Chromogranin A (CgA): structure, biological function, pre-analytical, analytical, and clinical aspects of its measurement in blood

Chromogran♫n A (CgA): budowa, funkcja biologiczna, przedanalityczne, analityczne i kliniczne aspekty oznaczania jej we krwi

INTRODUCTION

Chromogranin A (CgA) is present in some endocrine cells of adrenals, pituitary, pancreas, thyroid and in cells of diffuse endocrine system (DES) of gastrointestinal and respiratory system. It is co-secreted and co-released together with some amines and peptides, that are present in the neurosecretory granules. In functionally active, and non-active neuroendocrine tumors (NETs) blood CgA level is often elevated, therefore, it is accepted as a main nonspecific marker of NETs (1, 2).

CHROMOGRA♥N A: STRUCTURE AND BIOLOGICAL FUNCTION

Chromogranin A is an acidic protein with a molecular weight of 48 kDa that is composed of 439 amino acids (3). The human CgA gene (CHGA) is located on chromosome 14 (4). There are 10 dibasic sites in human CgA, which are potential sites for proteolytic cleavage (5). CgA occurs in two main conformations: random coil (60-65%) and alpha-helix (25-40%). Alteration of CgA conformation is pH and calcium ions dependent (6, 7). CgA is a protein binding Ca²⁺ ions.

Summary

Chromogranin A (CgA) is a main nonspecific neuroendocrine tumour (NET) marker. Currently few commercial assays are available: RIA, IRMA, ELISA, CLIA, TRACE. There are many factors: in vivo, in vitro and coexisting diseases which can influence the CgA blood concentration. Elevated CgA levels in blood can be usually detected in: gastroenteropancreatic neuroendocrine tumours (GEP-NET), pheochromocytoma, neuroblastoma, MEN syndromes, bronchopulmonary NETs, medullary thyroid carcinoma, small-cell lung carcinoma, and some other very rare NETs. CgA measurement became a routine investigation in the diagnosis of GEP-NET, but is especially helpful in monitoring the effects of their treatment. CgA can be considered as a complementary investigation in the diagnostic procedure of pheochromocytoma. In patients with multiple endocrine neoplasia (MEN) investigation of CgA level may be used in monitoring eventual coexistence or appearance with time of carcinoid, pancreatic neuroendocrine tumour or pheochromocytoma.

Streszczenie

Chromogranina A (CgA) jest głównym, niespecyficznym markerem guzów neuroendokrynnych (NET). Obecnie dostępnych jest kilka komercyjnych testów: RIA, IRMA, ELISA, CLIA, TRACE. Istnieje wiele czynników: in vivo, in vitro i współistniejących chorób, które mogą wpływać na stężenie CgA we krwi. Podwyższone stężenie CgA we krwi obserwujemy zwykle w: guzach neuroendokrynnych przewodu pokarmowego (GEP-NET), pheochromocytoma, neuroblastoma, zespołach MEN, guzach neuroendokrynnych oskrzeli, raku rdzeniastym tarczycy, raku drobnokomórkowym płuc oraz w innych rzadkich guzach NET. Pomiar stężenia CgA jest obecnie rutynowo stosowany w diagnostyce guzów GEP-NET, ale szczególnie jest on przydatny w monitorowaniu efektów leczenia. CgA może być dodatkowym badaniem w diagnostyce pheochromocytoma. U pacjentów z zespołami mnogich nowotworów endokrynnych (MEN) i u członków ich rodzin oznaczanie stężenia CgA może służyć ich monitorowaniu, aby jak najwcześniej wykryć pojawienie się w toku obserwacji guza neuroendokrynnego trzustki, rakowiaka albo pheochromocytoma.
Many regions of CgA are homologous with Ca²⁺ binding protein – calmodulin (8). CgA is a member of the chromogranin family. The granin family consists of eight proteins including: CgA, chromogranin B (CgB), secretogranin II, secretogranin III, secretogranin IV, secretogranin V, secretogranin VI, VGF (9). Posttranslational processing of CgA molecule leads to the formation of smaller biologically active peptides, such as: vasostatin I, vasostatin II, chromacin, pancreastatin, WE-14, parastatin, catestin (10). These CgA-derived peptides due to their influence on the secretion of other hormone, play an indirect role in the metabolism of lipids, carbohydrates, calcium homeostasis, catecholamine secretion, and possess some activities on the cardiovascular system (e.g. vasoconstriction, vasodilation). They participate also in regulation of secretion of some hormones (e.g. insulin, glucagon, leptin, LH, FSH, PTH), and play some role in the defense mechanism of the respiratory system (antimicrobial activity against bacteria, fungi) (11-15).

