Original article

Impact of chromogranin A measurement in the work-up of neuroendocrine tumors

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Summary

Since the development of the first immunoassay for circulating chromogranin A in 1984, a lot of studies have evaluated its clinical impact in neuroendocrine tumors. Initially studied in pheochromocytoma patients, the clinical impact of chromogranin A has rapidly extended to most neuroendocrine tumors, sometimes in combination with other eutopic or ectopic secretions. In our experience, CgA demonstrates a variable sensitivity between NET primary and a high specificity. Our results suggest that CgA should be routinely screened in foregut-derived NET and abandoned in the routine screening of medullary thyroid carcinoma. In addition, in phaeochromocytoma and ileum-NET patients, CgA demonstrates a comparable sensitivity with urinary reference markers and its impact on the follow-up will form a key point when recommending routine screening. Both tumor burden and secretory activity should be taken into account when interpreting CgA results.

Key words: chromogranin A, neuroendocrine tumors

Neuroendocrine tumors (NET) constitute a tumor network scattered in the body. They are characterized by common features including secretion of hormones, association as part of hereditary syndrome, impact of functioning imaging and, in one subgroup, poor aggressiveness. Secretion of hormones is a main feature of neuroendocrine tumors (NET) with multiple consequences for the diagnosis and prognosis of these tumors. Since 1984, the chromogranin A (CgA), a glycoprotein of the core of storage vesicles, has emerged as a potential general marker of these tumors [1]. CgA plays major roles in the storage and secretion of several hormones within the neuroendocrine cell vesicles. Furthermore, its impact on the pathological diagnosis of NET is well established [2]. This review article summarizes our experience with CgA measurement in the diagnostic biological work-up of NET.

Sensitivity of CgA

The methods used for CgA measurement in body fluids are a major concern when analyzing literature results. Indeed, CgA glycoprotein is considered as a preprohormone with multiple proteolytic sites allowing production of multiple peptides with various physiological function [3]. To date, competitive immunoassays based on polyclonal antibodies for CgA measurement have been used in most studies [4]. These assays are able to detect various molecular proteolytic forms of CgA as well as derived peptides. Recently, a ‘two-site sandwich’ immunoradiometric assay was developed which uses monoclonal antibodies directed against the median domain of CgA. This immunoassay is dramatically less affected by proteolysis [5]. The different strategies used for CgA measurement may partly explain the discrepancies between previous studies.

When considering the complete NET network, review of the literature suggests that the sensitivity of CgA varies between less than 10% and 100%, depending on the primary and tumor burden.

Indeed, a high prevalence of CgA secretion (above 80%) was described in pheochromocytoma [6-11], neuroblastoma [8, 12, 13] or gastrinoma [14], an intermediate one (above 60%) in ileum-NET patients, CgA demonstrates a comparable sensitivity with urinary reference markers and its impact on the follow-up will form a key point when recommending routine screening. Both tumor burden and secretory activity should be taken into account when interpreting CgA results.

On the other hand, a positive correlation between tumor burden and CgA levels was found in pheochromocytoma [6, 7], neuroblastoma [12, 13], medullary thyroid carcinoma (MTC) [19], small cell lung carcinoma [17, 18] and ileum NET [11, 15]. In our previous study...
of 128 NET patients of various origin, we found increased CgA levels in 29% and 67% of patients with locoregional or metastatic NET, respectively [15].

In order to progress in the understanding of increased CgA levels in NET patients, we investigated whether the CgA level was related to the tumor burden or tumor secretory activity in a group of 128 NET patients, including MTC, phaeochromocytoma, ileum, foregut-derived NET, including mainly, bronchial and non-functioning pancreatic NET primaries [15]. Of note, few patients with functioning pancreatic NET were enrolled and those with small cell lung tumor and neuroblastoma were not included. We demonstrated that both parameters were independently associated with CgA levels. In fact, CgA levels were increased in 26% and 73% of NET patients demonstrating an absence or presence of other secretion. When both parameters were taken into account, it appeared that in the patients with locoregional disease, CgA levels were increased in 41% (7 of 17) of patients with other secretions, compared to 10% (1 of 10) in the absence of other demonstrable secretion. Concerning patients with extensive disease, CgA levels were increased in 81% (59 of 73) of patients with other secretions, compared to 36% (10 of 28) in the absence of other demonstrable secretions. Finally, when the type of secretions was analyzed, only biogenic amine and peptidic, but not glycoprotein secretions were found to be significantly associated with elevated CgA levels. From a clinical point of view, these data suggest that: (1) both the tumor burden and the secretory activity of NET should be taken into account when analyzing CgA results; (2) CgA sensitivity is low in non-functioning locoregional NET; (3) finally, the influence of NET primary on CgA sensitivity may be partly explained by the NET secretory activity.

CgA sensitivity compared to other NET biological secretions

A wide spectrum of secretions of so-called 'eutopic or ectopic' hormones has been reported in NET depending on each primary [21]. In order to assign a definite role of CgA in the biological work-up of NET, its sensitivity should be compared with reference markers of each NET primary. Furthermore, CgA sensitivity should be compared to another general marker of NET: the neuron-specific enolase (NSE).

