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FROM THE MEDICAL DEPARTMENT AND LUDWIG INSTITUTE FOR CANCER RESEARCH, UNIVERSITY HOSPITAL, UPPSALA, SWEDEN.

PEPTIDE HORMONES AS TUMOR MARKERS IN NEUROENDOCRINE GASTROINTESTINAL TUMORS

B. ERIKSSON and K. ÖBERG

Abstract

Since the development of the radioimmunoassay, the determination of serum or plasma levels of peptides has improved the diagnostic possibilities in neuroendocrine gastrointestinal and pancreatic tumors. Once the diagnosis of a carcinoid or an endocrine pancreatic tumor has been established the analyses of various peptides can help to characterize the tumor. Up till now there has been no 'screening' marker for the early detection of these tumors. The development of radioimmunoassay for analyses of chromogranins in plasma might imply such possibilities. In the management of the patients it is also important to have tumor markers that can give prognostic information. Recent findings might indicate that CA-50 could be an indicator of malignant disease and possibly also correlate with survival.

Key words: Carcinoids, endocrine pancreatic tumors, tumor markers, chromogranins, CA-50.

Gastrointestinal tumors constitute a small but important group of tumors, the diagnostic and therapeutic methods of which have improved much during the last thirty years. Ever since the establishment of the radioimmunoassay in 1960 (1) a large number of peptides have been discovered and this has in turn facilitated the identification of clinical syndromes associated with over-production of hormones from the tumors. Assessment of circulating peptide levels is now a prerequisite in the diagnosis and management of patients with neuroendocrine gastrointestinal tumors.

In the following, the use of basal and stimulated peptide levels as diagnostic tools in carcinoid and endocrine pancreatic tumors will be discussed. Special emphasis will be given to the new tumor markers, the chromogranins, with respect to diagnostic sensitivity and correlation to other tumor markers during treatment. In addition, indicators of malignant disease will be discussed and especially the possible use of carcino-embryonic antigens, CA-50 and CA 19-9, as prognostic markers in endocrine pancreatic tumors.

For practical reasons, gastrointestinal endocrine tumors are divided into carcinoid tumor and endocrine pancreatic tumor, although there is no clear-cut distinction between them. Most of the tumors produce multiple hormones, but usually the excessive secretion of one hormone predominates the clinical picture and causes a clinical syndrome. The carcinoid tumors can be divided into foregut, midgut and hindgut carcinoids according to embryonic origin (2) and the clinically most important type is the midgut carcinoid due to its high tendency of metastasizing into the liver (3). The carcinoid syndrome consisting of symptoms of flush, diarrhea and bronchoconstriction, usually arises when liver metastases have developed. Clinical syndromes associated with endocrine pancreatic tumors are in order of frequency: the insulinoma syndrome (4), the gastrinoma syndrome (5), the watery diarrhea hypokalemia achlorhydria (WDHA) syndrome (6), the glucagonoma syndrome (7) and the somatostatinoma syndrome (8). About one-third of EPT are so-called non-functioning tumors, i.e. not associated with any hormone-related symptoms (9). In rare cases Cushing's syndrome and acromegaly might be caused by ectopic hormone production from both carcinoids and EPT (10).

Peptide tumor markers can be divided into specific markers; i.e. markers associated with specific symptoms and syndromes, and general markers, i.e. markers not associated with any particular symptom.

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Carcinoids

Increased synthesis and metabolism of serotonin or 5-hydroxytryptamin remain the most important diagnostic biochemical features of carcinoid tumors and the breakdown product urinary 5-hydroxyindole acetic acid (5-HIAA) is the most commonly used tumor marker in this type of tumor. In a recent study by Norheim et al. (11), 88% of patients with metastatic carcinoids had increased 24-h excretion of 5-HIAA (Table 1). The higher levels were found in midgut carcinoids. Foregut carcinoids could have moderately increased excretion, whereas hindgut carcinoids rarely do. It has been proposed that serotonin could be the mediator of diarrhea in the carcinoid syndrome.