PRE-ANALYTICAL AND ANALYTICAL ASPECTS OF CHROMOGRANIN A (CgA) MEASUREMENT IN BLOOD

Measurement of blood CgA concentration appeared possible despite the presence in blood of circulating CgA fragments induced by proteolysis (16).

The first radioimmunoassay for measurements of chromogranin A was introduced by O’Connor and Bernstein in 1984 (17). The next generation assays were based on sandwich methods with the use of monoclonal or polyclonal antibodies (18). Currently few commercial assays are available: IRMA (CgA-RIA CT, CIS Bio International-Schering, Gif-sur-Yvette, France), DAKO chromogranin A ELISA kit (DAKO A/S, Glostrup, Denmark), RIA (EuroDiagnostica, Malmo, Sweden), TRACE (Kryptor System; B-R-A-H-M-S GmbH, Thermo Scientific, Germany). These assays differ in test structure, use different antibodies and differently calibrated standards. The applied in these assay monoclonal or polyclonal antibodies recognize different epitopes of CgA molecule and bind also some CgA fragments (19-21). Main characteristics of the mentioned method for determination of CgA are presented in table 1.

Lack of the recognized international standard for CgA, differences of methodology and specificity of the antibodies used, cause that individual CgA measurements performed with different CgA assays cannot be directly compared (22-23).

Using two commercial assays (IRMA and ELISA), we were able to show that CgA concentration is markedly higher in plasma (EDTA, heparin) than in serum (difference 12-70%), therefore different reference ranges should be applied for serum and for plasma samples. Our finding may be explained by the known fact that CgA can partially aggregate at high concentration of free Ca²⁺ ions. In plasma samples (EDTA) anticoagulation occurs through the binding of Ca²⁺ ions, therefore absence of free Ca²⁺ ions may protect CgA from partial aggregation (24). Apart of the tendency for aggregation CgA is a relatively stable protein, although one recent study has indicated that storage of samples over 24 hrs and 48 hrs at +4°C might decrease CgA concentration by 15% and 44% respectively (25). No significant differences were found between CgA levels in men and women (26, 27). In one study, however, authors noted higher concentrations of CgA in males than females, but in another paper CgA levels were higher in females than men, regardless of age (28, 29).

Table 1. Comparison of the currently available methods for determination of CgA concentration.

<table>
<thead>
<tr>
<th>Method</th>
<th>Kit producer</th>
<th>Antibody</th>
<th>Standard</th>
<th>Unit</th>
<th>Sort of biological material (according to the kit producer)</th>
<th>Upper reference range (according to the kit producer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoradiometric (IRMA)</td>
<td>CIS Bio</td>
<td>2 monoclonal</td>
<td>rh CgA</td>
<td>ng/ml</td>
<td>serum plasma</td>
<td>serum 98 ng/ml plasma?</td>
</tr>
<tr>
<td>ELISA</td>
<td>CIS Bio</td>
<td>2 monoclonal</td>
<td>rh CgA</td>
<td>ng/ml</td>
<td>serum plasma</td>
<td>serum 98 ng/ml plasma?</td>
</tr>
<tr>
<td>ELISA</td>
<td>DAKO</td>
<td>2 polyclonal</td>
<td>23 kDa C-terminal fragment of CgA</td>
<td>U/l</td>
<td>plasma (EDTA, heparin)</td>
<td>serum? plasma 2-18 U/l</td>
</tr>
<tr>
<td>Radioimmunoassay (RIA)</td>
<td>EuroDiagnostica</td>
<td>1 polyclonal</td>
<td>CgA fraction purified from urine (patients with carcinoid tumours)</td>
<td>nmol/l</td>
<td>serum and plasma ≤ 3 nmol/l</td>
<td>serum and plasma ≤ 3 nmol/l</td>
</tr>
<tr>
<td>ELISA</td>
<td>ALPCO</td>
<td>2 polyclonal</td>
<td>rh CgA</td>
<td>ng/ml</td>
<td>plasma (EDTA)</td>
<td>serum? plasma 100 ng/ml</td>
</tr>
<tr>
<td>Automated immunoassay (Kryptor)</td>
<td>B-R-A-H-M-S</td>
<td>2 monoclonal</td>
<td>rh CgA</td>
<td>µg/l</td>
<td>serum</td>
<td>serum male: 84.7 µg/l female: 43.2 µg/l plasma?</td>
</tr>
</tbody>
</table>

rh CgA – human recombinant CgA
fluctuation of blood CgA concentration can be approximately 20-25%, and higher levels are observed in the late afternoon and at night (30, 31). Moderate exercise may exert a minor effect on CgA concentration (32). The CgA levels may also be influenced by ingestion of a meal, therefore blood for its measurement should be collected on fasting preferably in the morning (33, 34).

