0021-972X/97/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 82, No. 8
Printed in U.S.A.

Chromogranin A as Serum Marker for Neuroendocrine Neoplasia: Comparison with Neuron-Specific Enolase and the α-Subunit of Glycoprotein Hormones

FRANK R. E. NOBELS, DIK J. KWEKKEBOOM, WILLY COOPMANS, CHRISTIAAN H. H. SCHOENMAKERS, JAN LINDEMANS, WOUTER W. DE HERDER, ERIC P. KRENNING, ROGER BOUILLON, AND STEVEN W. J. LAMBERTS

Departments of Medicine (F.N., W.D.H., S.L.), Nuclear Medicine (D.K., E.K.), and Clinical Laboratory (C.S., J.L.), University Hospital Dijkzigt, Rotterdam, The Netherlands; and the Laboratory of Experimental Medicine and Endocrinology, University Hospital Gasthuisberg (W.C., R.B.), Leuven, Belgium

ABSTRACT

Chromogranin A (CgA) is gaining acceptance as a serum marker of neuroendocrine tumors. Its specificity in differentiating between neuroendocrine and nonneuroendocrine tumors, its sensitivity to detect small tumors, and its clinical value, compared with other neuroendocrine markers, have not clearly been defined, however. The objectives of this study were to evaluate the clinical usefulness of CgA as neuroendocrine serum marker. Serum levels of CgA, neuron-specific enolase (NSE), and the α-subunit of glycoprotein hormones (α-SU) were determined in 211 patients with neuroendocrine tumors and 180 control subjects with nonendocrine tumors. The concentrations of CgA, NSE, and α-SU were elevated in 50%, 43%, and 24% of patients with neuroendocrine tumors, respectively. Serum CgA was most frequently increased in subjects with gastrinomas (100%), pheochromocytomas (89%), carcinoid tumors (80%), nonfunctioning tumors of the endocrine pancreas (69%), and medullary thyroid carcinomas (50%). The highest levels were observed in subjects with carcinoid tumors. NSE was most frequently elevated in patients with small cell lung carcinoma (74%), and α-SU was most frequently elevated in patients with carcinoid tumors (39%). Most subjects with elevated α-SU levels also had elevated CgA concentrations. A significant positive relationship was demonstrated between the tumor load and serum CgA levels ($P < 0.01$, by χ^2 test). Elevated concentrations of CgA, NSE, and α-SU were present in, respectively, 7%, 35%, and 15% of control subjects. Markedly elevated serum levels of CgA, exceeding 300 μg/L, were observed in only 2% of control patients (n = 3) compared to 40% of patients with neuroendocrine tumors (n = 76). We conclude that CgA is the best general neuroendocrine serum marker available. It has the highest specificity for the detection of neuroendocrine tumors compared to the other neuroendocrine markers, NSE and α-SU. Elevated levels are strongly correlated with tumor volume; therefore, small tumors may go undetected. Although its specificity cannot compete with that of the specific hormonal secretion products of most neuroendocrine tumors, it can have useful clinical applications in subjects with neuroendocrine tumors for whom either no marker is available or the marker is inconvenient for routine clinical use. (*J Clin Endocrinol Metab* **82:** 2622–2628, 1997)

CHROMOGRANIN A (CgA) is a protein that is present in the secretory dense core granules of neuroendocrine tissues (1). It is widely used as an immunohistochemical marker of neuroendocrine tumors. It can also serve as a serum marker, as it is cosecreted with the amines and peptides that are present in the neurosecretory granules (2). It is at present considered to be a very sensitive and specific serum marker of neuroendocrine tumors. The concentrations often remain increased in cases of less well differentiated tumors of neuroendocrine origin that do not secrete known hormones (3). The publications on CgA as a serum marker of neuroendocrine neoplasia deal with rather small numbers of patients and small numbers of control subjects with nonneuroendocrine neoplasms (2, 4, 5). Most neuroendocrine tumors included are far advanced, with a large tumor volume. Data on the specificity of CgA for the differentiation between neuroendocrine and nonneuroendocrine tumors and on its sensitivity for the detection of small neuroendocrine tumors are sparse or lacking. There are also no large studies available in which CgA is compared with other neuroendocrine markers, such as neuron-specific enolase (NSE) and the α-subunit of glycoprotein hormones (α-SU).

We investigated the roles of the serum concentrations of CgA, NSE, and α-SU in a large study group of patients with neuroendocrine tumors, including tumors with a small volume, and in a control group consisting of patients with several nonendocrine tumors. The results suggest that the determination of CgA is useful in selected clinical conditions when either no known specific peptide markers are available or when the available markers are inconvenient for routine clinical practice.

Received September 18, 1996. Revision received April 23, 1997. Accepted May 2, 1997.

Address all correspondence and requests for reprints to: Dr. F. Nobels, Department of Endocrinology, Onze Lieve Vrouw Hospital, 164 Moorselbaan, 9300 Aalst, Belgium.

