Validation of Serum Versus Plasma Measurements of Chromogranin A Levels in Patients With Carcinoid Tumors

Lack of Correlation Between Absolute Chromogranin A Levels and Symptom Frequency

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Objective: Chromogranin A (CGA) levels are used to confirm the diagnosis and monitor the course of patients with neuroendocrine tumors. Chromogranin A levels are significantly reduced when patients are acutely treated with octreotide; however, limited data are available that correlates octreotide long-acting repeatable (LAR) dose or steady state octreotide blood levels to the absolute value of serum or plasma CGA.

Methods: Plasma, serum, and clinical information on carcinoid syndrome symptoms were collected anonymously from 40 patients treated with long-term octreotide LAR therapy for carcinoid syndrome.

Results: We found a strong positive linear relationship exists between serum and plasma CGA levels ($r = 0.9858$, $P < 0.0001$). No correlation existed between plasma octreotide levels or LAR dose and the static, absolute plasma/serum CGA levels. Although, higher mean CGA values were seen in the group whose diarrhea was "not under optimal control" than for the group "under optimal control," these results did not reach statistical significance ($P = 0.24$). Contrary to our hypotheses, a statistically significant inverse relationship was found between the frequency of flushing and the CGA levels ($P = 0.0372$). Higher mean CGA values were observed in the "under optimal control" group with flushing symptoms.

Conclusions: Either serum or plasma can be used to measure CGA levels. Absolute (static) CGA levels do not positively correlate with symptom intensity during LAR therapy. Dynamic (serial) measurements of CGA are necessary to monitor the effectiveness of medical or surgical therapy.

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Chromogranin A (CGA) is a major component of the secretory granules found in neuroendocrine tumor cells and is involved in granulogenesis. Chromogranin A acts as a prohormone and is degraded into a variety of active peptides. The serum concentration of CGA has been shown to reflect the mass of neuroendocrine tumors.[1–5] Because serum levels of CGA closely correlate with tumor burden in patients with neuroendocrine tumors, especially in those with carcinoid tumors, serial CGA measurements are often used to confirm the diagnosis and subsequently monitor the clinical course and outcome of treatment in patients with neuroendocrine tumors.[6–10] Little is known about the relationship of the absolute (static) value of CGA to the volume of tumor in an individual or the intensity of the symptoms of carcinoid syndrome.

Treatment of neuroendocrine tumors with the long-acting somatostatin analog (octreotide long-acting repeatable [LAR]) has been shown to relieve symptoms produced by carcinoid syndrome including wheezing, diarrhea, and flushing.[11] Studies have also shown that dynamic changes in plasma CGA levels occur when patients are acutely treated with octreotide; however, no data are available that correlates chronic octreotide LAR dose or steady state octreotide blood levels to the static or absolute serum/plasma CGA levels.[1] We hypothesized that the absolute CGA serum/plasma levels in patients treated with long-term octreotide LAR at various doses should negatively correlate with the octreotide LAR doses and plasma octreotide levels. We also hypothesized that the absolute serum/plasma CGA levels should positively correlate with symptoms of carcinoid syndrome.

MATERIALS AND METHODS

Patient Information

Patients volunteering for this study were part of an internet support group for patients with carcinoid tumors (www.yahoo.com carcinoid group). Subjects' heights and weights were obtained. Patients also provided information

