Utility of combined use of plasma levels of chromogranin A and pancreatic polypeptide in the diagnosis of gastrointestinal and pancreatic endocrine tumors

F. Panzuto[1], C. Severi[1], R. Cannizzaro[2], M. Falconi[3], S. Angeletti[1], A. Pasquali[1], V. D. Corleto[1], B. Annibale[1], A. Buonadonna[2], P. Pederzoli[3], and G. Delle Fave[1]

[1]Digestive and Liver Disease Unit, II School of Medicine, University "La Sapienza", Rome; [2]Centro di Riferimento Oncologico, Aviano; [3]Department of Surgery, University of Verona, Verona, Italy

ABSTRACT. Background: Chromogranin A (CgA) is considered the most accurate marker in the diagnosis of gastro-entero-pancreatic (GEP) endocrine tumors. Pancreatic polypeptide (PP) has also been proposed to play this role, but then not used due to its low sensitivity. The aim of the present study was to determine whether the assessment of PP would improve the diagnostic reliability of CgA in patients with GEP tumors. Patients and methods: Both markers were assessed in 68 patients [28 functioning (F), 40 non functioning (NF)]. Twenty-seven patients disease-free (DF) after surgery, and 24 with non-endocrine tumors (non-ETs) were used as control groups. Results: CgA sensitivity was: 96% in F, 75% in NF, 74% in pancreatic, and 91% in gastrointestinal (GI) tumors. Specificity was 89% vs DF, and 63% vs non-ETs. PP sensitivity was: 54% in F, 57% in NF, 63% in pancreatic, and 53% in GI tumors. Specificity was 81% vs DF, and 67% vs non-ETs. By combining the two markers a significant gain in sensitivity vs CgA alone was obtained: overall in GEP tumors (96% vs 84%, *p*=0.04), in NF (95% vs 75%, *p*=0.02), and in pancreatic (94% vs 74%, *p*=0.04). More specifically, a 25% gain of sensitivity was obtained in the subgroup of NF pancreatic tumors (93% vs 68%, *p*=0.04). Conclusion: The combined assessment of PP and CgA leads to a significant increase in sensitivity in the diagnosis of GEP tumors, particularly in pancreatic NF.

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INTRODUCTION

On clinical grounds, gastro-entero-pancreatic (GEP) endocrine tumors are usually classified into two groups: functioning (F) and non-functioning (NF). In the NF group, in the past reported to be approximatively 1/3 of endocrine tumors (1) [but that accounted for 3/4 of GEP tumors in a recent survey performed in our country (2)], no specific associated syndrome is present and symptoms are related only to the "mass effect" caused by the tumor growth, diagnosis often being an incidental finding. This classification plays a critical role in the biochemical diagnosis. In F tumors, the hormone responsible for the associated syndrome is, in fact, a useful specific diagnostic marker with well established high sensitivity, whilst for those tumors with no associated syndrome, only general serum markers are available (3). Of these, Chromogranin A (CgA), which is considered an "On/Off" switch controlling the dense-core secretory granule biogenesis in endocrine and neuroendocrine cells (4), is the most reliable, since other general markers, such as Neuron Specific Enolase and the α subunit of glycoprotein have been demonstrated to be not quite sensitive (5-7). However, CgA sensitivity ranges from 50 to 100% [80-100% for F (5, 6, 8, 9) and 50-70% for NF tumors (5-7, 10)]. Nevertheless, very few studies have been performed on homogeneous populations of tumors arising only in the digestive system, and even in these, few NF tumors were included. Thus, the real diagnostic impact of CgA in GEP tumors, particularly in NF, remains to be clearly established.

Key-words: Chromogranin A, pancreatic polipeptide, neuroendocrine tumors, pancreas, non functioning.

Correspondence: G. Delle Fave, M.D., Digestive and Liver Disease Unit, II School of Medicine, University "La Sapienza", Ospedale Sant'Andrea, via di Grottarossa 1035 Roma, 00189, Italy.

E-mail: gianfranco.dellefave@uniroma1.it

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Another possible general tumor marker for GEP endocrine tumors might be pancreatic polypeptide (PP), a 36 amino acids product of a distinct type of endocrine cell located primarily in the islets of Langerhans in the pancreatic head (11), whose physiological role remain to be fully elucidated. This peptide has already been proposed as a potential neuroendocrine tumor marker, but, to date, it has not been extensively used on account of its reported low diagnostic accuracy (12, 13).
This prospective study was aimed at determining whether the assessment of PP too could improve the diagnostic reliability of CgA in a series of naïve patients with endocrine tumor arising from the gastrointestinal tract or pancreas.

