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Chromogranin A and Neuroendocrine Tumors

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1. Introduction

Neuroendocrine tumors (NETs) are neoplasms that arise from cells of the endocrine and nervous systems. Many are benign, while some are cancers. They most commonly occur in the intestine, but are also found in the lung and in the rest of the body.

A neuroendocrine tumor is suspected when classical clinical symptoms occur but the large majority of NETs does not show any specific symptomatology (Oberg et al., 1999). Accordingly, the biochemical diagnosis is of great value, with the validation of radio-immunoenzymatic assays for various circulating peptide hormones in the last decade, clinical awareness and ability to diagnose NET is increased . However, due to the relative low incidence of NETs and the very large number of measurable hormones, clinicians need to know which measurable variables have an established clinical value and are cost effective (Giuffrida et al., 2006)

Neuroendocrine tumors can be functional and nonfunctional.

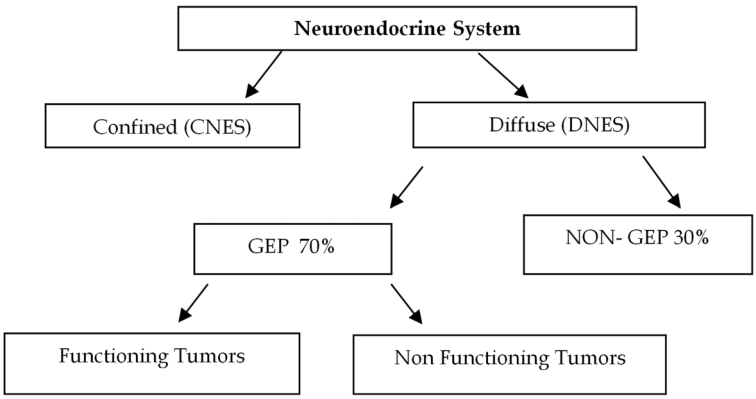

Fig. 1. Neuroendocrine System GEP: Gastroenteropancreatic

In the case of funtional NETs signs and symptoms include:

- Flushing of the face and neck (appearance of deep red color, usually with sudden onset)
- Diarrhea, nausea, vomiting, rapid heart rate

- Wheezing, coughing, difficulty breathing

2. Tumor markers

Symptoms that are exhibited in the functional NETs is related to the release of circulating hormones and peptides such as catecholamines, insulin, 5-hydroxyindoleacetic acid (5-HIAA), gastrin, calcitonin and others. Although there are many kinds of NETs, they are treated as a group because the cells of these neoplasms share common features, such as looking similar, having special secretory granules, and often producing biogenic amines and polypeptide hormones 5-hydroxytryptamine (5-HT) or serotonin is product by functional neuroendocrine tumors (NETs) originating from the midgut. Serotonin is a tryptophan-derived biogenic amine involved in smooth muscle contraction, blood pressure regulation and both peripheral and central nervous system neurotransmission. Approximately 2% of dietary tryptophan is converted into serotonin. Serotonin is synthesized and stored in enterochromaffin cells of the gastrointestinal tract (80% of total body serotonin content), in dense granules of platelets (storage only) and in the serotoninergic neurons of the central nervous system. The urinary breakdown metabolite of serotonin is 5-hydroxyindole acetic acid (5 - HIAA) which is particularly useful in the diagnosis and follow-up of NETs with carcinoid syndrome. Serum measurements of serotonin are possible in these patients; however, large individual variation makes them unreliable for diagnosis and in follow-up. Universally, 5-HIAA is the most frequently performed assay in the clinical setting of the carcinoid syndrome (O'Toole et al., 2009).

The generic markers of NETs are Neurone Specific Enolase (NSE) and Cromogranine A. Neurone-Specific Enolase is an useful immunohistochemical marker of NETs. Neverthelles ,its serum mesurament has not, except for patients with small cell lung cancer and neuroblastoma, because of relatively low sensitivity and specificity of the marker (Giovannella et al., 1999).

3. Chromogranin

Chromogranin A is an acidic glycoprotein expressed in the secretory granules of most normal and neoplastic neuroendocrine cell types, where it is released togheter with peptide hormones and biogenic amines. In humans, chromogranin A protein is encoded by the CHGA gene (Helman et al., 1988)

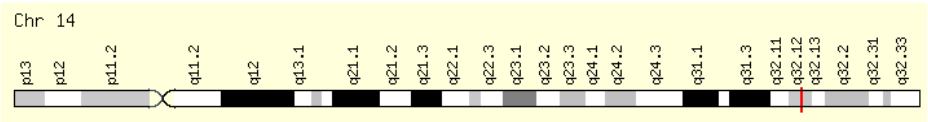

Fig. 2. CHGA structure

The chromogranin family consists of at least three different watersoluble acidic glycoproteins (CgA, CgB, and secretogranin II, sometimes called chromogranin C). Upon stimulation, CgA and other peptide hormones and neuropeptides are released. CgA is also secreted from neuroendocrine derived tumors including foregut, midgut and hindgut gastrointestinal NETs, pheochromocytomas, neuroblastomas, medullary thyroid carcinomas, some pituitary tumors, functioning and non-functioning pancreatic NETs and other amine precursor uptake and decarboxylation tumors.