Subjects and Methods

Patients

Serum samples were obtained from 211 subjects with the following neuroendocrine neoplasms: carcinoid tumor (n = 62), medullary thyroid carcinoma (n = 26), paraganglioma (n = 25), pheochromocytoma (n = 9), neuroblastoma (n = 3), small cell lung carcinoma (n = 23), insulinoma (n = 21), gastrinoma (n = 9), nonfunctioning pancreatic islet cell tumor

(n = 13), Merkel cell tumor (n = 4), clinically nonfunctioning pituitary adenoma (n = 10), and GH-secreting pituitary adenoma (n = 6). All diagnoses were made histologically, except in a few patients with small tumors of the neuroendocrine pancreas. In these cases the following diagnostic criteria were used: paradoxical rise in gastrin levels after stimulation by iv injection of secretin in gastrinoma, and hypoglycemia with inappropriate hypersecretion of insulin and C peptide during a diagnostic fast in insulinoma. All plasma samples were obtained before operation.

Serum samples were also obtained from 180 subjects with a variety of "control" neoplasms of nonendocrine origin, both benign and malignant, including hematological and neurological tumors. This control group consisted of patients with breast carcinoma (n = 64), nonsmall cell lung cancer (n = 24), pancreatic adenocarcinoma (n = 21), adenocarcinoma of unknown origin (n = 12), non-Hodgkin lymphoma (n = 25), Hodgkin lymphoma (n = 13), multiple myeloma (n = 7), meningioma (n = 10), and astrocytoma (n = 4). All of these diagnoses were confirmed by histological examination.

Immunoassays

CgA was measured in serum samples, stored at −20 C, by a polyclonal RIA, using human CgA isolated from pheochromocytomas as tracer and standard, as previously described (6). The within-assay coefficients of variation were 6.5% and 8.6% for mean concentrations of 95 and 1160 ng/mL (n = 18), respectively. The between-assay coefficients of variation were 6.9% and 6.3% for mean concentrations of 90 and 698 ng/mL (n = 38), respectively. The detection sensitivity was 1.6 μg/L. The CgA immunoreactivity remained stable whether the serum samples were immediately frozen or kept at 4 C or at room temperature for 24 h, or whether blood was centrifuged immediately to obtain serum or after 24-h storage at room temperature. The reference value in 568 normal subjects of both sexes, aged 6–50 yr, is 90 ± 24 μg/L (range, 35–176); in 33 normal men older than 50 yr, it is 106 ± 22 μg/L (range, 70–159); in 249 normal postmenopausal women older than 50 yr, it is 110.1 ± 35.5 μg/L (range, 54–220). In men and premenopausal women, 175 μg/L was chosen as the upper cut-off value, and in postmenopausal women, 220 μg/L was used, to avoid overlapping values with normal subjects. This corresponds to slightly more than 3 SD above the mean.

NSE was measured by RIA. The upper cut-off value is 12.5 μg/L.

α-Subunit was measured by RIA using antibodies purchased from UCB (Brussels, Belgium). The upper cut-off values are 1.1 μg/L in men, 2.3 μg/L in premenopausal women, and 4.0 μg/L in postmenopausal women.

Determination of tumor mass

The number of neuroendocrine tumor localizations was counted using computed tomography scan images and [^{111}In-DTPA-D-Phe^1]octreotide scanning (7). The tumor load was considered limited when one or two localizations were found; it was considered extensive when more than three localizations were demonstrated.

Statistical analysis

Results are reported as the mean ± SD. To compare the different markers, χ^2 tests and Spearman rank correlations were used. To study the effect of tumor load on circulating concentrations of the markers, χ^2 tests were used.

Results

Serum concentrations of the markers CgA, α-SU, and NSE were determined in 211 patients with neuroendocrine tumors and compared to levels in a control group, consisting of 180 patients with nonendocrine neoplasms. The study and control groups showed comparable age distributions (53 ± 14 and 54 ± 13 yr, respectively). The sex distribution showed a higher male/female ratio for the study group (1.67 *vs.* 0.68), which can be ascribed to the high number of patients with breast carcinoma in the control group.

Because renal failure can increase circulating CgA concentrations (4), we evaluated whether this could cause falsely elevated levels. In the control group a significant relationship was demonstrated between a serum creatinine level higher than 133 μmol/L and increased levels of CgA ($P < 0.001$, by χ^2 test). A creatinine concentration above 133 μmol/L was found in seven patients in the control group. Elevated serum concentrations of CgA (maximum, 371 μg/L) were present in six of these patients. A creatinine level above 133 μmol/L was also present in three subjects in the study group (all with carcinoid tumor). One of these patients had normal and one had slightly increased CgA concentrations (268 μg/L). The third patient, with an extensively metastasized carcinoid tumor, had a creatinine level of 220 μmol/L and very high levels of CgA (188, 160 μg/L). Although these extreme elevations probably cannot be attributed to the diminished renal function (4), these three study patients and seven control subjects with creatinine levels above 133 μmol/L were eliminated for further analysis of the data. Slightly elevated CgA concentrations can also occur in cases of severe liver dysfunction (4). This was not encountered in any of our study or control patients.

The results are summarized in Tables 1 and 2 and Fig. 1. The serum concentrations of CgA were elevated in 103 of 208 patients with neuroendocrine tumors. They were more frequently increased (in 50% of the subjects) than the concentrations of NSE and α-SU (in 43% and 24% of the subjects, respectively). The highest elevations of CgA were observed in subjects with carcinoid tumors (up to a maximum of 52,340 μg/L). Very high levels (>1,000 μg/L) were also seen in subjects with nonfunctioning pancreatic islet cell tumor, medullary thyroid carcinoma, pheochromocytoma, paraganglioma, small cell lung carcinoma, gastrinoma, and Merkel cell tumor. The levels were most frequently elevated in subjects with gastrinoma (100%), pheochromocytoma (89%), and carcinoid tumor (80%). In subjects with pituitary adenoma (13%), insulinoma (10%), and paraganglioma (8%), elevated CgA levels were only rarely present (Tables 1 and 2 and Fig. 1).