Carcinoid tumors can produce several other biologically active substances, contributing to the syndrome. In the same study (11), it was shown that plasma concentrations of neuropeptide K (NPK) were increased in all patients with metastatic midgut carcinoids. NPK belongs to the tachykinins, a family of peptides with vasoactive properties, and probably contributes to the flush in many patients. The very first member of this family to be described was substance P, which also can be found in increased amounts in the plasma of carcinoid patients. Usually the concentrations of substance P are lower than those of NPK, probably due to the shorter half-life of substance P. Furthermore, the patients may display different spectra of tachykinins during flushing.

Stimulating flush reaction with pentagastrin and analysing plasma NPK can be employed to increase the diagnostic accuracy in patients with suspected midgut carcinoid tumors but normal basal NPK.

Other peptide markers with peripheral hormonal activity which can be found, especially in carcinoids originating in the foregut region, are histamine and gastrin which can produce symptoms of flush and ulcer disease respectively. In rare cases corticotropin-relating factor (CRF) or growth hormone-releasing factor (GRF) are produced by the tumors, leading to Cushing's syndrome and acromegaly respectively (10). Among general tumor markers without any clinical symptoms human chorionic gonadotropin (HCG-) α and β, pancreatic polypeptide (PP) calcitonin and somatostatin can be mentioned.

Endocrine pancreatic tumors

In a recent report, we reviewed biochemical findings in 84 patients with endocrine pancreatic tumors (12). Among specific tumor markers, serum insulin was increased in 37/80 patients (46%), which should be compared to the number of patients presenting the insulinoma syndrome; 23 (Table 2). Similarly, the number of patients displaying elevated serum gastrin level 52/84 (62%) was much higher than the number presenting the gastrinoma syndrome; 25. Interestingly, only 6/14 (43%) patients with the WDHA syndrome had elevation of plasma VIP, whereas 9/14 (64%) had increased serum calcitonin. In our patient material there was only one individual with the glucagonoma syndrome but 18/53 patients displayed increased level of glucagon.

The most useful general tumor markers—if one excepts chromogranins, which will be discussed separately—appear to be serum PP, which was increased in as many as 74% of the patients. The HCG-subunits, which have been

Table 1

Tumor markers in carcinoid tumors

Specific
U-5-HIAA
p-NPK
p-substance P
s-gastrin
U-histamine
p-GRF
p-CRF
General
s-PP
s-HCG-α
s-HCG-β
s-calcitonin
p-somatostatin

HIAA = hydroxyindole acetic acid; NPK = neuropeptide K; GRF = growth hormone releasing factor; CRF = corticotropin releasing factor; PP = pancreatic polypeptide; HCG = human choronic gonadotropin.

Table 2

Tumor secretory products in 84 patients with endocrine pancreatic tumors at first evaluation

'Tumor marker'	Patients		Patients with syndrome
	n	%	n
Specific			
Insulin c-peptide Pro-insulin	37/80	46	23
Gastrin	52/84	62	25
VIP	11/84	23	14
Calcitonin	25/60	42	14
Glucagon	18/53	34	1
Somatostatin	7/33	21	1
General			
Chromogranin A + B	46/49	94	
PP	56/76	74	
Neurotensin	12/18	67	
HCG-α	33/81	41	
HCG-β	24/80	30	

VIP = vasoactive intestinal polypeptide; PP = pancreatic polypeptide; HCG = human choronic gonadotropin.

proposed to be indicators of malignant disease in neuroendocrine gastrointestinal tumors (13), were increased in 40% (HCG-α) and 30% (HCG-β) of individuals respectively. All except three patients with increased levels of these markers had malignant tumors, and so there is a clear correlation with malignancy. Unfortunately, normal HCG-subunits do exclude the possibility of a malignant tumor.

Stimulatory tests

Stimulatory tests are used in EPT to increase the diagnostic sensitivity although the reliability of certain tests have been questioned. None of them has a 100% sensitivity but despite that fact, most clinicians consider them useful.