Chromogranin A might promote the generation of secretory granules. Chromogranin A is the precursor to several functional peptides including vasostatin, pancreastatin, catestatin and parastatin. These peptides negatively modulate the neuroendocrine function of the releasing cell (autocrine) or nearby cells (paracrine). Other peptides derived from chromogranin A with uncertain function include chromostatin, WE-14 and GE-25.

Chromogranin A concentrations are normally low. An increased level in a symptomatic person may indicate the presence of a tumor but not what type it is or where it is. The quantity of CgA is not associated with the severity of the symptoms but with the mass and the functional activity of the tumor (Wu et al., 2000)

The possibility to measure Chromogranin A (CgA) plasma levels by means of radio- or immunoenzymatic assay represents a tremendous step forward in the management of patients with NETs.

3.1 Chromogranin: Laboratory test

Chromogranin can be dosed. The IRMA method is based on two monoclonal antibodies raised against the unprocessed central domain of the human CgA, allowing sensitive detection of total human CgA. Recombinant human CgA was used as calibrator and the standard curve concentration ranged from 22 to 1200 ng/ml, with a minimal detectable level of 10ng/ml. Inter-assay coefficients of variation were 3.4 and 4.5% at 124.7and 355.2 ng/ml, respectively. Intra-assay coefficients of variation were 5.1, 3.0, and 7.8% for the following ranges 15-25, 90-110, and 500-700ng/ml, respectively.

The ELISA assay is based on two polyclonal rabbit antibodies directed toward a 23 kDa carboxyl-terminal fragment of human CgA, therefore measuring more human CgA fragments . The calibrators were extracted from urine of patients with carcinoids and the standard curve concentraction ranged from 5 to 650 U/l, with a minimal detectable level of 5U/l. Inter-assay coefficients of variation were 3.4, 3.9 and 6.8 at 11.5, 52.7, and 358U/l, respectively. Intra-assay coefficients of variation were 4.5, 3.8 and 8.5% for the following ranges 5-10, 15-25 and 250-450 U/l, respectively (Zatelli et al., 2007). The three most commonly available employed assays for CgA measurement, has been compared in a group of NET patients and has been found that sensitivities vary between 67 and 93%, while specificities were 1 to 85% for all three (Stridsberg et al., 2003). A recent multicenter prospective comparison between two methods, immunoradiometric and ELISA, found a 36% clinical discordance rate. These results were mirrored with a difference of 5-fold inter-laboratory variation rate in a recent Italian study aimed at assessing CgA detection performance as applied to immunoradiometric and ELISA assays (Janson et al., 1997). A further prospective analysis underlined CgA to be a practical marker in patients with NET, however, with limited diagnostic power. A cut-off of 53 ng/ml for IRMA and 16 U/l for ELISA for discriminating between healthy controls and NET patients yielded only moderate sensitivities (71.3 and 83%, respectively) and specificities (71 and 85%, respectively).

3.2 Chromogranin related to net

The Chromogranin A test is used often as a tumor marker. It may be ordered in combination with or in place of 5-HIAA to help diagnose carcinoid tumors. It is also used to help monitor the effectiveness of treatment and detect recurrence of this tumor. Sometimes it may be ordered with specific hormones, such as catecholamines, to help diagnose and monitor a

pheochromocytoma. It may also be used to detect the presence of other neuroendocrine tumors, even those that do not secrete hormones . Plasma CgA levels (2-18 u/l) were found elevated in a variety of NETs, including pheocromocytoma, carcinoid tumors, pancreatic islet cell tumors, medullary carcinoma of the thyroid, small-cell lung cancer and so forth (Verderio et al., 2007).

Positive Cromogranin A related to Neuroendocrine Tumors:

- Gastroenteropancreatic NETs
- Anterior Pituitary tumors
- Parathyroid tumors
- Medullary Thyroid Carcinoma
- Merkel Cell Tumor
- Ectopic Adrenocorticotropic Hormone Producing Tumors
- Ganglioneuroma / Neuroblastoma
- Pheocromocytoma
- Small Cell Lung Cancer
- Prostate Cancer

Table 1. CgA and Neuroendocrine Tumors

The sensitivity and specificity of circulating CgA in any NETs vary between 70% and 95%. The highest accuracy has been observed in tumors characterized by an intense secretory activity, but its specificity and sensitivity remain very high also in non-functioning tumors.