MATERIALS AND METHODS

Patients

A total of 68 consecutive naïve patients (never submitted, before enrollment, either to medical or surgical treatment) with a diagnosis of GEP endocrine tumor were enrolled (40 males/28 females, median age 53 yr, range 18-74). Another 27 patients, disease-free after surgery (DF) (17 males, median age 48 yr), and 24 with histologically diagnosed non-endocrine tumors (non-ETs: gastric, colorectal, and pancreatic carcinomas) (13 males, median age 57 yr), were used as control groups to define specificity. DF status was defined when, after radical surgery, at 1-yr follow-up, imaging [helical CT (hCT) and somatostatin receptor scintigraphy (SRS)] and specific biochemical markers were negative, in F tumors, as well as only imaging, in NF. Of the patients with GEP tumors, 41% (no.=28) had an associated syndrome (F tumors), whereas 59% (no.=40) had no associated syndrome (NF tumors). Fifty-two percent (no.=35) were localized in the pancreas, 32% (no.=22) in the gastrointestinal (GI) tract, 7% (no.=5) presented a multiple localization, while in 6 patients the localization of the primary tumor was unknown [5 patients had Zollinger Ellison syndrome (ZES) with no lesions detected by imaging procedures, whereas in the remaining patients with carcinoid syndrome, diagnosis was based on histological examination of liver lesion biopsy] (Table 1). Tumor load was assessed by SRS and hCT as previously described (14), and was considered limited when no liver metastases were found, otherwise it was considered extended. The diagnoses of GEP endocrine tumor were confirmed by histological examination of a surgical or needle-biopsy specimen.
This study was approved by the Local Ethics Committee, and all patients gave informed consent before enrolment.

Methods

Blood samples were collected in the morning in fasting conditions. Plasma was obtained by collecting blood in aprotinin/ ethylenediamine-tetraacetic-acid-containing vials and by subsequent +4 C centrifugation. Samples were then stored at –20 C until the day of the assay. All samples were collected prior to surgery and/or before any specific medical treatment was started. Patients with plasma creatinine >1.5 mg/dl, systemic inflammatory diseases, liver failure, proton pump inhibitors therapy, were excluded from this study. CgA and PP levels were measured on the same blood sample.
Healthy subjects (20 male, 13 female, median age 46 yr) were used as reference populations to define normal ranges, and the values corresponding to the upper 95th percentile (mean + 1.654 standard deviation) were taken as cut-off values (22 U/l and 42 pmol/l for CgA and PP, respectively).

Immunoassays

CgA was assessed by enzyme-linked immunoadsorbent assay (ELISA) kit (Dako A/S, Glostrup, Denmark). The intra- and inter-assay coefficients of variation (CV) were 4.1 and 5.3, respectively. PP was assessed by radioimmunoassay (RIA) kit (Euro-Diagnostica AB, Sweden). Intra and inter-assay CV were 2.6% and 3.5%, respectively.

Statistical analysis

CgA and PP values are expressed as median (95% CI for the median), and, due to the wide range, are reported as natural or log-transformed data as appropiate. Mann-Whitney rank test was used to compare plasma values in the various groups. Fisher exact test was used to compare percentages and p levels <0.05 were considered statistically significant. In this study, a combined finding was considered when at least one of the two markers was increased.
In NF pancreatic tumors, sensitivity and specificity of CgA and PP were calculated for every value of each marker, and ROC (Receiver Operating Characteristic) curves (graphs of the sensitivity against 100 - specificity) were then constructed. When both markers were considered together, a discriminant function was obtained by calculating the multiple logistic regression of cases/controls of the

Table 1 - *General features of patients population.*

Characteristics	No. (%)
GEP tumors	68
Type	
Functioning	28 (41)
Gastrinoma	14
Gastrinoma-MEN I	5
Carcinoid	6
Somatostatinoma	2
Glucagonoma	1
Non functioning	40 (59)
Primary tumor localization	
Pancreas	35 (52)
Functioning	7
Non functioning	28
GI tract 22 (32)	
Functioning	10
Non functioning	12
Multiple	5 (7)
Unknown	6 (9)
Load	
Limited	40 (59)
Extended	28 (41)
DF	27
Non-ETs	24

GEP: gastro-entero-pancreatic; MEN I: multiple endocrine neoplasia type 1; GI: gastrointestinal; DF: disease free.

natural logarithms of CgA and PP [-6+(2.76logCgA) + (2.02logPP)] and the sensitivity and specificity were again used to construct a ROC curve.