The highest levels of NSE were recorded in patients with small cell lung carcinoma and Merkel cell tumor (up to a maximum of 558 μg/L). NSE was more frequently elevated than CgA in subjects with small cell lung carcinoma (in 74% and 39%, respectively), Merkel cell tumor (in 50% and 25%, respectively), insulinoma (in 38% and 10%, respectively), paraganglioma (in 36% and 8%, respectively), and neuroblastoma (in 67% and 33%, respectively).

The levels of α-SU were most frequently elevated in patients with carcinoid tumors (39%). Very high levels (up to a maximum of 353 μg/L) were found in these patients. α-SU concentrations higher than 10 μg/L were found in 7 of 59 subjects with carcinoid tumors (12%), whereas they were never encountered in subjects with other neuroendocrine neoplasms.

Elevated levels of CgA, α-SU, and NSE were present in respectively 9 (69%), 4 (31%), and 3 (23%) of 13 patients with nonfunctioning pancreatic islet cell tumors (Table 3). In 7 (54%) of these 13 patients, CgA levels were markedly elevated (>300 μg/L).

Elevated levels of CgA, α-SU, and NSE were present in,

TABLE 1. Serum levels of CgA, NSE, and α-SU in patients with neuroendocrine tumors and in controls with nonendocrine tumors

Type of tumor	No. of subjects	CgA (μg/L)		NSE (μg/L)		α-SU (μg/L)	
		Median	Range	Median	Range	Median	Range
Neuroendocrine tumors (n = 208)							
Carcinoid tumor	59	688	33–52,340	12	6–156	1.5	0.6–353.0
Medullary thyroid carcinoma	26	184	80–13,900	11	4–146	1.1	0.6–3.7
Paraganglioma	25	106	50–11,590	10	6–35	0.9	0.6–2.0
Pheochromocytoma	9	275	110–4,674	11	1–19	0.9	0.6–1.8
Neuroblastoma	3	133	117–238	36	12–100	0.6	0.3–3.0
Small cell lung carcinoma	23	149	45–2,948	27	6–511	1.2	0.5–2.5
Insulinoma	21	105	63–236	12	5–19	1.1	0.5–3.4
Gastrinoma	9	772	289–1,933	13	8–23	1.6	0.5–4.7
NF pancreatic islet cell tumor	13	306	85–14,750	10	5–91	1.1	0.7–2.1
Merkel cell tumor	4	109	84–1,056	23	7–558	1.0	0.6–2.5
Clinically NF pituitary adenoma	10	131	85–240	10	8–12	0.9	0.6–3.6
GH-secreting pituitary adenoma	6	71	53–115	8	6–10	0.9	0.5–1.7
Nonendocrine tumors (n = 173)							
Breast carcinoma	62	96	55–8,307	11	4–26	1.5	0.4–13.0
Nonsmall cell lung cancer	23	95	47–219	11	5–41	1.1	0.6–6.7
Pancreatic adenocarcinoma	20	116	51–395	11	6–24	1.1	0.5–2.9
Adenocarcinoma uo	12	119	68–154	12	7–44	1.3	0.7–2.7
Non-Hodgkin lymphoma	24	99	67–185	10	5–22	1.1	0.5–2.3
Hodgkin lymphoma	13	75	22–161	10	6–52	1.0	0.7–1.4
Multiple myeloma	5	162	98–215	13	9–15	1.1	0.8–1.7
Meningioma	10	89	57–134	11	6–32	1.0	0.4–2.6
Astrocytoma	4	86	66–121	6	5–52	0.8	0.6–0.8

NF, Nonfunctioning; uo, unknown origin.

TABLE 2. Presence of elevated serum levels of CgA, NSE, and α-SU in patients with neuroendocrine tumors and controls with nonendocrine tumors

Type of tumor	No. of subjects	↑ CgA		↑ NSE		↑ α-SU	
		n	%	n	%	n	%
Neuroendocrine tumors							
Carcinoid tumor	59	47	80	28	47	23	39
Medullary thyroid carcinoma	26	13	50	11	42	5	19
Paraganglioma	25	2	8	9	36	3	12
Pheochromocytoma	9	8	89	4	44	1	11
Neuroblastoma	3	1	33	2	67	1	33
Small cell lung carcinoma	23	9	39	17	74	8	35
Insulinoma	21	2	10	8	38	0	0
Gastrinoma	9	9	100	4	44	3	33
NF pancreatic islet cell tumor	13	9	69	4	31	3	23
Merkel cell tumor	4	1	25	2	50	0	0
Clinically NF pituitary adenoma	10	2	20	0	0	2	20
GH-secreting pituitary adenoma	6	0	0	0	0	0	0
Total	208	103	50	89	43	49	24
Nonendocrine tumors							
Breast carcinoma	62	5	8	23	37	6	10
Nonsmall cell lung carcinoma	23	1	4	9	39	4	17
Pancreatic adenocarcinoma	20	3	15	7	35	2	10
Adenocarcinoma uo	12	0	0	6	50	2	17
Non-Hodgkin lymphoma	24	1	4	5	21	5	21
Hodgkin lymphoma	13	0	0	3	23	4	31
Multiple myeloma	5	2	40	3	60	2	40
Meningioma	10	0	0	4	40	1	10
Astrocytoma	4	0	0	1	25	0	0
Total	173	12	7	61	35	26	15

n, Number of subjects with elevated levels.

respectively, 7%, 15%, and 35% of control subjects with nonendocrine neoplasms.