regarding specific symptoms of their carcinoid syndrome (flushing, diarrhea, and wheezing), frequency of symptoms (number of episode per day and the number of days per week), and severity. Aqueous octreotide "rescue" usage patterns were recorded, including the number (and dose) of rescue shots used per week. To test these hypotheses, 52 patients receiving stable doses (3 months or longer) of octreotide LAR for control of symptoms associated with carcinoid tumors volunteered for this study. Forty of these patients provided information on their symptoms, their monthly dose of octreotide LAR and information on the frequency and dose of rescue (aqueous) octreotide injections. Patient information was sent to an independent analyst who coded the questionnaire and individual serum/plasma specimen containers. These coded materials and the collection containers were sent to the patient along with a prepaid mailer, which ensured that no patient identifiers were included on the mailing labels. All blood specimens were drawn immediately before the patients' next LAR injection. Thus, all levels are trough levels. After the collection of the blood specimens, the mailers were express mailed to the laboratory at ambient temperature. We had previously determined that both octreotide and chromogranin are stable in serum or plasma at ambient temperatures. All patient identifiers were kept confidential from the investigators and laboratory performing the CGA and octreotide assays; code numbers were used to identify patients and to link patient questionnaires with laboratory values. Individuals also provided serum and plasma samples for CGA and octreotide acetate assays. Plasma and serum CGA levels, as well as plasma/serum octreotide acetate levels, were measured by highly specific and sensitive radioimmunoassays developed at Inter Science Institute (Inglewood, Calif).[11] This data/specimen collection scheme was discussed with the Louisiana State University Health Sciences Center's Institutional Review Board who concurred with the study design and allowed the study to be done without obtaining individual patient consents.

Chromogranin A Assay Information

Plasma and serum CGA levels are measured by a 2-site chemiluminescence immunometric assay using 1 biotinylated monoclonal antibody clone no. 5E8 (University of California at San Diego, Calif) conjugated with biotin and the other monoclonal antibody labeled with an acridinium ester ACE (10X) clone no. 3H 12 (University of California) and an avidin-coated bead.[12] The immunosandwich is bound to the bead by the avidin-biotin interaction, and the nonbound materials are washed away. The bound acridinium ester is detected in a luminometer using triggering reagents that cause light to be produced. The amount of light emitted (relative light units) is proportional to the amount of CGA in the sample. Plasma/serum octreotide acetate levels were measured by highly specific and sensitive radioimmunoassays developed at Inter Science Institute (Inglewood, Calif).[11]

Limits of Detection and Limits of Quantification, Interassay, and Intra-Assay Variations in CGA Assays

The limit of detection and the limit of quantification of plasma CGA in this assay is less than 2 ng/mL. Interassay and intra-assay variation studies were undertaken at CGA concentrations of 8.5 ng/mL, 20.5 ng/mL, and 75.4 ng/mL. Intra-assay variation at these levels were 3.6%, 3.8%, and 5.6%, respectively (n = 10 each). Inter-assay variations at these levels were 0.6%, 1.6%, and 7.8%, respectively (n = 32 each). Normal values were determined from noncarcinoid patient specimens and commercially obtained normal plasma and serum.

