# The Role of Chromogranin A in Adrenal Tumors

**ALEXANDRA MIRICA[1], IOANA ANCA BADARAU[2], ANA MARIA STEFANESCU[1], RADU MIRICA[3]*, SORIN PAUN[2,4], DACIANA ANDRADA COSTINA STEFAN[2], DIANA LORETA PAUN[1,2]**

[1] National Institute of Endocrinology, C.I.Parhon, 34-36 Aviatorilor Blvd., 11863, Bucharest, Romania

[2] University of Medicine and Pharmacy, Carol Davila, 8 Eroii Sanitari Blvd., 050474, Bucharest, Romania

[3] Emergency Hospital 'Saint John, 13 Vitan-Barzesti Str., 042123, Bucharest, Romania

[4] Emergency Clinical Hospital, 8 Calea Floreasca, 014461, Bucharest, Romania

*Chromogranin A (CgA) is part of the family of granins, which are acidic glycoproteins that represent an important part of secretory dense core granules. They are specific to various neuroendocrine and endocrine tissues, as components of diffuse neuroendocrine system and endocrine glands. CgA is co-secreted and co-released in the circulation along with hormones, bioamines and peptides secreted from the neuroendocrine cells . In the last decade, studies have emphasized the major importance of serum CgA in the diagnosis and follow-up of neuroendocrine tumors such as gastroenteropancreatic tumors, pheochromocytoma, medullary thyroid carcinoma. But its diagnostic value for adrenocortical adenomas or for adrenal malignancy, is still controversial. The current study aims to provide a comprehensive review , for synthesizing current knowledge regarding corelations between plasma CgA concentration and various adrenal tumors. Furthermore, there will be also analyzed and synthesized the clinical applicability and the diagnostic usefulness of dosing CgA in adrenal pathology, both medullary and cortical benign and malignant lesions.*

*Keywords: chromogranin A, pheochromocytoma, adrenocortical secretory adenomas, non-functioning adrenocortical tumors, adrenal neoplasia*

Granins are a family of water-soluble glycoproteins localised in the secretory vesicles of a wide heterogeneity of neuroendocrine and endocrine cells. This grouped of proteins is composed of the following granins: chromogranin A (also called parathyroid secretory protein 1), chromogranin B (also named secretogranin I ), C (secretogranin II), secretogranin III, IV, V, VI..The most representative and numerous components of this family are chromogranins A, B and C (CgA, CgB, CgC). These proteins have an important role in the formation, storage and release of secretory granules comprising neurotransmitters, biologically active amines, neuropeptides and hormones [1].

The first chromogranin described was CgA, that was initially discovered in adrenal medulla catecholamine secretion granules. CgA has a molecular mass of 49 kd and is composed of 439 amino acids [2,3]. It is produced and co-secreted by exocytosis together with the hormones within secretory granules. From a physiological point of view, this protein also acts as a prohormon, because after its cleavage into various peptides, it can perform autocrine and paracrine roles, regulating the functions of the tissue . But the exact roles and the complete biological functions are still under study. Subsequently it was observed that serum CgA increases in patients with pheochromocytoma and paragangliomas, as well as in other hormone-secreting or non-hormone secreting neuroendocrine tumors (NETs) such as gastroenteropancreatic tumors, medullary thyroid carcinoma, pituitary tumors (except prolactinomas), neuroblastomas [4]. Therefore, CgA dosing is recommended by the majority of endocrine societies such European NeuroEndocrine Tumor Society (ENETS), North American Neuroendocrine Tumor Society (NANETS) as a general serum biomarker for the diagnosis and monitoring of NETs [5].

Moreover, increased levels of serum CgA have been associated with neoplasia with epithelial embryological origin, such as breast, prostate, ovary, hepatic and pancreas cancers. These epithelial tumors can gain the biological ability to secrete CgA , consisting of a phenotypic neuroendocrine transformation that is associated with a unfavorable prognosis.

The largest amount of CgA exists within chromaffin neuroendocrine cells of the adrenal medulla and inside the storage granules of sympathetic nerves.The cortical and medullary parts of the adrenal gland are vascularly and hormonally interconnected, even though they are differentiated by embryological origins, histological and secretory characteristics. Thus, the adrenal medulla consists of chromaffin cells that are specialised in the synthesis of catecholamines, along with other substances including CgA. In addition, chromaffin cells can also be found in the adrenal cortex, especially in the glomerular and subcapsular regions. Complementary, cortical cells can also be localised in the adrenal medulla.Therefore, the connections between the two cell types are very tight, providing a functional interrelation, whose exact roles are not yet fully understood and described [6].

Due to the widespread of neuroendocrine and endocrine cells in the human body, CgA levels were also found elevated in other medical situations. The most frequent cause reported in the literature, was the use of proton pump inhibitors, that can rise the CgA serum levels two to four times higher [7].

In addition, kidney dysfunction, cardiac and hepatic insufficiency, inflammatory bowel disease, rheumathoid arthritis and atrophic gastritis were also correlated with an increase in CgA serum levels [1,4].