When these control subjects were used as reference population, the sensitivities of CgA, NSE, and α-SU for the diagnosis of peripheral neuroendocrine tumors (pituitary adenomas excluded) were, respectively, 53%, 46%, and 26%, with specificities of 93%, 65%, and 85%. We applied, however, a rather high upper cut-off value for CgA, corresponding to slightly more than 3 SD above the mean, to avoid overlapping values with normal subjects. Usually 2 SD above the mean is used as the upper cut-off level, increasing the risk of overlap. When we reanalyzed our data using 2 SD as the upper cut-off level, the sensitivity hardly improved to 58% with a specificity of 90%. When using 300 μg/L as the upper

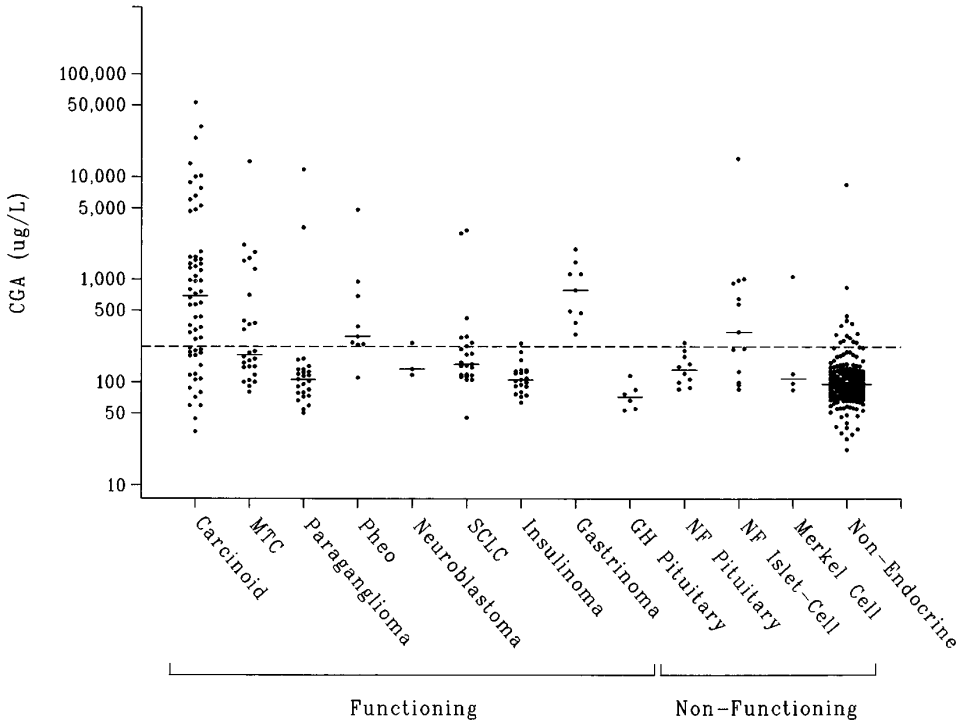

FIG. 1. Serum concentrations of CgA in patients with neuroendocrine tumors and in controls with nonendocrine tumors. Individual levels are presented as *dots*; median levels as *lines*. The *dashed line* represents the upper cut-off level of 220 μg/L. The results are plotted logarithmically to accommodate extreme values. MTC, Medullary thyroid carcinoma; Pheo, Pheochromocytoma; SCLC, Small cell lung carcinoma; GH, GH-producing; NF, Nonfunctioning.

TABLE 3. Serum levels of CgA, NSE, α-SU in patients with nonfunctioning tumors of the endocrine pancreas

Patient no.	Sex	Age (yr)	CgA (μg/L)	NSE (μg/L)	α-SU (μg/L)
1	M	56	969[a]	11.4	1.9[a]
2	V	67	306[a]	4.6	1.7
3	M	23	999[a]	5.6	1.1
4	V	55	14,750[a]	10.3	2.1
5	V	51	94	12.6[a]	2.0
6	M	72	641[a]	6.8	1.3[a]
7	M	70	207[a]	15.5[a]	1.0
8	V	46	99	11.0	1.0
9	M	51	910[a]	90.8[a]	1.1
10	M	64	211[a]	8.1	1.0
11	M	59	85	5.3	0.7
12	M	51	126	15.6[a]	1.2[a]
13	M	63	567[a]	8.0	0.7

[a] Elevated levels.

Upper cut-off values: CgA (micrograms per L), 175 in men and premenopausal women, 220 in postmenopausal women; NSE (micrograms per L), 12.5; α-SU (micrograms per L), 1.1 in men, 2.3 in premenopausal women, and 4.0 in postmenopausal women.

cut-off concentration for CgA, elevated levels were found in only 3 of 173 patients (2%) with nonneuroendocrine control tumors compared to 76 of 192 patients (40%) with peripheral neuroendocrine tumors (sensitivity, 40%; specificity, 98%). Thus, finding an excessively elevated level of CgA firmly suggests the presence of a neuroendocrine tumor.

