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# Serum Chromogranin-A Assay in Differential Diagnosis of Incidentally Discovered Adrenal Masses

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**Abstract.** *Adrenal incidentalomas are defined as asymptomatic adrenal masses occasionally discovered during high-resolution imaging procedures such as computed tomography (CT) or magnetic resonance (MR). Pheochromocytoma, a potentially lethal chromaffin tumour, must be excluded before any invasive diagnostic procedures to avoid massive catecholamines release. Chromogranin A (CgA) is a member of the granin family contained in secretory vescicles of chromaffin adrenal cells. Consequently, serum CgA increases in patients affected by pheochromocytoma and other diseases of the chromaffin system. This study investigated the performance of serum CgA assay in diagnosis of pheochromocytoma among patients affected by adrenal incidentaloma. Additionally, we evaluated the role of the CgA assay in selection of patients for $^{123}$I-metaiodobenzylguanidine (MIBG) scintigraphy, a very accurate but high-cost and time-consuming imaging procedure. We enrolled 104 patients affected by adrenal incidentally discovered masses and 100 healthy blood donors as controls. Serum CgA was assayed by a specific immunoradiometric method (IRMA) and $^{123}$I-MIBG scan was performed in all patients. A cytological or histological diagnosis was obtained in all cases. Circulating CgA assay was positive in 12 out of 12 patients with pheochromocytoma and negative in 92 out of 92 patients with non-chromaffin adrenal nodules. Serum levels of CgA clearly increased from blood donors and patients with non-chromaffin adrenal nodules to patients with pheochromocytoma ($p<0.0001$). All patients with negative CgA assay showed a negative $^{123}$I-MIBG scan. Serum CgA assay is effective in evaluating the presence of chromaffin tumour among patients with adrenal incidentaloma. A negative serum CgA assay rules out successive $^{123}$I-MIBG imaging.*

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Adrenal incidentalomas are defined as asymptomatic adrenal masses occasionally discoverd during high-resolution imaging procedures such as computed tomography (CT) or magnetic resonance (MR). Most adrenal nodules are benign and do not secrete clinically significant amounts of hormones: however, in some cases, definitive cytological or histological diagnosis is required to exclude malignancy (1).

Pheochromocytoma is a rare, potentially lethal, catecholamines-producing tumour derived from adrenomedullary chromaffin cells. Consequently, pheochromocytoma must be rapidly treated if present and must be excluded before any invasive diagnostic procedures to avoid massive catecholamines release after puncture or manipulation of the adrenal gland (2).

The screening of pheochromocytoma in patients with adrenal incidentaloma is traditionally based on 24-hour urinary cathecolamines, metanephrines (MNs) and vanillymandelic acid (VMA) assay (3). However, a lot of substances and drugs, as well as physical exertion or stress, can give false-positive results. Additionally, very accurate urine collection and conservation are mandatory and dedicated HPLC technology is required (4). Due to the relatively low specificity of urine markers, most patients underwent accurate but high-cost and time-consuming chromaffin-cell scintigraphy by $^{123}$I-metaiodobenzylguanidine ($^{123}$I-MIBG) to confirm/exclude the presence of a chromaffin tumour (5).

Human chromogranin A (CgA), a 48-kDa protein encompassing 439 amino acids, distributed in large dense core granules of neuroendocrine cells, was initially recognized in adrenal medullary catecholamine storage vesicles (6, 7). Consequently, circulating CgA increases in patients affected by pheochromocytoma and paragangliomas, as well as in other neuroendocrine tumours (8, 9). Recently, we found that CgA immunoradiometric assay (IRMA) correctly identified 15 out of 15 pheochromocytoma patients showing higher diagnostic accuracy than urine markers. A significant relationship was found between tumour weight and serum CgA ($r=0.908$, $p<0.0001$). The CgA assay also detected 3 asymptomatic non-functioning pheochromocytomas and ruled-out pheochromocytoma in 100% of patients affected by essential hypertension

Table I. *Pathological results.*

| Diagnosis | n° |
|---|---|
| - Pheocromocytoma | 12 |
| - Non-chromaffin | 92 |
| Adenoma *(cortisol)* | 28 |
| Adenoma *(aldosteron)* | 6 |
| Adenoma *(androgens)* | 2 |
| Adenoma *(non-functioning)* | 19 |
| Hyperplasia | 26 |
| Adrenal TBC | 3 |
| Metastasis | 8 |

and non-chromaffin adrenal incidentaloma (10, 11). The present study was undertaken to evaluate the diagnostic sensitivity and specificity of CgA IRMA assay in diagnosis and exclusion of pheochromocytoma among patients affected by asymptomatic adrenal incidentaloma and to compare serum CgA and $^{123}$I-MIBG scan.

## Patients and Methods

*Patients.* We enrolled 104 consecutive patients (57 males, 47 females; age: mean 48 years, range: 18-76 years) affected by adrenal incidentaloma (diameter>20 mm) discovered by abdominal CT or MR and candidates for fine-needle biopsy or surgery.

All patients underwent $^{123}$I-MIBG imaging as well as serum CgA assay before biopsy or surgery. Thirty-two patients were submitted to surgical removal of the affected adrenal gland and 72 to adrenal CT-guided fine-needle aspiration biopsy (FNAB). The diagnosis was confirmed by a pathologist particularly involved in the endocrine pathology field: chromaffin-cell-derived tumours were found in 12 cases: no patients showed either histological signs of malignancy or micro-vascular or capsular invasion. In the remaining 92 cases, cytological or histological diagnosis excluded chromaffin-cell-derived lesions (Table I).

One hundred healthy blood donors (48 males, 52 females; age: mean 29 years, range: 18-56 years) were employed as controls.

