region-specific antibodies. J Histochem Cytochem 2002;50:1487–92.
[11] Pregun I, Herszenyi L, Juhasz M, Miheller P, Hritz I, Patocs A, et al. Effect of proton-pump inhibitor therapy on serum chromogranin A level. Digestion 2011;84:22–8.
[12] Mosli HH, Dennis A, Kocha W, Asher LJ, Van Uum SH. Effect of short-term proton pump inhibitor treatment and its discontinuation on chromogranin A in healthy subjects. J Clin Endocrinol Metab 2012;97:E1731–5.
[13] Popovici T, Moreira B, Schlageter MH, Bories PN. Automated two-site immunofluorescent assay for the measurement of serum chromogranin A. Clin Biochem 2014;47:87–91.
[14] Dittadi R, Meo S, Gion M. Biological variation of plasma chromogranin A. Clin Chem Lab Med 2004;42:109–10.
[15] Braga F, Ferraro S, Mozzi R, Dolci A, Panteghini M. Biological variation of neuroendocrine tumor markers chromogranin A and neuron-specific enolase. Clin Biochem 2013;46:148–51.
[16] Portela-Gomes GM, Grimelius L, Stridsberg M. Immunohistochemical and biochemical studies with region-specific antibodies to chromogranins A and B and secretogranins II and III in neuroendocrine tumors. Cell Mol Neurobiol 2010;30:1147–53.
[17] Spigel DR, Hainsworth JD, Greco FA. Neuroendocrine carcinoma of unknown primary site. Semin Oncol 2009;36:52–9.
[18] Dittadi R, Bertoli I. Evaluation of an ELISA method for the measurement of chromogranin A and comparison with an immunoradiometric method. Int J Biol Markers 2013;28:114–6.
[19] Pedersen L, Nybo M. Preanalytical factors of importance for measurement of Chromogranin A. Clin Chim Acta 2014;436:41–4.
[20] Yoo SH, Lewis MS. Effects of pH and Ca^{2+} on heterodimer and heterotetramer formation by chromogranin A and chromogranin B. J. Biol Chem 1996;271:17041–6.
[21] Hutton JC, Davidson HW, Peshavaria M. Proteolytic processing of chromogranin A in purified insulin granules. Formation of a 20 kDa N-terminal fragment (betagranin) by the concerted action of a Ca^{2+}-dependent endopeptidase and carboxypeptidase H (EC 3.4.17.10). Biochem J 1987;244:457–64.
[22] Wickus GG, Smith MJ. Rapid loss of lactate dehydrogenase isoenzyme activity in serum by cold-induced formation of immunoglobulin G-lactate dehydrogenase complex. Clin Chem 1984;30:11–7.
[23] Franks F, Hatley RH, Friedman HL. The thermodynamics of protein stability. Cold destabilization as a general phenomenon. Biophys Chem 1988;31:307–15.
[24] Hatley RH, Franks F. The cold-induced denaturation of lactate dehydrogenase at sub-zero temperatures in the absence of perturbants. FEBS Lett 1989;257:171–3.