Relationships among the general neuroendocrine markers CgA, NSE, and α-SU

In subjects with peripheral neuroendocrine tumors (pituitary adenomas excluded), a statistically significant relationship was demonstrated between the presence and absence of elevated serum levels of CgA and α-SU ($P < 0.001$, by χ^2 test), but not between CgA and NSE or between α-SU and NSE (Table 4). A weak, but significant, relationship was present between the presence and absence of elevated serum concentrations of CgA and α-SU ($P = 0.05$, by χ^2 test) in subjects with carcinoid tumors, who frequently had elevated α-SU levels. In patients with small cell lung carcinoma, who frequently had elevated NSE levels, no significant relationship could be shown between CgA and NSE concentrations.

Relationship with other neuroendocrine markers

Measurements of 24-h urinary 5-hydroxyindole acetic acid (5-HIAA) excretions were available in 46 of 59 patients with carcinoid tumors. Increased levels (>40 μmol/24 h) were present in 31 patients (67%). Elevated serum concentrations of CgA were demonstrated in 30 of these 31 subjects (97%; $P < 0.01$, by χ^2 test). A significant correlation was also present between the absolute values of serum CgA and 24-h urinary 5-HIAA excretion (Spearman rank correlation test; r = 0.65; $P < 0.01$). No significant relationships were demonstrated between α-SU and NSE concentrations, on the one hand, and urinary 5-HIAA excretions, on the other hand ($P > 0.05$, by χ^2 tests).

Determinations of serum calcitonin and carcinoembryonic antigen (CEA) concentrations were available in, respectively, 20 and 21 of 26 subjects with medullary thyroid carcinoma. Calcitonin was elevated (>0.14 μg/L) in 18 of 20 patients (90%), and CEA (>10 μg/L) was elevated in 18 of 21 patients (86%). Elevated CEA levels were present in the 2 patients with normal calcitonin levels. In 1 of these 2 subjects, slightly elevated concentrations of CgA (192 μg/L) and α-SU (1.5 μg/L) were found. CgA, α-SU, and NSE levels were not increased in the 3 patients with normal CEA levels. Significant correlations were demonstrated between serum CgA, on the one hand, and calcitonin (by Spearman rank correlation test: r = 0.79; $P < 0.01$) and CEA (r = 0.84; $P < 0.01$),

TABLE 4. Relations between serum levels of CgA, NSE, and α-SU in subjects with neuroendocrine tumors

$\chi^2 = 18.10$ ($P < 0.001$)	CgA		No. of subjects	$\chi^2 = 1.08$ (P = NS)	CgA		No. of subjects	$\chi^2 = 0.05$ (P = NS)	NSE		No. of subjects
	Normal	Elevated			Normal	Elevated			Normal	Elevated	
α-SU				NSE				α-SU			
Normal	93	66	159	Normal	64	56	120	Normal	92	67	159
Elevated	12	38	50	Elevated	41	48	89	Elevated	28	22	50
No. of subjects	105	104	209		105	104	209		120	89	209

NS, $P > 0.05$.

on the other hand, as well as between NSE, on the one hand, and calcitonin (r = 0.71; $P < 0.01$) and CEA (r = 0.82; $P < 0.01$), on the other hand. α-SU showed a correlation with calcitonin (r = 0.63; $P < 0.01$), but no significant correlation with CEA (r = 0.33; $P > 0.05$).

Determinations of 24-h urinary excretions of vanilmandelic acid (VMA) were only available in five of nine patients with pheochromocytoma. The highest levels of CgA were found in the patients with the highest urinary VMA excretion, although the small number of cases did not permit statistical evaluation.

Relationship with tumor load

Using computed tomography scan images and octreotide scintigrams, information on tumor volume could be obtained in subjects with the following neuroendocrine neoplasms: 60 carcinoid tumors, 26 medullary thyroid carcinomas, 25 paragangliomas, 11 small cell lung carcinomas, 9 gastrinomas, and 12 nonfunctioning pancreatic islet cell tumors. Tumor load was considered to be limited when 1 or 2 localizations were found and was considered extensive when more than 3 localizations were demonstrated. A highly significant positive relationship was demonstrated between the tumor load and serum CgA levels ($P < 0.01$, by χ^2 test). Such a relationship could not be shown for α-SU or NSE. In the individual neuroendocrine neoplasms, the relationship between tumor load and CgA levels was only significant in subjects with carcinoid tumors. Because they represent the largest subgroup, statistical significance is more easily reached. Gastrinomas form an exception to the rule that small neuroendocrine tumors have low CgA levels; elevated CgA levels were detected in all patients with gastrinomas, although they all presented with limited neoplastic disease.

Discussion

We evaluated the clinical usefulness of CgA, NSE, and α-SU as serum markers of neuroendocrine neoplasia in general. Serum concentrations were measured in a large group of patients with several neuroendocrine tumors and compared with those in a large control group with a variety of nonendocrine tumors.

The highest concentrations of CgA, with values up to 250 times the upper limit of normal, were observed in subjects with carcinoid tumors, medullary thyroid carcinomas, pheochromocytomas, and some tumors of the endocrine pancreas. This confirms the results of previous smaller studies (2, 4, 5). Elevated levels were also frequently encountered in subjects with peripheral (nonpituitary) neuroendocrine tumors without detectable hormonal secretion. These so-called chromogranimomas were first described by Sobol and coworkers (3). Serum concentrations of CgA are only rarely increased, however, in cases of clinically nonfunctioning pituitary adenomas. This is probably due to the small volume of these adenomas (6).