There are two groups of drugs which strongly influence CgA concentration: proton pump inhibitors (PPI) and \( H_2 \)-receptor antagonists (\( H_2 \)-RA) (35). Long-term use of PPI, and in some cases even a short-term treatment too (1-2 weeks), cause a significant increase of CgA concentration in blood, reaching sometimes levels like those observed in advanced NET tumours with metastases (36, 37). To avoid the effect of PPIs on CgA, if only possible, proton pump inhibitors should be discontinued for two weeks, before blood sampling, or PPI should be temporarily replaced by \( H_2 \)-RA, and this later drug should be discontinued 3 days before blood collection (38, 39). Treatment with corticosteroids may increase the concentration of CgA about 2-fold. The hypotensive drugs, have a little or no effect on CgA concentration (40, 41). During chemotherapy CgA levels may increase temporarily due to the tissue damage and/or nephrotoxic effect of such drugs (42, 43). The list of drugs and pathological conditions which can have influence on CgA concentration is presented in the table (tab. 2).

**Table 2.** Drugs and pathological conditions which can have influence on the CgA blood level.

<table>
<thead>
<tr>
<th>High or moderate increase of the blood CgA level</th>
<th>Moderate or little increase of the blood CgA level</th>
</tr>
</thead>
<tbody>
<tr>
<td>– proton-pump inhibitors</td>
<td>– inflammatory bowel disease</td>
</tr>
<tr>
<td>– histamine ( H_2 ) receptor-blockers</td>
<td>– untreated essential hypertension</td>
</tr>
<tr>
<td>– chronic atrophic gastritis</td>
<td>– acute coronary syndrome</td>
</tr>
<tr>
<td>– impaired renal function</td>
<td>– cardiac insufficiency</td>
</tr>
<tr>
<td>– prostate cancer and BPH</td>
<td>– impaired liver function</td>
</tr>
<tr>
<td>– untreated essential hypertension</td>
<td>– hepatocellular carcinoma</td>
</tr>
<tr>
<td>– chronic renal failure</td>
<td>– pancreatic adenocarcinoma</td>
</tr>
<tr>
<td>– untreated hypertension</td>
<td>– hyperthyroidism</td>
</tr>
<tr>
<td>– acute renal failure</td>
<td>– hyperparathyroidism</td>
</tr>
<tr>
<td>– impaired liver function</td>
<td>– rheumatoid arthritis (RF IgM)</td>
</tr>
<tr>
<td>– hepatocellular carcinoma</td>
<td>– Parkinson disease</td>
</tr>
<tr>
<td>– cardiac insufficiency</td>
<td>– pregnancy</td>
</tr>
</tbody>
</table>

Practical recommendations for monitoring the concentration of CgA are presented in table 3.

**Table 3.** Practical recommendations for monitoring the concentration of CgA.

- The same method, preferably in the same laboratory
- Use the same type of biological material (serum, EDTA-plasma, heparin-plasma)
- Results refer to the reference range established for serum or plasma
- Order blood collection preferably in the morning on fast (> 8 hr after last meal)
- Withdraw drugs (proton pump inhibitors for 2 weeks, \( H_2 \)-receptor blockers for 3 days)
- Consider other non-specific factors which can have influence on CgA blood level

**CHROMOGRANIN A – NEUROENDOCRINE TUMOURS MARKER**

Neuroendocrine tumours are considered as rare, heterogeneous neoplasms. Some of these tumours secrete into the blood various substances, such as: hormones, pre-hormones, amines, various peptides which measurements have been applied for diagnostic purposes, assessment of treatment efficacy, and disease prognosis.

CgA is a main nonspecific neuroendocrine tumour marker. Elevated blood CgA level can be usually detected in: gastroenteropancreatic neuroendocrine tumours (GEP-NET), pheochromocytomas, neuroblastomas, MEN syndromes, bronchopulmonary NETs, medullary thyroid carcinoma, small-cell lung carcinoma, and some other very rare NETs (44). Dimension of CgA level elevation depends of the cell type, number of secretory granules, tumour volume, localization and stage of the disease (metastases) (45).