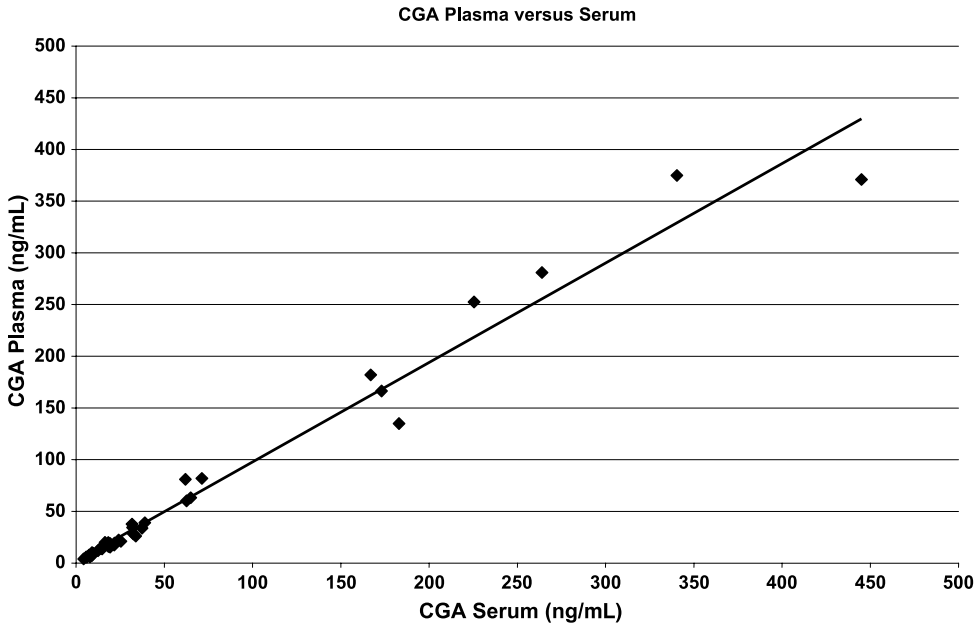

FIGURE 1. This graph represents the results of the regression analysis of serum versus plasma CGA values ($r = 0.9858$, $P < 0.0001$).

Linearity and Parallelism

Plasma samples were serially diluted, and the expected versus observed CGA levels were determined. Dilutions (n = 4 each) were performed at 1:1, 1:2, 1:4, 1:8, 1:16, and 1:32. Regression analysis demonstrated a high degree of correlation ($r^2 = 0.9993$, $P = 0.0001$).

Recovery of CGA from Spiked Samples of Plasma

Recovery experiments demonstrated that essentially all of the CGA added to serum or plasma specimens was recovered in this assay. Chromogranin A concentrations tested included specimens spiked with 0, 25, 50, 75, and 100 ng/mL (n = 4 each). Observed versus expected CGA values were tested by regression analysis ($r^2 = 0.9997$ and $P = 0.0001$).

Statistics

A simple linear regression was performed to test for a correlation between serum CGA levels and plasma CGA levels. To test for a difference between the various group mean values of CGA serum and plasma, nonparametric median tests were used for 2 group comparisons and Brown-Mood k-sample median tests were used for 3 or more groups. Nonparametric tests were used because of the small sample sizes in some groups and the skewed nature of the data. LAR dose groups were categorized into 3 groups corresponding to LAR dose levels of 20, 30, and 60 mg. Trough aqueous octreotide plasma level groups were categorized into 4 groups corresponding to plasma levels less than 2000 pg/mL, between 2000 and 5000 pg/mL, between 5001 and 10,000 pg/mL, and more than 10,000 pg/mL. For diarrhea and flushing, good symptom control was categorized as 14 or fewer episodes per week (averaged across weeks) and poor symptom control greater than 14 episodes per week. Fourteen or less episodes of diarrhea or flushing per week were classified as "under control," whereas more than 14 episodes per week were classified as "not under control." To test for a relationship between LAR dose levels and carcinoid symptom control, the Fisher exact test was used. Tests for wheezing were not performed because only 3 patients indicated experiencing any wheezing episodes during the study period. One subject from the LAR-20 group indicated experiencing an average of 5 wheezing episodes per week; one subject from the LAR-30 group indicated experiencing an average of 7 wheezing episodes per week; and one subject from the LAR-60 group indicated experiencing an average of 1 wheezing episode per week.

RESULTS

The results of the regression analysis for trough serum and plasma CGA levels revealed a strong positive linear relationship between these 2 measures ($r = 0.9858$, $P < 0.0001$). Every unit (ng/mL) increase in the CGA serum level resulted in a corresponding increase of one unit in the CGA plasma level (Fig. 1). F1

Contrary to our hypothesis, mean absolute CGA values for the 3 LAR dose groups, LAR-20, LAR-30, and LAR-60 (mg/mo), demonstrated a general increasing trend in CGA serum and plasma levels with increasing LAR dosage; however, these results did not reach statistical significance for either CGA serum or plasma levels ($P = 0.9388$ and 0.5450, respectively). Similarly, mean absolute CGA values for the 4 plasma octreotide level groups (<2000 pg/mL, 2000–5000 pg/mL, 5001–10,000 pg/mL, and more than 10,000 pg/mL) showed a general increasing trend (up to 10,000 pg/mL) in CGA serum and plasma levels with increasing octreotide plasma levels, but, again, these results did not reach statistical significance for either the absolute CGA serum or plasma levels ($P = 0.9847$ and 0.9092, respectively) (Table 1A and B). T1

