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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.