## Experimental part

### *Matherials and methods*

We performed a comprehensive general review synthesizing data from recent relevant studies. In this direction we have collected data from the following medical electronic databases: Pubmed, Embase, Ebsco, Medline. We searched through medical literature without

* email: mirica_rm@yahoo.com; Phone: 0740992324

any restrictions in terms of language and publication status, all relevant studies published in the last decade,which focused on the study of serum levels of Cg A in adrenal tumors.

**Results and discussions**

The collected data were grouped into four distinct parts, discussed in detail in the following paragraphs, depending on the specific adrenal tumor type. Each paragraph will also emphasize the importance and clinical usefulness of dosing CgA.

*CgA as a general biomarker of neuroendocrine tumors*

Neuroendocrine tumors (NETs) consists of a group of rare and heterogeneous cancers that are originating from neuroendocrine cells of the nervous and endocrine tissues. These tumors are sparsed throughout the entire human body including the stomach, lung, intestine, pancreas, pituitary, adrenals, sympathetic nerves ganglia, thyroid and parathyroid glands. Their classification comprises two categories depending on the secretory profile: functional tumors , usually with symptoms specific to carcinoid syndrome or nonfunctional tumors characterized by hormonal inactive tumors. CgA can be used to diagnose both functioning and non-functioning NETs.

The CgA can be secreted by a wide variety of NETs, including pheochromocytoma, paraganglioma, medullary thyroid carcinoma, pituitary tumors except prolactinomas, parathyroid adenomas, pulmonary neuroendocrine neoplasia including small cell lung cancer and gastroenteropancreatic NETs.

In comparative studies, CgA assessment was established to be more sensitive and specific for the diagnosis of NETs than measurements of other neuroendocrine biomarkers such as urinary 5-HIAA, carcino-embryonic antigen (CEA) and neuron specific enolase (NSE) [8].

Recent guidelines are recommending that all patients with NETs should have their CgA levels determined as a diagnostic and prognostic factor, before starting medical, surgical, chemo or radiotherapy treatment [9] .

The largest amount of CgA serum levels have been recorded in gastroenteropancreatic NETs, particularly in the small intestine or midgut tumors and also in pancreatic NETs. In such patients, it were described a 100 to 1000 fold increase in CgA levels.

Moreover, CgA levels are significantly increased in the case of disseminated neuroendocrine disease especially in the cases associated with hepatic metastasis. In this regard, studies reported higher CgA levels in the case of more than 5 metastatic lesions compared to the situation of less than 5 liver metastasis or disseminated neoplastic disease located only in the lymph nodes. Thus, high CgA levels were correlated with a large tumor burden , tumor relapse and with progressive disease , all leading to a poor prognosis and a short survival rate for the patient . Nevertheless, CgA serum level is recommended to be used in order to monitor the treatment, knowing that a beneficial treatment should reduce its serum levels by half. With respect to long-term patient monitoring, it was noticed that regular plasmatic assessments of CgA could be a more useful clinical tool than repeated imagistic procedures, taking into account that the values of this biomarker are the first to increase in the case of tumor recurrence [10].

*Pheochromocytomas and CgA levels*

Pheochromocytomas represent rare neuroendocrine catecholamine-producing tumors, developing inside the adrenal gland, from the chromaffin cells of the adrenal medulla. Moreover, tumors developing from cromaffin cells of sympathetic or parasympathetic nerve ganglia, are termed paragangliomas and are located beyond the adrenal gland. The majority of these neuroendocrine tumors, occur sporadically but recent genetic studies demonstrated that aproximatelly 30-40% could have a genetic background, such in endocrine familial syndromes as multiple endocrine neoplasia type 2, neurofibromatosis type I or von Hippel Lindau disease [11,12].

Catecholamines are transformed inside the chromaffin cells to metanephrines, thus norepinephrine is conversed to normetanephrine and epinephrine to metanephrine. Subsequently to sympathetic or adrenal stimulation, CgA is released by exocytosis from the storage granules together with the catecholamines into the bloddstream. Therefore, CgA levels corresponds to the norepinephrine and epinephrine serum levels, in the context of an adrenal tumor [13]. In line with these, current guidelines recommendations are that the diagnosis and monitoring for pheochromocytoma or paraganglioma must include evaluation of plasmatic and urinary fractionated metanephrines (normetanephrines and metanephrines) [14]. It is to be mentioned that the plasma metanephrines showed superiority over the urinary metanephrines in some studies [15,16]. On the other hand, there were also medical reports pointing out that elevated plasma metanephrines levels have not always identified the presence of a pheochromocytoma.

European society clinical guide recommend assaying Cg A preoperatively in patients with normal plasma or urinary levels of metanephrines, normetanephrines and 3-methoxytyramine (3MT) as an alternative marker of functional activity of the tumors. Furthermore, after 2 to 6 weeks after recovery from surgery, it is also important to measure CgA in patients with normal preoperatively fractioned metanephrines and 3-methoxytyramine levels and elevated preoperatively CgA levels. Regarding long term follow-up, it is suggested to evaluate serum CgA every year in patients operated for pheochromocytoma or paraganglioma if they had no increase in fractioned metanephrines or 3MT preoperatively, together with the imagistic screening [17,18].