The standardized meal stimulation test developed by Skogseid et al. (14) is a very important diagnostic tool in demonstrating the involvement of the endocrine pancreas in multiple endocrine neoplasia (MEN) type 1, but can also be used in sporadic disease. Hence, in our study 94% of individuals with familial EPT and 70% of those with sporadic EPT had a positive test.

The secretin test (15) is the most widely used test to confirm the diagnosis of a gastrinoma. Pure natural GIH secretin, either a standard dose or an individualized dose, is administered as a bolus injection intravenously and serum gastrin measured at 5-min intervals for 30 min. Some authors recommend that calcium should be given immediately before the secretin injection to increase the sensitivity of the test (16). The definition of a positive test might also be discussed. Some investigators claim that an absolute value of the increase in serum gastrin, i.e. 100 pmol/l, should be used. However, in most endocrinological stimulatory tests a two-fold increase of the prestimulatory level is required for the test to be considered as positive. By the latter definition 80% of our gastrinoma patients had a positive secretin test (12).

A 12–72 h fast (17) with analyses of blood glucose, serum insulin, c-peptide and proinsulin may be used in the diagnosis of insulinomas. A blood glucose level below 2.2 mmol/l and/or and insulin/glucose ratio 5.4 or c-peptide and/or proinsulin levels above the reference range are considered abnormal. As many as 88% of our insulinoma patients had a positive fast.

The insulin suppression test is another adjunct in the assessment of the insulinoma diagnosis (18), in which c-peptide levels are followed after the injection of exogenous insulin, a positive test being defined as a $<50\%$ suppression of c-peptide levels. In our hands, 57% of the insulinoma patients had a positive test. The false negative results in some individuals could be explained by difficulties in achieving adequate hypoglycemia and the 'normal' suppression of c-peptide levels in benign insulinomas.

Chromogranins

The chromogranins are a family of secretory proteins with a widespread distribution in neuroendocrine granules of normal and neoplastic cells (19). Three proteins have been identified, chromogranin A, B and C, each protein having a unique amino acid sequence but they also have many biochemical properties in common. The distribution of the three proteins is overlapping but not identical. Immunohistochemistry has shown that the chromogranins are present in neuroendocrine tumors of the pituitary, medullary thyroid carcinomas, neuroendocrine tumors of the lung, pancreas and small intestine and pheochromocytomas (19). They can also be demonstrated in increased levels in the plasma of patients with neuroendocrine tumors (20).

Because of their abundance and universal presence in neuroendocrine granules many suggestions for a physiological role have been proposed. Since all the chromogranins contain multiple dibasic sites for potential cleavage, they have been suggested to be precursors of other smaller peptides (21). Chromogranin A for instance is the precursor of pancreastatin, a smaller biologically active peptide (22). Secretory protein I, which is identical to chromogranin A, binds calcium (23) and as calcium binding proceeds the protein aggregates. This aggregation of the protein leads to a condensation of granule contents, which in turn could lower the osmotic pressure of the granules (24). Chomogranin A has also been proposed to regulate the proteolytic processing of certain hormones from prohormones (25).

In a previous report we could demonstrate that plasma chromogranin A + B were elevated in all patients with manifest carcinoid tumors, endocrine pancreatic tumors and pheochromocytomas, while half of the patients with small cell lung cancer had increased levels (26). Patients with non-endocrine tumors displayed normal levels.

Unpublished observations indicate that chromogranins can be sensitive markers also in patients with limited disease. We examined 30 patients with carcinoid tumors of different origin, including three ECL-omas, two rectal carcinoids and 25 midgut carcinoids, who had been operated upon and after surgery had small residual tumors in the gastric mucosa or in regional lymph nodes (Table 3). As is shown in Fig. 1, all these patients had elevated plasma chromogranin A + B levels, whereas only three showed elevations of urinary 5-HIAA.