Although CgA specificity cannot compete with that of the specific hormonal products secreted by many NETs, this molecule has very useful clinical applications in subjects with NETs for whom either no marker is available or the marker is inconvenient for routine clinical use generally, if concentrations of CgA are elevated prior the treatment and then fall, the treatment is likely to have been effective. CgA concentrations may be elevated but not monitored with conditions, such as liver disease, inflammatory bowel disease, renal insufficiency, and with stress. These possible causes for elevated CgA levels should be considered when interpreting test results, as false positive.

Overall CgA has been found to be clinically informative and moderately sensitive in the majority of studies devoted to this topic. CgA was found of a large mixed NET patient cohort, CgA was more sensitive than neurone-specific enolase (Baudin et al., 1998) . While performances have been limited in low-level cut-offs due to the overlap with control populations, very high levels of serum CgA are rarely found outside the setting of NETs with the exception of patients on gastric acid secretory blockers, especially PPIs (Sanduleanu et al., 2001) or those with hypergastrinaemia. Specificity of CgA in the diagnosis of NETs depends on the tumor type and burden (100% specificities have been reported in patients with metastatic disease), the quality of the control populations used and the cut-off values employed. Elevated CgA was found to be more sensitive than high urinary 5- HIAA levels in patients with metastatic midgut lesions (87 vs. 76%, respectively). A significant positive relation between the serum levels of CgA and the tumor mass in NETs, has been demonstrated; however, the distinction between high and low tumor volume may be open to question, infact, high CgA concentrations were found in all patients with gastrinoma, although tumor was small in volume (Nobels et al., 1997). In a mixed series of 128 patients with NET, increased CgA levels were found in 29% and 67% of patients with locoregional or

metastatic disease, respectively. Nonetheless, the prognostic value of CgA in patients with NET has not been confirmed to date.

False-positive elevation of CgA may occur in the following circumstances:

- Impaired renal function
- Parkinson disease
- Untreated hypertension
- Pregnancy
- Chronic atrophic gastritis (type A)
- Treatment with anti-secretory medications, expecially PPIs

Chronic elevation of gastrin levels provokes hyperplasia of the neuroendocrine cells of the stomach, and these cells are able to secrete CgA (D'Adda et al., 1990) . In patients with chronically elevated CgA and Zollinger Ellison Syndrome (ZES), has been demonstrated that the CgA concentrations can be normalized by gastrectomy alone, without resection of the gastrin producing tumor. A more recently described case report of false-positive CgA was due to the presence of heterophile antibodies (HAb), which can bind to animal antigens and may be present in up to 40% of the normal population (Levinson et al., 2007); in the CgA immunometric assays, HAb interferences may be circumnavigated by using a Habblocking tube.

CgA laboratory tests that have been developed and validated will all be slightly different, and their results will not be interchangeable. For this reason, if someone is having more than one CgA test performed (such as for monitoring), all test are sent to the same laboratory.

The very frequent elevation of CgA in patients with pheochromocytomas/paragangliomas confirms that it may be the marker of choice for these diseases, being more convenient than catecholamines either measured in plasma or in urine.

The highest CgA levels were noted in patients with metastatic carcinoid tumors and neuroendocrine carcinomas of gastrointestinal origin. Conversely, the lowest values were found in patients with advanced SCLC. Some data support the notion that CgA is less useful in undifferentiated neuroendocrine neoplasms (Blanco, 2007; Stivanello, 2011).

It is noteworthy that elevated plasma CgA levels cannot differentiate between neuroendocrine and non neuroendocrine neoplasms. Slightly elevated CgA levels, in fact, were identified in more than 40% of patients with advanced non-endocrine tumors, a proportion that was not so different from that of patients with SCLC (Nobels, 1997, Stivanello, 2001). The detection of elevated plasma CgA in non-endocrine tumors mainly indicates that there is a neuroendocrine differentiation and a proliferation of neuroendocrine cells at advanced stage of many carcinomas.

Drugs that stimulate secretion of neuroendocrine cells can lead to artifactual chromogranin A elevations. In particular, proton pump inhibitors (e.g., omeprazole), which are used in the treatment of esophageal and gastroduodenal ulcer disease and dyspepsia, will result in significant elevations of serum chromogranin A levels, often to many times above the normal range. If medically feasible, proton pump inhibitor therapy should be discontinued drug week of serum chromogranin A levels.