When we evaluated diarrhea symptom control, higher mean absolute (static) CGA values were seen in the group "not under optimal control" than for the group "under optimal control" for both CGA serum and plasma levels; however,

TABLE 1. Chromogranin A Serum and Plasma Levels by Groups

A. Chromogranin A Serum Levels by Group

	n	Mean Serum CGA	SD	*P*
LAR dose (mg)				
20	8	53.1	87.06	NS
30	19	65.8	94.16	
60	13	70.7	120.18	
OA plasma level (pg/mL)				
<2000	4	32.1	23.26	NS
2000–5000	15	48.4	72.97	
5001–10,000	11	92.6	116.68	
>10,000	10	72.0	132.48	
Diarrhea				
Control	27	69.9	102.52	NS
No Control	8	77.2	119.9	
Flushing				
Control	33	66.6	95.67	0.0372
No Control	3	10.6	5.27	

B. Chromogranin A Plasma Levels by Group

	n	Mean Plasma CGA	SD	*P*
LAR dose (mg)				
20	8	56.6	93.79	NS
30	19	66.2	99.95	
60	13	65.2	102.85	
OA plasma level (pg/mL)				
<2000	4	36.4	32.22	NS
2000–5000	15	49.0	76.48	
5001–10,000	11	94.8	125.98	
>10,000	10	63.4	109.69	
Diarrhea				
Control	27	68.1	95.13	NS
No control	8	80.3	130.28	
Flushing				
Control	33	64.5	89.11	0.0372
No control	3	11.3	7.68	

These tables demonstrate the statistical analysis of plasma and serum CGA levels by monthly LAR dose, plasma octreotide levels, and frequency of symptoms (diarrhea and flushing) control. The large SDs seen in this study are felt to be the result of the wide variations seen in CGA levels in endothelials with varying tumor burdens. Other studies have shown that CGA levels are proportional to tumor burden.[7]

NS indicates not significant; OA, aqueous octreotide.

these results did not reach statistical significance ($P = 0.2410$). In stark contrast, and contrary to our hypothesis, there was a significant statistical difference between the flushing "not under optimal control" and "under optimal control" groups for both absolute CGA serum and plasma levels ($P = 0.0372$) with higher mean values observed in the "under control" group.

Finally, tests of association between LAR dose groups and symptom control for both diarrhea and flushing revealed no significant associations between these factors. For diarrhea symptom control, 0% of the subjects in the LAR-20 group were out of control, compared with 27.8% and 30.8% in the LAR-30 and LAR-60 groups, respectively ($P = 0.3182$). For flushing symptom control, 0% of the subjects in the LAR-20 group were out of control compared with 11.1% and 7.1% in the LAR-30 and LAR-60 groups, respectively ($P = 1.0000$) (Table 1A and B).

DISCUSSION

Chromogranin A is a matrix protein found in dense core vesicles of neuroendocrine cells. It acts as a prohormone and is involved in processing, regulation, secretion, and stabilizing vesicles. Several peptide fragments are contained within the CGA molecule including β-granin, chromostatin, vasostatin, and pancreastatin. One study found dynamic (serial) measurements of pancreastatin to be a more reliable marker for carcinoid tumor progression than serial measurements of the intact CGA molecule itself.[12] Nehar et al[13] showed that measurements of CGA and pyrophosphate levels, in combination, enhance the sensitivity to detect a response to treatment in both functioning and nonfunctioning tumors to greater than 95%.