RESULTS

Plasma levels of CgA and PP (Fig. 1 and 2)

Median CgA values in patients with GEP endocrine tumors [166 U/l (104-270)] were higher than those in DF controls and in non-ETs [7 U/l (6-12) and 14 U/l (6-77), respectively], the difference being statistically significant ($p<0.0001$). Median CgA values in F tumors [270 U/l (165-533)] were statistically higher ($p=0.005$) than in NF tumors [107 U/l (54-169)]. Similar CgA levels were observed with respect to the primary tumor localization, being 161 U/l (62-317) and 160 U/l (57-572) respectively in pancreatic and GI tract tumors. No significant difference in CgA levels was observed in relationship to the tumor load, since median CgA values were 162 U/l (106-294) in patients with limited disease (no liver metastases), and 170 U/l (72-415) in those with extended disease (with liver lesions). Furthermore, median PP values, were higher in GEP tumors [56 pmol/l (35-93)] than in the two control groups [22 pmol/l (12-40) and 23 pmol/l (6-54) in DF and non-ETs, respectively], the difference being statistically significant ($p<0.02$). The highest levels were observed in pancreatic tumors [82 pmol/l (33-169)].

Sensitivity and specificity of CgA and PP (Table 2)

Raised CgA levels were observed in 57/68 patients with GEP tumors, and more specifically in 27/28 F and 30/40 NF, resulting in a sensitivity of 84% in the overall GEP tumors group, 96% in F and 75% in NF. As far as specificity is concerned, increased CgA levels were observed in 11% of the DF patients and in 37% of the non-ET patients, with a resulting specificity of 89% and 63%, respectively. PP sensitivity was lower than that observed with CgA, both in the overall group of GEP tumors and in F and NF tumors, being approximately 55% in each group. Specificity, however, was similar to that observed with CgA, being 81% vs DF and 67% vs non-ETs controls.

By combining CgA and PP, a 12% increase was observed in sensitivity, in the overall group of GEP tumors, CgA or PP being elevated in 96% of cases ($p=0.04$ vs CgA alone). A greater improvement in sensitivity, by combining the two markers, was observed in the subgroup of NF tumors in which the combined assessment of the two markers improved sensitivity from 75% of CgA alone to 95% with the association ($p=0.02$).

As far as the primary tumor localization is concerned, CgA was elevated in 74% of pancreatic, and 91% of GI tract, tumors. PP sensitivity, again, was lower than that of CgA in both groups of tumors, being 63% and 53%, respectively. By combining the two markers, an increase in sensitivity was observed, in pancreatic tumors, with a gain of 20% vs CgA alone (94% vs 74%, $p=0.04$). This increase in sensitivity was further enhanced when the subgroup of pancreatic NF tumors was considered. In this group of patients, sensitivity rose from 68% for CgA alone to 93% when both markers were assessed ($p=0.04$).

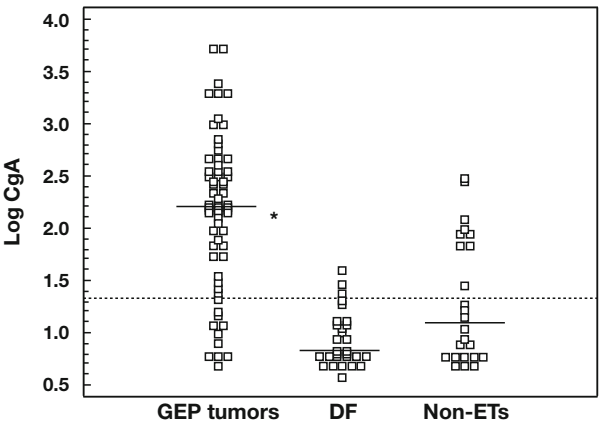

Fig. 1 - *p<0.0001 vs both control groups (DF and non-ETs).
CgA plasma levels in GEP tumors and control groups (DF and non-ETs). Values are log-transformed. Continuous line: median value. Dotted lines: normal upper cut-off level (22 U/l).
CgA: chromogranin A; GEP: gastro-entero-pancreatic; DF: disease free; Non-ETs: non endocrine tumors.