Chromogranin A and its peptide fragments are cleared by a combination of hepatic metabolism and renal excretion. In patients with significant impairment of liver or kidney function, serum chromogranin levels are often substantially elevated and single

chromogranin A measurements are uninterpretable. Serial measurements may have some value in selected patients if the disturbance in hepatic or renal function remains stable, but results must be interpreted with extreme caution. There is no universal calibration standard for serum chromogranin A assays. In addition, different chromogranin A assays, which use different antibodies or antibody combinations, will display different cross-reactivity with the various chromogranin A fragments. Therefore, reference intervals and individual patient results differ significantly between different chromogranin A assays and cannot be directly compared. Serial measurements should be performed with the same assay, or, if assays are changed, patients should be rebaselined. As with all immunometric assays, there is a low but definite possibility of false-positive results in patients with heterophile antibodies.

These antibodies are rarely found in the normal population, but are observed at increased rates in persons with autoimmune disease or after prior sensitization to rodent proteins (patients who have received diagnostic or therapeutic mouse monoclonal antibodies). Blocking reagents have been added to this assay to minimize the likelihood of heterophile antibody interference. However, test results that do not fit the clinical picture should always be discussed with the laboratory.

A "hook effect" can occur at extremely high chromogranin A concentrations, resulting in a lower measured chromogranin A concentration than is actually contained in the specimen. This is not expected to impact the utility of the assay for initial diagnosis, as levels will typically remain significantly above the reference range, even in the presence of hooking. However, hooking may complicate the interpretation of serial chromogranin A measurements in rare patients with extremely high levels. Normally it would be useful to dose dilute and remeasure all specimens >625 ng/mL to minimize the risk of this occurring. However, if there is the clinical suspicion of hooking, then retesting after further specimen dilution should be requested.

There are some pitfalls in the interpretation of CgA levels. Among them, renal impairment is one of the most important. All the patients with chronic renal failure presented very high levels of CgA, thus suggesting that serum creatinine should always be measured concomitantly with plasma CgA (Stridsberg et al., 2003)

Circulating CgA was found to be a reliable marker for the follow-up of patients with neuroendocrine tumors. CgA levels were with not evident disease (NED) , CgA levels were within normality. In advanced cases submitted to systemic treatment, a clear relationship was found between changes in CgA levels and disease response. This marker decreased in all patients showing a tumour shrinkage after cytotoxic treatment, increased in the great majority of patients showing progressive disease, and did not change in most cases depicting a disease stabilization. Discrepancies between tumor and biochemical changes in non-responding patients are attributable to the concomitant administration of somatostatin analog (Campana et al., 2007)

The correlation between CgA levels and tumor mass is lost during treatment with somatostatin so that CgA may not be used as a marker of tumor response when a cytotoxic regimen is administered in combination with a somatostatin analog (O'Toole et al., 2009).

4. Conclusion

Many data confirm the general view that CgA is the best circulating neuroendocrine marker available up to now. Its clinical application involves all differentiated NETs, irrespective of

tumor location and functional status. In gastrointestinal neuroendocrine tumours the measurement of general and specific markers offers important information for the clinician treating patients. This information is useful for the initial diagnosis and during the follow-up for monitoring patients with non functional disease and under medical treatment. Several of the markers are good prognostic markers for both carcinoid and pancreatic disease (Ardill & Erikkson , 2003).

This marker seems to be less useful in undifferentiated tumors such as Small Cell Lung Cancer. Elevated CgA plasma levels allow the identification of the coexistence of neuroendocrine differentiation in the context of non-endocrine malignancies and this could have diagnostic, prognostic, and possibly therapeutic implications. A dynamic evaluation of this marker in the follow-up of NETs provides useful information on the disease recurrence in NED cases or on the treatment efficacy in advanced cases submitted to cytotoxic or biologic therapy (Zatelli et al., 2007)

CgA: General Remarks and Assays

- Elevated CgA can occur in normal individuals and in patients with non-NET tumors although the levels are usually lower than in patients with NET
- CgA is the most practical and useful general serum tumor marker in patients with NET
- Sensitivity of elevated CgA varies according to NET tumor type and volume
- Reference laboratories should be preferred for clinical samples assays
- Reference intervals and individual patient results differ significantly between different chromogranin A assays and cannot be directly compared
- Serial measurements should be performed using the same assay
- If assays are changed, patients should undergo a new baseline measurement
- False-positive results are possible in patients with hypergastrinaemia (especially on anti- secretory medications or chronic atrophic gastritis type A) and in the presence of heterophile antibodies (care in patients autoimmune disease or those sensitized to rodent proteins (mouse monoclonal antibodies))
- Where possible, proton pump inhibitors should be interrupted, leaving a clearance of at least 3 half-lives, prior to CgA plasma sampling.

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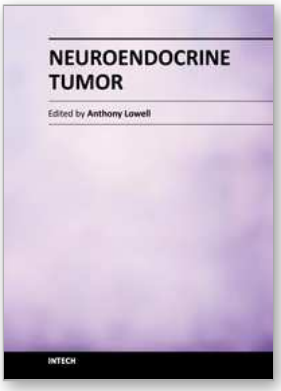

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