*Blood sampling and CgA assay.* The blood sampling was performed after fasting overnight, in rest conditions. Serum was separated and stored at -20°C until the assay (mean 3 days, range 1-8 days). Three months after surgery, serum CgA was re-evaluated in all patients affected by pheochromocytoma.

Circulating CgA was measured in duplicate by the CGA RIA CT® immunoradiometric method (Schering-Cis BioInternational, France) involving two monoclonal antibodies against the sequence 145-245 of the molecule. Assay of CgA should be carried out directly on serum or plasma: in the latter case, values will be consistently higher (12). In our laboratory, CgA assay is performed on serum. The CGA RIA CT® method proved to have analytical sensitivity of 2.1 ng/mL and showed intra- and inter-assay imprecision between 0.06-0.08 and 0.06-0.11, respectively, in the range of concentrations between 39 and 280 ng/mL (13). Quality control was ensured by assaying two levels of control sera in each series and by re-assessing all sera showing a CV exceeding 10%.

Table II. *Distribution of serum CgA in incidentaloma patients [pheo vs non-pheo] [Mann-Whitney U-test].*

| | Controls (100) | Non-chromaffin (92) | Pheochromocytoma (12) |
|---|---|---|---|
| CgA (ng/mL) | 36 (25 – 74) | 44 (24 – 85) | 395 (174 – 1230) |
| Mann-Whitney | | ns | p<0.0001 |

Patients and controls presenting serum creatinine concentrations exceeding 180 μmol/L and serum bilirubin exceeding 50 μmol/L, or taking proton-pump inhibitors and steroids, were excluded to avoid aspecific CgA increase.

*$^{123}$I-MIBG scintigraphy.* Whole-body and abdominal planar images, as well as single-photon emission tomography (SPET), were acquired 6 and 24 hours after *i.v.* administration of 370 MBq of $^{123}$I-MIBG by a large-field of view dual-head gamma camera equipped by medium energy parallel-hole collimators.

*Statistics and cut-off selection.* Statistical analysis was performed assuming non-parametric distribution and the Mann-Whitney *U*-test was employed to compare markers levels in patients and controls. A $p$ value less than or equal to 0.05 was considered statistically significant. The cut off-value of 100 ng/mL was selected at a specificity of 99% in healthy blood donors group.

*Ethics.* Imaging studies and serum sampling were performed in accordance with the regulations of the local ethics committee. Informed consent was obtained from each patient and control subject.

## Results

*Diagnostic performance and serum CgA distribution.* The serum CgA level increased in 12 patients affected by pheochromocytoma, while it remained in the normal range in 92 patients with non-chromaffin nodules. Serum CgA significantly increased from controls and patients with non-chromaffin adrenal nodules (ns) to patients affected by pheochromocytoma ($p<0.0001$) (Table II).

The $^{123}$I-MIBG scintigraphy was positive in 12 patients affected by pheochromocytoma and negative in all 92 cases of non-chromaffin-cell-derived tumours. No patient with pheochromocytoma showed extra-adrenal involvement (Table III).

## Discussion

Measurement of 24-hour urinary catecholamines and their metabolites are the routine methods for the diagnosis of the

Table III. *$^{123}$I-MIBG scan result and CgA serum levels in patients affected by pheochromocytoma.*

| Patients | $^{123}$I-MIBG scintigraphy | CgA | Diagnosis |
|---|---|---|---|
| 1 | positive | 236 | pheo |
| 2 | positive | 550 | pheo |
| 3 | positive | 384 | pheo |
| 4 | positive | 196 | pheo |
| 5 | positive | 1230 | pheo |
| 6 | positive | 398 | pheo |
| 7 | positive | 290 | pheo |
| 8 | positive | 448 | pheo |
| 9 | positive | 204 | pheo |
| 10 | positive | 324 | pheo |
| 11 | positive | 174 | pheo |
| 12 | positive | 762 | pheo |

pheochromocytoma (14). However, high accuracy is required in the pre-analytical phase and aspecific causes can falsely increase urinary levels of catecholamines and metabolites (15-17). Recent studies focused on the diagnostic relevance of the increase of free MNs in plasma, due to enhanced intra-tumoral metabolism of catecholamines: this approach requires an accurate control of the environmental circumstances of sampling and dedicated assay technology (18-20).

Chromogranin A is widely expressed in adrenal medulla and its circulating levels are increased in patients affected by pheochromocytoma (21). We previously obtained a 100% sensitivity and 96% specificity by employing the IRMA CgA assay to differentiate patients with pheochromocytoma from patients with essential hypertension and found a linear relationship between pheochromocytoma mass and serum CgA levels (10). In the present study, we found that the CgA assay correctly detected 12 chromaffin-tumours among 104 adrenal incidentally discovered nodules and correctly predicted the $^{123}$I-MIBG scan results in all cases. Our data are also in very good agreement with those of d'Herbomez *et al.*, who found a sensitivity of 0.90 and a specificity of 0.92 in 89 patients submitted to $^{131}$I-MIBG scan to confirm (41 cases) or refute (48 cases) the diagnosis of pheochromocytoma (22).

We did not perform plasma MNs assay and this constitute a major limitation of our work: on the other hand, MNs assay is not always available and requires adequate technology and very accurate pre-anaytical control. In this instance, further comparative evaluations in larger number of patients are needed to evaluate whether serum CgA assay is a reliable alternative to plasma metanephrine assay in diagnosis and, particularly, in ruling out pheochromocytoma.

Hovewer, in our opinion a negative CgA assay in patients affected by adrenal incidentally disovered masses can safely reassure the clinician about the absence of chromaffin-tissue disease and avoid further examination by $^{123}$I-MIBG scan.

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