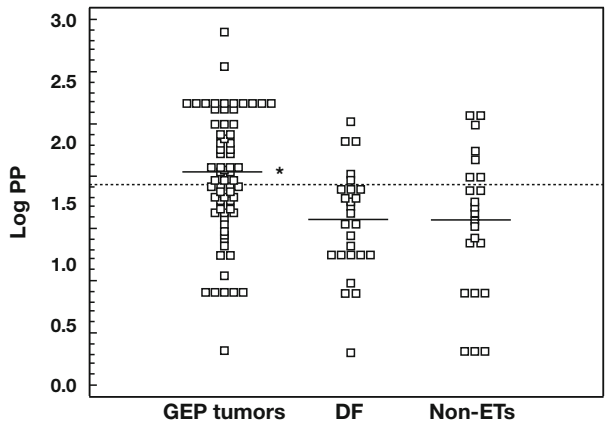

Fig. 2 - *p<0.02 vs both control groups (DF and non-ETs).
PP plasma levels in GEP tumors and control groups (DF and non-ETs). Values are log-transformed. Continuous line: median value. Dotted lines: normal upper cut-off level (42 pmol/L).
CgA: chromogranin A; PP: pancreatic polypeptide; GEP: gastro-entero-pancreatic; DF: disease free; Non-ETs: non endocrine tumors.

Table 2 - *High CgA and PP in GEP tumors and control groups.*

Patient group	No. pts	↑ CgA (%)	↑ PP (%)	↑ CgA or PP (%)
GEP tumors	68	84	56	96*
Type				
Functioning	28	96	54	96
Non functioning	40	75	57	95^
Load				
Limited	40	80	65	90
Extended	28	89	54	100
Controls				
DF	27	11	19	33
Non-ETs	24	37	33	54

*p=0.04, ^p=0.02 vs CgA alone.
GEP: gastro-entero-pancreatic; CgA: chromogranin A; PP: pancreatic polypeptide; DF: disease free.

ROC curves were then constructed for each marker and for their combination in the subgroup of pancreatic NF tumors, using DF patients as a control group. As shown in Figure 3, a larger area under the curve was obtained with the combination of the two markers compared to that of CgA alone (0.845 vs 0.917), reflecting the improvement in the discriminating ability. Finally, as far as tumor load is concerned, both CgA and PP showed similar sensitivity values regardless of disease extension.

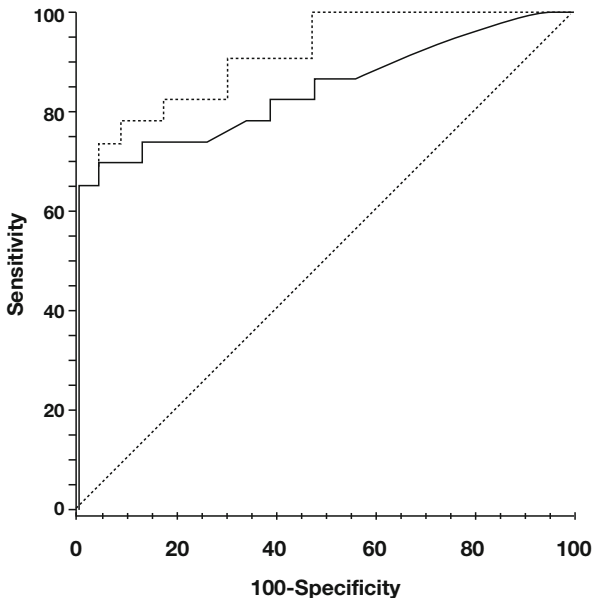

Fig. 3 - Receiver operating characteristic (*ROC) curve of chromogranin A (CgA), alone or combined with pancreatic polypeptide (PP), in non functioning (NF) pancreatic tumors. Continuous line: CgA alone. Dotted line: combined CgA and PP. Diagonal line corresponds to the value of area under curve=0.5, when the variables cannot discriminate between presence/absence of the disease.*