One important application of measuring hormone levels in patients with gastrointestinal neuroendocrine tumors is to monitor and evaluate the effects of various therapeutic procedures. We have compared chromogranin A + B levels to other tumor markers in our patients during causal medical treatment. It appears, as is illustrated in Figs 2 and 3, that changes in chromogranin A + B levels correlated well with changes in other markers, and can be used to monitor treatment.

Table 3

Characteristics of carcinoid patients with limited disease

Type of tumor	No. of patients	Mult. tumor	Op. tumor	Tumor burden			Symptoms	
				Residual primary	Mesenteric lymph nodes	Liver metastases	Flush	Diarrhea
ECL-oma	3	3	3	3	–	–	2	–
Midgut	25	5	24	1	25	3	2	8
Rectal carcinoid	2	–	2	–	–	2	–	–

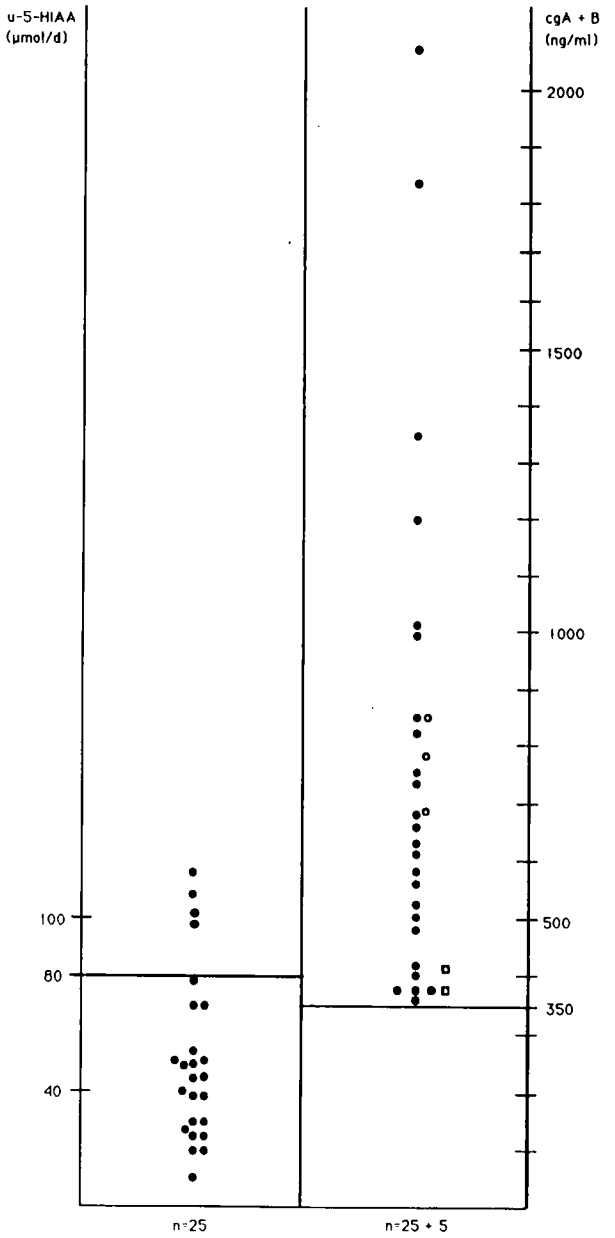

Fig. 1. Levels of plasma chromogranin A + B and urinary 5-HIAA in carcinoid patients with limited disease (25 midgut carcinoids, 3 ECL-omas and 2 rectal carcinoids). Upper reference level for urinary 5-HIAA: 80 µmol/24 h and for plasma chromogranin A + B: 350 ng/ml. ● = midgut carcinoids (n = 25); ○ = ECL-omas (n = 3); □ = rectal carcinoids (n = 2).