We first demonstrated that the sensitivity of CgA was higher than that of NSE in all subgroups of NET patients we studied (Figure 1) [15]. CgA and NSE displayed a 59% and 38% sensitivity, respectively. Furthermore, our findings did not support NSE as a direct marker of tumor burden and NSE levels fluctuated in half of the patients during follow-up (Figure 2). NSE was therefore dropped out from the routine biological screening of well-differentiated NET.

Our results concerning the comparison between CgA sensitivity and more specific biological markers of each NET primary, are updated in Figure 3 and can be summarized in three main groups:

Firstly, NET in which CgA sensitivity is higher than that of the reference biological markers: this situation is observed in foregut-derived NET including bronchial, thymic, head and neck primaries. CgA sensitivity was comparable to the sensitivity of the three sensitive biological markers analyzed together. These three markers, 5-hydroxyindolacetic acid (5HIAA), calcitonin (CT) and glycoprotein alpha subunit (GPα), were demonstrated to be the most sensitive in this group of patients in a previous study [21]. Moreover, in the subgroup of non-functioning pancreatic NET, CgA sensitivity was higher than 5-HIAA, CT and GPα analyzed together. The update of our results gives a sensitivity of 57%, 48%, 28% and 32% for CgA, 5-HIAA, CT, and GPα respect-
Figure 2. Evolution of circulating levels of CgA, NSE and GPa in a 54-year-old woman with progressive liver metastases of a non clinically-functioning pancreatic NET. * Levels divided by ten, ° levels below the normal value [15].

Figure 3. Comparison of sensitivities in 177 NET of CgA (CgA; CGA-RIACT, CIS Bio International, normal < 100 µg/l) and Calcitonin (CT) (ELSA-hCT, CIS-Bio International; normal, <10 pg/ml), 24-hour urinary metanephrines (HPLC; metanephrine, normal < 400 nmol/mmol creatinine; normetanephrine, normal < 500 nmol/mmol creatinine), glycoprotein hormone α-subunit (GPa; house specific assay; normal, men and premenopausal women < 1 ng/ml, postmenopausal women < 3 ng/ml) and 24-hour urinary 5-hydroxyindolacetic acid (5-HIAA reagent kit, Bio-rad, Munich, Germany; normal, <42 umol/24h or <10 umol/mmol creatinin/24h). MTC: medullary thyroid carcinoma, phaeo: eutopic or ectopic phaeochromocytoma, FG: foregut-derived NET excluding pancreatic NET; NF-Pa NET: non-functioning pancreatic NET; MG: midgut-derived NET. In MTC, phaeo. and midgut-NET CgA sensitivity is respectively compared to CT, 24-h urinary metanephrines and 24-h 5-HIAA. In FG and NF-Pa NET CgA sensitivity is compared to CT, GPa and 24-h 5-HIAA results analysed together. Results of different biological markers are analysed as positive or negative and compared with CgA results measured at the same time.

Inforegut-derived NET (excluding non-functioning pancreatic NET). In non-functioning pancreatic NET, a 69%, 5%, 9% and 35% sensitivities for CgA, 5-HIAA, CT, and GPaα respectively, were found. Few functioning pancreatic NET were enrolled in our study and the results are not given here. Other studies have demonstrated a high sensitivity of CgA in gastrinoma but a low sensitivity in insulinoma. Our data suggests that CgA is the single most sensitive biological marker in foregut-derived NET.

Secondly, NET in which CgA sensitivity is comparable to that of the reference biological markers: CgA sensitivity was similar to that of 24-hours-urinary 5-HIAA in ileum-derived NET and 24 hours-urinary metanephrines in phaeochromocytoma. It should be noted that our phaeochromocytoma included both eutopic and
ectopic tumors which may explain the lower sensitivity of CgA compared to previous studies. The interest of CgA as a follow-up marker of these NET may be the key point in order to decide the routine screening of CgA. Indeed, serum CgA measurement is more convenient than repeated 24-hours urinary collections.

Finally, NET in which CgA sensitivity is lower than the reference biological markers: this situation was found in the medullary thyroid carcinoma in which calcitonin sensitivity is much higher than that of CgA-precluding routine CgA measurement in these patients (Baudin et al. submitted).

**Specificity of CgA**

The specificity of CgA varies between 68% and 100% depending on the upper threshold of the normal range and of the control group. In our preliminary study, we found a 68% specificity of CgA studying patients with other non-neuroendocrine tumors as a control group. Using a cut-off value fixed at 160 μg/l, CgA attained a specificity of 95% but sensitivity then fell to 38% [15]. Renal insufficiency and hypergastrinemia are the main causes of false positive CgA results [4, 22]. A major concern is increased CgA levels associated with mixed tumors [11]. This hypothesis should always be raised, especially when the clinical history is not typical of NET.

In conclusion, CgA demonstrates a variable sensitivity among NET primary and a high specificity. Our results suggest that CgA should be routinely screened in foregut-derived NET. In addition, in phaeochromocytoma and ileum-NET patients, CgA demonstrates a comparable sensitivity to 24-hour urinary metanephrine and 5-HIAA measurements, respectively, and may be useful as a follow-up marker in these patients. Both tumor burden and the secretory activity should be taken into account, when interpreting CgA results.

**References**


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