DISCUSSION

In the present study, the additional assessment of PP significantly increased the diagnostic reliability of CgA, particularly in the group of pancreatic NF tumors, in which sensitivity increased from 68% to 93%. CgA has been confirmed to have a good diagnostic accuracy, in agreement with data reported by others (3, 5-9). However, most of these investigations have been performed on populations including patients both before and after surgical and/or medical treatment was performed, affected by neoplasms arising from different systems, and usually including few NF tumors. In our study, on the contrary, blood samples were always collected in all patients with endocrine tumors arising from the digestive system, before any surgical or medical treatment was started (naïve patients). CgA sensitivity was confirmed to be higher in patients with a hormone-related syndrome (F) than in those without specific syndromes (NF). Baudin et al. (6) observed that more elevated CgA levels were associated with the presence of other peptide secretions, and Kim et al. (4) demonstrated that hormone secretion in endocrine and neuroendocrine cells is strictly dependent upon CgA. However, in F tumors, the high CgA sensitivity could be considered relatively less important, since the availability of other specific markers (i.e. gastrin in ZES, and 5-HIAA in carcinoids) as useful tools for reliable biochemical diagnosis in these tumors. On the contrary, in the NF group, the absence of typical symptoms, the relatively low CgA sensitivity (50-70%) reported in the literature (5-7, 10) and confirmed by our findings, as well as the lack of other specific circulating markers, make early diagnosis extremely difficult, as suggested by the high frequency of advanced disease at the time of diagnosis. In these patients, this lack of CgA diagnostic ability could be filled by the assessment of PP, which, in our study,

has been demonstrated to increase sensitivity of CgA alone to values similar to those observed in F tumors. As far as the primary tumor site is concerned, the combined use of the two markers in pancreatic tumors was found to be more effective than CgA alone, improving sensitivity by 20%. On the contrary, the additional information provided in patients with tumors arising in the GI tract was negligible. Even if these findings would appear to be expected, given the greater physiological concentration of PP-releasing cells both in the islets of Langerhans and in the endocrine cells of the ventral pancreas (11), they are worthy of further comment. In fact, we observed increased PP levels also in 53% of patients with primary GI tract tumor, confirming, as previously suggested by others (13, 15), that also non-pancreatic endocrine tumors can produce PP. Thus, the increase in sensitivity obtained by the association of the two markers seems to be related primarily to the relatively lower sensitivity of CgA alone in the group of patients with pancreatic tumors, rather than to the specificity of PP in these patients. This finding seems to be confirmed by the analysis of the ROC curve in NF pancreatic tumors, which shows an increase in the area under the curve of the two markers combined vs CgA alone, demonstrating the better reliability of the combination to distinguish between the presence and absence of a pancreatic NF tumor. However, the difference between the areas of the two curves did not reach statistical significance, in the present investigation, probably due to the relatively small number of cases studied.

No difference in CgA levels was observed in the different groups of patients analyzed with respect to the presence of metastatic lesions suggesting the absence of a direct correlation between CgA levels and disease extension. However, other factors need to be taken in account when interpreting these data, such as tumor functional status, specific diagnosis, and the presence of other secretory activity (16). In our opinion, the possible correlation between tumor load and circulating CgA levels still remains a controversial issue, and our data add to the conflicting results previously reported, with CgA correlating with tumor extent in some studies (5, 6, 17, 18) but not in others (8, 19).

As far as the specificity of the two markers is concerned, we used both DF and non-ET patients as control groups. Both markers showed a high specificity vs DF patients even if, as already suggested, the use of such patients as controls has some limits. In fact, even if a DF status is defined when the patient underwent radical surgery, there was no evidence of disease at imaging procedures and specific tumor markers returned to normal (F tumors), it is possible that such a patient has a non-detectable disease (8). Therefore, it cannot be excluded that DF patients presenting abnormal tumor markers might develop recurrence in the future. On the other hand, neither CgA nor PP has been demonstrated to discriminate the endocrine origin of the neoplasm, as both showed low specificity vs the non-ET control group. However, this finding seems not surprising, since, as is well known, neuroendocrine cells are present in most tumors of non-endocrine origin (20-22). In conclusion, CgA has been confirmed to be an accurate circulating marker for the diagnosis of GEP endocrine tumors, with high sensitivity (84%) and specificity (89%). The addition of PP assessment provides a significant increase in sensitivity, playing a critical role in tumors with no associated syndrome, mostly arising from the pancreas.

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