**GEP-NET**

CgA measurement became a routine investigation in the diagnosis of GEP-NET (46), but is especially helpful in monitoring the effects of treatment. In small NETs, however (e.g. insulinoma) CgA level may not be elevated (47), therefore in general the sensitivity of CgA in GEP-NET is 10-100%, and specificity 60-100%. In patient with metastasis, the sensitivity is 60-100%. In some cases CgA level can increase by 100-1000 times above the cut-off value (the highest values are observed in carcinoids with liver metastases). CgA is considered as an independent prognostic factor of survival in patients with midgut GEP-NET (48). The highest sensitivity (80-100%) of CgA is observed in: gastrinoma, carcinoid tumour, and glucagonoma. Concentration of circulating CgA is associated with the degree of differentiation of neuroendocrine tumors (in poorly differentiated tumours CgA level are lower than in the highly differentiated tumors). According to the established recommendation in poorly differentiated tumours, CgA level should be measured every 2-3 months, whereas in other cases, at 6-12 months intervals (49).

**PHEOCHROMOCYTOMA AND OTHER ADRENAL TUMOURS**

CgA is secreted by the neuroendocrine cells of adrenal medulla. CgA levels correlate with tumour mass and secretion of catecholamines, but this correlation disappears in large tumours (50). The sensitivity of CgA is 80-96% (51, 52). CgA can be considered as a complementary investigation in the diagnostic procedure of pheochromocytoma (together with catecholamines the sensitivity is approx. 100%). In preliminary differential diagnosis of adrenal tumours a markedly increased CgA level might be a useful additional marker of pheochromocytoma, however there are some cases of “silent” pheochromocytoma in which CgA level is not elevat-
ed. On the other side we should know that there are some cases of hormonally active adenomas and adrenal carcinomas secreting cortisol in which blood CgA levels may be moderately increased (53).

It is important to note that pheochromocytoma should be excluded before any invasive diagnostic or therapeutic procedure is undertaken to avoid the development of catecholaminergic crisis. It has been reported that in clinically silent cases of pheochromocytoma, after administration of glucocorticoids in a dose exceeding 1 mg of dexamethasone, there is a potential threat of severe hypertensive crisis, with life-threatening symptoms (low dose DST – 1 mg orally – has not been associated with such crisis) (54). Therefore, special care is necessary while planning the performance of a pre-treatment before CT or MRI in patients allergic to contrast dye. For the above reasons, an additional test is warranted.

For patients with an asymptomatic adrenal tumour seems warranted. It is important to note that pheochromocytoma should be excluded before any invasive diagnostic or therapeutic procedure is undertaken to avoid the development of catecholaminergic crisis. It has been reported that in clinically silent cases of pheochromocytoma, after administration of glucocorticoids in a dose exceeding 1 mg of dexamethasone, there is a potential threat of severe hypertensive crisis, with life-threatening symptoms (low dose DST – 1 mg orally – has not been associated with such crisis) (54). Therefore, special care is necessary while planning the performance of a pre-treatment before CT or MRI in patients allergic to contrast dye. For the above reasons, an additional test is warranted.

**MEN SYNDROMES**

In patients with multiple endocrine neoplasia (MEN) investigation of CgA level may be used for monitoring eventual coexistence or appearance with time of carcinoid, pancreatic neuroendocrine tumours or pheochromocytoma (55). Sensitivity of CgA in patients with MEN-1 is approximately 60% (56).

**DETERMINATION OF THE BLOOD CgA CONCENTRATION IN SOME OTHER DISEASES**

During long-term gastric acid inhibition serum CgA levels correlate with serum gastrin and reflect the presence and severity of fundic enterochromaffin (ECL) cells proliferative changes. Therefore it is proposed that monitoring blood CgA levels might be a useful screening test for ECL cell hyperplasia. It is suggested, that in cases with markedly increased CgA values an indication for endoscopic and histological examination of the gastric mucosa should be considered (57). CgA level may increase slightly also in patients with liver cirrhosis, chronic hepatitis, pancreatitis, inflammatory bowel diseases, obstructive pulmonary disease, sepsis, and in extreme physical stress (58).


45. Feldman SA, Eiden LE: The chromogranins: Their roles in secretion from neuroendocrine cells and as markers for neuroendocrine neoplasia. Endocrine Pathology 2003; 14: 3-23.


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