AQ3

However, serum CGA remains the pivotal tumor marker for the diagnosis and follow-up of functioning and nonfunctioning neuroendocrine tumors[1,12,14,15] and in carcinoid tumors of diverse origin, proves to be more sensitive than serotonin or 5-hydroxyindole acetic acid (Vinik et al, Endotext.org). Four recent consensus and "guideline" papers for the diagnosis and management of neuroendocrine tumors rely on CGA levels to help confirm the existence of a tumor and, when used sequentially over time, to monitor its progression. The absolute concentration of plasma CGA levels in patients with neuroendocrine tumors reflects the degree of neuroendocrine differentiation, the total tumor burden and the effect of ongoing, steady state octreotide therapy. When CGA measurements are used in a serial fashion, they can help assess the acute effects of medical or surgical interventions.[1–8]

Changes in serial CGA levels are good predictors of the efficacy of medical or surgical intervention. Sondenaa et al[2] found a correlation between tumor weight and absolute plasma CGA levels in nude mice with carcinoid tumors. In this study, the octreotide-treated mice had significantly reduced plasma CGA levels when compared with untreated animals. Another study showed CGA levels returned to normal in patients after radical resection of neuroendocrine tumors. A normal CGA level in these patients corresponded to a negative postoperative octreotide scan.[16]

Octreotide LAR therapy significantly reduces the severity and frequency of diarrhea and flushing in most of the carcinoid patients but has a much more limited effect on tumor growth. Treatment of patients with octreotide LAR leads to a significant reduction in CGA levels; however, these relatively acute changes in marker levels do not reflect acute changes in tumor burden. Rohaizak and Farndon[17] found octreotide and long-acting lanreotide were useful palliative treatments for the control of symptoms in patients with unresectable carcinoid tumors, but there was no evidence of tumor regression on computed tomography scans. Therefore, some authors suggest that short-term changes in CGA levels can only be interpreted in the context of the current clinical scenario and other diagnostic tests.[12,14] An acute decrease in CGA after octreotide therapy does not correlate with a significant acute change in tumor volume, but merely the tumor's output of amines. In our series, patients were on a stable dose of octreotide LAR for at least 3 months before measurement of octreotide and CGA levels. Thus, these absolute CGA levels should reflect steady state rather than acutely changing levels of the octreotide peptide or the CGA amine. Based on our observations, we suggest that in patients on LAR therapy, the absolute CGA level will not be a good indicator of symptom severity, rather serial CGA measurement will be required to determine the efficacy of therapy.

In a previous study of this group of patients, we attempted to correlate octreotide blood levels with the symptom severity scores for flushing, diarrhea, and wheezing. In that study, we found that there was an unexpected correlation between higher octreotide levels and more intense symptoms of carcinoid syndrome.[11] We attributed this correlation to the use of doses higher than the 30-mg dose approved by the Food and Drug Administration in those individuals whose carcinoid syndrome was more pronounced. Similar relationships may be present in this study because we showed that the absolute CGA levels were not inversely associated with octreotide dose or to the plasma octreotide levels. This may imply that those individuals with higher tumor burdens and/or higher amine output may be treated with higher doses of LAR to control tumor growth or symptoms of carcinoid syndrome. Moreover, we saw that higher CGA levels were associated with those individuals whose flushing was considered to be "under optimal control" and lower CGA levels were seen in those individuals whose flushing assessment showed that this symptom was less well controlled ($P < 0.04$). This was clearly an unexpected result. In contrast, those individuals whose diarrhea was less well controlled had the highest CGA values, whereas those with better control of their diarrhea had lower mean plasma or serum CGA values (P = not significant). Although this was not statistically significant, the differences in the CGA levels in individuals with flushing and diarrhea implies that different mechanisms, peptides, or amines may be responsible for these 2 syndrome complexes. More importantly, we have shown that for a group of individuals, the absolute CGA value does not positively correlate with symptom intensity or clinical control of diarrhea or flushing. Dynamic (serial) measurement of CGA levels should better predict the clinical and biochemical effectiveness of medical or surgical therapy. This concept will be tested in a future prospective multi-institutional trial.

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