Furthermore, numerous studies found a positive interdependence between CgA and tumor volume in the adrenal medulla derived tumors [19] .

Moreover, malignant pheochromocytoma is defined as the presence of distant metastasis consisting in chromaffin cells in non chromaffinic organs, while the most frequent sites for metastases are liver,lungs, lymph nodes and bones. In cases of malignant tumors, CgA levels are 15 times higher, being accompanied by other predictive factors of neoplasia such as increased serum levels of 3MT and the presence of the SDHB gene mutation [20,21].

*Adrenocortical secretory adenomas and CgA levels*

Adrenal cortex is composed of specialized steroidogenic cells that produce mineralocorticoids, glucocorticoids and androgens. Depending on the origin cells, tumorigenesis can induce cellular hyperfunction determining specific clinical syndromes such as Cushing syndrome, Conn syndrome, virilization in case of woman patients or feminization in case of male patients.

It is well known that CgA is specific to the cromaffine cells, that are characteristic for the adrenal medulla area, but as mentioned above there may be islets of chromaffine cells also in the adrenal cortex. Therefore, serum measurements of CgA can be usefull in order to distinguish

the clinical causes of elevated cortisol levels, between hypophysis, adrenal or ectopic sources. Thus, it was reported that in case of a Cushing syndrome caused by an ectopic neuroendocrine secretion of ACTH , the serum levels of CgA are appreciably higher compared to the other cases.

Studies regarding plasma concentration of CgA in adrenocortical adenomas are contradictory. Some studies have reported that CgA is not expressed at immunohistochemical level and have not registered significantly elevated plasma levels associated with adrenocortical tumors, concluding that this molecule does not appear to be involved in cortical tumorigenesis [22, 23]. In opposition, other researchers showed a significant correlation between adrenocortical adenomas and elevated serum CgA levels, without associating them with immunohistochemical staining of the protein . In addition, they have noted higher serum CgA values for cortisol secreting adenomas, but further research is needed in order to elucidate this aspect. Furthermore, they concluded that elevated CgA levels are not a reliable biomarker of malignant potential of adrenocortical adenomas[17, 24].

CgA assesment were also evaluated as a reliable marker in estimating the selectivity in adrenal venous sampling in cases of primary aldosteronism. However, the conclusion have offered no support regarding the usefulness of using CgA instead of cortisol venous sampling for that pathology [25].

*Non-functioning adrenocortical tumors, adrenal incidentaloma and CgA*

Adrenal incidentalomas are characterized by an expected diagnosis of an adrenal tumor, frequently localised by imaging techniques as computed tomography, magnetic nuclear imaging or echography. These tumors can be hormonally functional generating specific symptomatology or non-functional , clinically silent. Most of the adrenal incidentalomas, approximately 80% of them, are benign non functional adrenal adenomas [26].

Once more, the data from the literature are contradictory. Several studies reported that patients usually do not have elevated serum CgA levels, especially in adrenal non functional incidentaloma [27]. Whereas, others sustain the opposite, namely that serum CgA levels of patients with non-secretory, benign cortical adenomas were with 38% higher than in healthy participants [17].

However, there is unanimity in one clinical aspect. Thus, for a patient who presents with an adrenal incidentaloma and with negative CgA serum values, it can be considered that there is a poor chance for diagnosing a neuroendocrine chromaffine tumor [28].

*Adrenal neoplasia and CgA levels*

Adrenocortical carcinomas are rare tumors of the adrenal cortical region that are recognized to have a unfavorable prognosis and a small survival rate, despite emerging therapeutic options. These tumors can be non-secretory and patients present with nonspecific mass effects symptoms or neoplasia related clinical signs, such as weight loss, asthenia, abdominal pain . However, the clinical picture can include signs and symptoms specific to hormonal abnormalities. The final diagnosis is based on the anatomopathological examination. In this context, immunohistochemical stains determine the evaluation and differential diagnosis of the adrenal neoplasia. Although in case of catecholamine producing tumors, CgA usually stains positive, in case of adrenal carcinoma , it has a negative staining [29]. However, positive immunohistochemical staining for CgA was reported in a case of adrenocortical oncocytoma [30].

Moreover, multiple studies stated that elevated circulating CgA levels did not represent a prognostic factor for neoplastic alteration of cortical adenomas. However, knowledge about this subject is scant in the medical literature, emphasizing the need for further studies [31].

## Conclusions

In this paper, we have highlighted current knowledge regarding the role of chromogranin A in various type of adrenal tumors.

Plasmatic dosage of CgA is recommended for diagnosis and monitoring of treatment and long-term evolution, both in digestive neuroendocrine tumors and also in pheochromocytomas.

Regarding nonsecretory adrenal incidentaloma, the negativity of serum samples for CgA is a good marker for the absence of a cromaffin tumor.

Future research on the exact biological roles of chromogranin A is needed, in order to establish its role in adrenal tumorigenesis.

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