We demonstrated a significant positive relation between the serum levels of CgA and the tumor mass of the neuroendocrine neoplasms. This confirms our earlier findings in Cushing's syndrome caused by ectopic ACTH production by extrapituitary neuroendocrine tumors (8) and the findings by O'Connor and Deftos (2) and Hsiao and co-workers (9) in pheochromocytomas. The serum concentrations of CgA are only rarely slightly elevated in subjects with small neuroendocrine tumors, such as insulinomas, paragangliomas, or pituitary adenomas (6, 8, 10). These tumors are usually detected at an early stage of oncological evolution, because they rapidly induce symptoms due to active hormonal secretion or compression of important surrounding tissues. The presence of CgA or its messenger ribonucleic acid can nearly always be demonstrated in the cells of these tumors by immunohistochemistry or *in situ* hybridization (6, 11, 12). Nevertheless, it must be assumed that the small amount of CgA released by these neoplasms usually fails to elevate the serum concentration above the physiological background level. Increased CgA concentrations were detected, however, in all of our patients with gastrinoma, although they all had a very limited tumor burden. It is well known that chronic elevation of gastrin levels provokes hyperplasia of the neuroendocrine cells of the stomach (13). As these cells are able to secrete CgA, they might be responsible for the elevated CgA concentrations. Stabile and co-workers demonstrated that the CgA concentrations can be normalized by gastrectomy alone, without resection of the gastrin-producing tumor (and thus without correction of the elevated gastrin levels) (14).

In our hands, CgA had a smaller sensitivity for the detection of neuroendocrine neoplasms than reported in previous studies (2, 4, 5). However, the technical characteristics of our RIA for CgA are very similar to those of the other assays used in frequently cited publications (4, 15). A small neuroendocrine tumor mass was present in a rather large percentage of our patients, in contrast to the hitherto published series, in which almost all tumors were extensively metastasized (2, 4, 5). This can probably be explained by the fact that patients tend to be transferred earlier in their oncological evolution, after the development of [^{111}In-DTPA-D-Phe^1]octreotide scanning for the visualization of neuroendocrine tumors in our hospital. Many patients with biochemical proof of a neuroendocrine tumor were trans-

ferred for somatostatin receptor scintigraphy after conventional radiography failed to elucidate the location of the tumor. The inclusion of a number of patients with these smaller tumors decreased the overall sensitivity of serum CgA in our series.

The specificity of elevated levels of CgA in the diagnosis of neuroendocrine tumors was also lower in our study than in previous ones (2, 4, 5). O'Connor and co-workers (2, 4) and Erikkson and co-workers (5) reported specificities of 100%. By contrast, we used a much larger control group, consisting of patients with a greater variety of nonendocrine tumors. After excluding patients with decreased renal function, elevated serum concentrations of CgA were demonstrated in 12 of 173 nonendocrine neoplasms (7%). The serum levels in these control patients were usually only slightly elevated. They exceeded 300 μg/L in only 3 of 173 patients (2%) compared to 76 of 208 patients (37%) with neuroendocrine tumors. Thus, finding an excessively elevated level of CgA firmly suggests the presence of a neuroendocrine tumor.

It is well established that many nonendocrine tissues contain neuroendocrine cells, belonging to the amino-precursor-uptake-decarboxylation system. A substantial body of data has accumulated in the literature during recent years, revealing that these cells are also present in most tumors of nonendocrine origin (16–20). They are either diffusely scattered throughout the tumor or multifocally located in small nests. In malignant tumors these neuroendocrine cells even participate in the neoplastic growth, as they show nuclear abberations and are present in locally invasive or metastatic tumor tissue. The number of tumors harboring these neuroendocrine cells or the percentage of neuroendocrine cells in a tumor depend on the tumor type, the number of neuroendocrine markers used, and the detection technique (histochemistry for argyrophilia, immunohistochemistry, or detection of messenger ribonucleic acid of neuroendocrine markers). These cells probably secrete CgA, as it is present in their dense core secretory granules. There are only scarce data available in the literature concerning serum levels of CgA in subjects with nonendocrine tumors. Elevated levels were reported in patients with carcinomas of the prostate gland (21, 22) and in cases of nonsmall cell lung cancer (23). Whether proliferation of neuroendocrine cells also occurs in hematological neoplasms is not known. One study reported the presence of scarcely distributed CgA-positive cells in the normal spleen, lymph nodes, and thymus (24).

As a general neuroendocrine marker, CgA cannot differentiate between different subtypes of neuroendocrine neoplasms. Most tumors of neuroendocrine origin release typical secretion products that can be used as specific serum markers. These markers usually provide a higher sensitivity and specificity than CgA, as illustrated by our data comparing calcitonin and CEA with CgA in subjects with medullary thyroid carcinoma. In these situations the usefulness of CgA is limited, because it does not provide additional information. By contrast, CgA can have interesting clinical applications in so-called nonfunctioning neuroendocrine tumors that are either not able to secrete hormonal products or release products that cannot be detected by current techniques. It can also be useful in neuroendocrine tumors in which other diagnostic procedures have their limitations (*e.g.* fluctuating levels of serum catecholamines in pheochromocytoma) or are inconvenient (*e.g.* 24-h urine collections for 5-HIAA determination in carcinoid tumors). Our data illustrate the value of CgA in these conditions: increased levels were found in 69% of nonpituitary, hormone-negative neuroendocrine tumors, 89% of pheochromocytomas, and 80% of carcinoid tumors. Very high concentrations were frequently encountered in these patients.

