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Letter to the Editor

Biological variation of plasma chromogranin A

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Chromogranin A (CGA) is an acidic protein composed of 439 amino acids, present in chromaffin granules of the neuroendocrine cells. CGA acts as a pro-hormone, which produces, after proteolytic cleavage, biologically active peptides that have different paracrine and autocrine functions (1).

The presence of CGA in cancer cells is related to the neuroendocrine origin of the tumor.

The use of the circulating CGA assay was first reported in the management of pheochromocytoma, then rapidly extended to other neuroendocrine cancers, particularly in carcinoid tumors, also showing satisfactory sensitivity and specificity in biochemically inactive neuroendocrine tumors (1).

Information on the biological variation of CGA is lacking, despite the importance of establishing both the analytical quality specification and the critical differences between serial measurements in monitoring of the disease.

Venous blood was obtained from 22 healthy subjects (10 male and 12 female; age range 41–60 years) once a month for 4 months (5 specimens per subject). Samples were collected by the same phlebotomist using vacuum collection tubes. Plasma-EDTA specimens, separated by centrifugation at 1500 *g* for 10 min, were stored at −80°C until analysis. All the specimens of the same subject were analyzed in duplicate in a single run.

CGA was assayed by a two-step immunoradiometric assay (CGA-RIA CT, CIS-Shering, Gif-sur-Yvette Cedex, France). The manufacturer's claimed within-assay and between-assay imprecision ranging from 2.2 to 6.0% and from 5.3 and 8.5%, respectively.

The analytical (CV_A), within-subject (CV_I) and between-subject (CV_G) components of variation were calculated by nested analysis of variance (2). The analytical goal for imprecision ($CV_A < 1/2CV_I$), indices of individuality (CV_I/CV_G) and critical differences required for significant ($p < 0.05$) changes in serial results [$2.77\ (CV_A^2 + CV_I^2)^{\frac{1}{2}}$] were also estimated (Table 1).

Table 1 Components of biological variation of chromogranin A and estimated statistical indices.

Mean value (range)	83 ng/ml (49–122 ng/ml)
CV_A*	2.7%
CV_I	12.8%
CV_G	26.3%
Desired analytical goal	6.4%
Index of individuality	0.49
Critical difference **	38.4–42.6%

*Intra-assay, **calculated using as CV_A the manufacturer's claimed range of between-assay imprecision (5.3–8.5%).

No significant differences were found between men and women (data not shown).

From the within-subject biological variation we can state that a between-assay coefficient of variation cannot exceed the minimum analytical performance of 9.6% ($\leq 0.75\ CV_I$,) (3). Nevertheless, apart from particularly low levels of CGA (<30 ng/ml), for which the CV was 8.5%, the between-assay imprecision both claimed by the manufacturer and evaluated from the results of our internal quality control at a level approaching the reference limit (mean 150 ng/ml, CV 5.2%) showed a better performance, remaining within the desirable analytical imprecision of 6.4% ($\leq 0.5\ CV_i$).

The high individuality of CGA (I.I:0.49) suggests that the reference interval could be of limited importance in the interpretation of the results, at least when serial specimens are evaluated. Data from biological variation allow for the determination of an objective value of critical difference. On this basis, a change of about 40% (Table 1) should be considered as significant ($p < 0.05$) in the comparison of serial results. This criterion could be useful in the evaluation and the possible improvement of the correlation of CGA levels with the response to treatment (4).

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