NSE is the neuron-specific isomer of the glycolytic enzyme 2-phospho-D-glycerate hydrolase or enolase (25). It is a widely used immunohistochemical and serum marker for neuroendocrine tissues and is especially known as a marker for small cell lung carcinoma (26). Our data confirm the frequent elevation of its serum concentrations in patients with several neuroendocrine tumors (27–29). The highest levels were encountered in small cell lung carcinoma and in the rare cases of Merkel cell tumors. Serum concentrations of NSE are more often elevated than those of CgA in subjects with these tumors and in those with insulinomas, paragangliomas, and neuroblastomas. The specificity of serum NSE for the diagnosis of neuroendocrine tumors is, however, much lower than that of serum CgA. Increased NSE levels were demonstrated in 61 of 173 subjects with nonneuroendocrine neoplasms (35%) compared to 89 of 192 with peripheral (nonpituitary) neuroendocrine tumors (46%). Unlike CgA, the specificity of NSE can hardly be improved by increasing the upper cut-off value. Therefore, NSE cannot be considered a good diagnostic marker for neuroendocrine tumors, but can be very useful as a follow-up marker, especially for small cell lung carcinoma and Merkel cell tumors.

The α-SU of the glycoprotein hormones is a well known marker of pituitary adenomas of gonadotroph origin (6). Recent studies suggest that determination of the serum concentrations of α-SU might also be of value in patients with peripheral neuroendocrine neoplasms (30–32). Our data confirm the presence of elevated serum levels in several subjects with these neoplasms. Again, as with CgA and NSE, the marker lacks specificity. Serum levels were elevated in 26 of 173 subjects with nonneuroendocrine neoplasms (15%) compared to 47 of 192 with peripheral neuroendocrine tumors (24%). Increasing the cut-off level again failed to improve the specificity. Very high levels were frequently detected in patients with carcinoid tumors; 7 of 59 subjects with carcinoid tumors (12%) had levels higher than 10 μg/L. Such high levels were only encountered once in the control group, in a patient with breast carcinoma. Thus, the finding of very high serum concentrations of α-SU suggests the presence of a carcinoid tumor when tumors of germ cell or trophoblastic origin are excluded. The clinical usefulness of α-SU as a marker for neuroendocrine tumors is limited, however, because most subjects with elevated levels also have elevated CgA concentrations.

In conclusion, CgA is the best general neuroendocrine serum marker available. It had the highest specificity for the detection of neuroendocrine tumors of the three tested markers. Unfortunately, it is not a very sensitive marker; its serum concentrations seem to rise relatively late in the evolution of the tumor. Although its specificity cannot compete with that of the specific hormonal secretion products of most neuroendocrine tumors, it can have useful clinical applications

in subjects with neuroendocrine tumors for which either no marker is available (so-called nonfunctioning neuroendocrine tumors) or the marker is inconvenient for daily clinical use (*e.g.* 24-h urinary 5-HIAA excretions and plasma catecholamines).

References

1. **Deftos LJ.** 1991 Chromogranin A: its role in endocrine function, and as an endocrine and neuroendocrine tumor marker. Endocr Rev. 12:181–187.
2. **O'Connor DT, Deftos LJ.** 1986 Secretion of chromogranin A by peptide-producing endocrine neoplasms. N Engl J Med. 314:1145–1151.
3. **Sobol RE, Memoli V, Deftos LJ.** 1989 Hormone-negative, chromogranin A-positive endocrine tumors. N Engl J Med. 320:444–447.
4. **O'Connor DT, Pandian MR, Carlton E, Cervenka JH, Hsiao RJ.** 1989 Rapid radioimmunoassay of circulating chromogranin A: *in vitro* stability, exploration of the neuroendocrine character of neoplasia, and assessment of the effects of organ failure. Clin Chem. 35:1631–1637.
5. **Eriksson B, Arnberg H, Öberg K, et al.** 1990 A polyclonal antiserum against chromogranin A and B–a new sensitive marker for neuroendocrine tumors. Acta Endocrinol (Copenh). 122:145–155.
6. **Nobels FRE, Kwekkeboom DJ, Coopmans W, Hoekstra R, Bouillon R, Lamberts SWJ.** 1993 A comparison between the diagnostic value of the measurement of gonadotropins, α-subunits and chromogranin A and their response to TRH in clinically nonfunctioning, α-subunit secreting and gonadotroph pituitary adenomas. J Clin Endocrinol Metab. 77:784–789.
7. **Lamberts SWJ, Bakker WH, Reubi J-C, Krenning EP.** 1990 Somatostatin-receptor imaging in the localization of endocrine tumors. N Engl J Med. 323:1246–1249.
8. **Nobels FRE, de Herder WW, Kwekkeboom DJ, et al.** 1994 Serum chromogranin A in the differential diagnosis of Cushing's syndrome. Eur J Endocrinol. 131:589–593.
9. **Hsiao RJ, Neumann HPH, Parmer RJ, Barbosa JA, O'Connor DT.** 1990 Chromogranin A in familial pheochromocytoma: diagnostic screening value, prediction of tumor mass, and post-resection kinetics indicating two-compartment distribution. Am J Med. 88:607–613.
10. **Deftos LJ, O'Connor DT, Wilson CB, Fitzgerald PA.** 1989 Human pituitary tumors secrete chromogranin-A. J Clin Endocrinol Metab 68:869–872.
11. **Weiler R, Fischer-Colbrie R, Schmid KW, et al.** 1988 Immunological studies on the occurrence and properties of chromogranin A and B and secretogranin II in endocrine tumors. Am J Surg Pathol. 12:877–884.
12. **Lloyd RV, Iacangelo A, Eiden LE, Cano M, Jin L, Grimes M.** 1989 Chromogranin A, and B messenger ribonucleic acids in pituitary and other normal and neoplastic human endocrine tissues. Lab Invest. 60:548–556.
13. **D'Adda T, Corleto V, Pilato FP, et al.** 1990 Quantitative ultrastructure of endocrine cells of oxyntic mucosa in Zollinger-Ellison syndrome. Gastroenterology. 99:17–26.
14. **Stabile BE, Howard TJ, Passaro E, O'Connor DT.** 1990 Source of plasma chromogranin A elevation in gastrinoma patients. Arch Surg. 125:451–453.
15. **O'Connor DT, Bernstein KN.** 1984 Radioimmunoassay of chromogranin A in plasma as a measure of exocytotic sympathoadrenal activity in normal subjects and patients with pheochromocytoma. N Engl J Med. 311:764–770.
16. **Sundaresan V, Reeve JG, Stenning S, Stewart S, Bleehen NM.** 1991 Neuroendocrine differentiation and clinical behaviour in non-small cell lung tumors. Br J Cancer. 64:333–338.
17. **Scopsi L, Andreola S, Pilotti S, et al.** 1992 Argyrophylia and granin (chromogranin/secretogranin) expression in female breast carcinomas. Their relationship to survival and other disease parameters. Am J Surg Pathol. 16:561–576.
18. **di Sant'Agnese PA.** 1992 Neuroendocrine differentiation in carcinoma of the prostate. Diagnostic, prognostic and therapeutic implications. Cancer. 70:254–268.
19. **De Bruïne AP, Wiggers T, Beek C, et al.** 1993 Endocrine cells in colorectal adenocarcinomas: incidence, hormone profile and prognostic relevance. Int J Cancer. 54:765–771.
20. **Pour PM, Permert J, Mogaki M, Fujii H, Kazakoff K.** 1993 Endocrine aspects of exocrine cancer of the pancreas. Their patterns and suggested biologic significance. Am J Clin Pathol. 100:223–230.
21. **Kadmon D, Thompson TC, Lynch GR, Scardino PT.** 1991 Elevated plasma chromogranin-A concentrations in prostatic carcinoma. J Urol. 146:358–361.
22. **Deftos LJ, Nakada S, Burton DW, di Sant'Agnese PA, Cockett AT, Abrahamsson PA.** Immunoassay and immunohistology studies of chromogranin A as a neuroendocrine marker in patients with carcinoma of the prostate. Urology 96;48:58–62.
23. **Sobol RE, O'Connor DT, Addison J, Suchocki K, Royston I, Deftos LJ.** 1986 Elevated serum chromogranin A concentrations in small-cell lung carcinoma. Ann Intern Med. 105:698–700.
24. **Hogue-Angeletti R, Hickey WF.** 1985 A neuroendocrine marker in tissues of the immune system. Science. 230:89–90.
25. **Schmechel D, Marangos PJ, Brightman M.** 1978 Neuron-specific enolase is a molecular marker for peripheral, and central neuroendocrine cells. Nature. 276:834–836.
26. **Akoun GM, Scarna HM, Milleron BJ, Bénichou MP, Herman DP.** 1985 Serum neuron-specific enolase. A marker for disease extent and response to therapy for small-cell lung cancer. Chest. 87:39–43.
27. **Oishi S, Sato T.** 1988 Elevated serum neuron-specific enolase in patients with malignant pheochromocytoma. Cancer. 61:1167–1170.
28. **Cunningham RT, Johnston CF, Irvine GB, Buchanan KD.** 1992 Serum neurone-specific enolase levels in patients with neuroendocrine and carcinoid tumors. Clin Chim Acta. 212:123–131.
29. **D'Alessandro M, Mariani P, Lomanto D, Carlei F, Lezoche E, Speranza V.** 1992 Serum neuron-specific enolase in diagnosis and follow-up of gastrointestinal neuroendocrine tumors. Tumor Biol. 13:352–357.
30. **Heitz PU, von Herbay G, Klöppel G, et al.** 1987 The expression of subunits of human chorionic gonadotropin (hCG) by nontrophoblastic, non-endocrine and endocrine tumors. Am J Clin Pathol. 88:467–472.
31. **Eriksson B, Oberg K, Skogseid B.** 1989 Neuroendocrine pancreatic tumors. Clinical findings in a prospective study of 84 patients. Acta Oncol. 28:373–377.
32. **Grossmann M, Trautmann ME, Poertl S, et al.** 1994 Alpha-subunit and human chorionic gonadotropin-beta immunoreactivity in patients with malignant endocrine gastroenteropancreatic tumors. Eur J Clin Invest. 24:131–136.