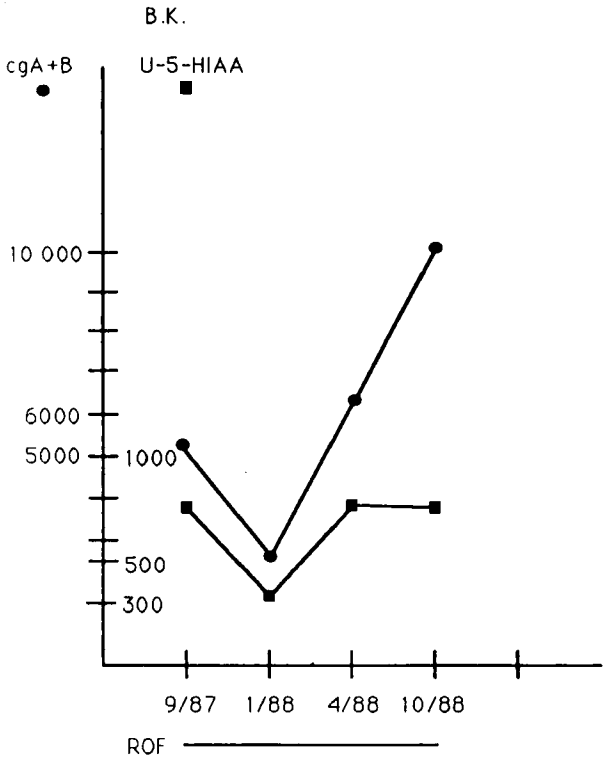

Fig. 2. Plasma chromogranin A + B levels and urinary 5-HIAA levels during Roferon (recombinant interferon α-2a) in a midgut carcinoid. At 3 months, neutralizing interferon antibodies developed.

In 7 patients with pheochromocytomas pre-, per- and postoperative plasma samples were analyzed for chromogranin A + B concentrations (27). The results demonstrate that all 7 patients had increased levels before the operation, chromogranin levels were not much perturbed during surgery and normalized in all patients postoperatively (Fig. 4). Hence, in this small patient material determination of plasma chromogranin A + B was as reliable as urine collections of catecholamines.

CEA, CA-50 and CA 19-9

Carcinoembryonic antigen (CEA), a glycoprotein mainly associated with the surface of tumor cells, has been widely investigated and utilized in the differential diagnosis

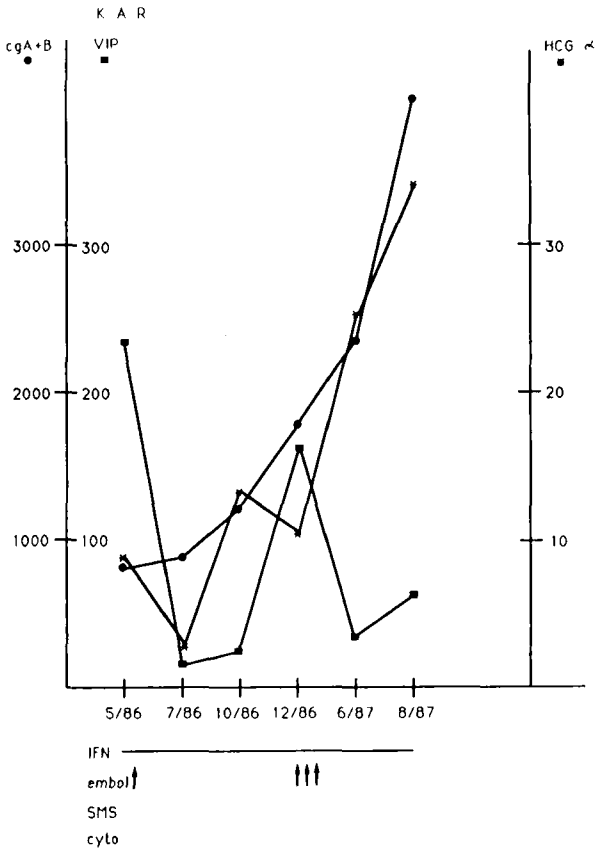

Fig. 3. Plasma chromogranin A + B, VIP and serum HCG-α levels during treatment of a patient with a malignant endocrine pancreatic tumor. Treatment with α-interferon, Sandostatin, chemotherapy as well as embolizations were attempted.

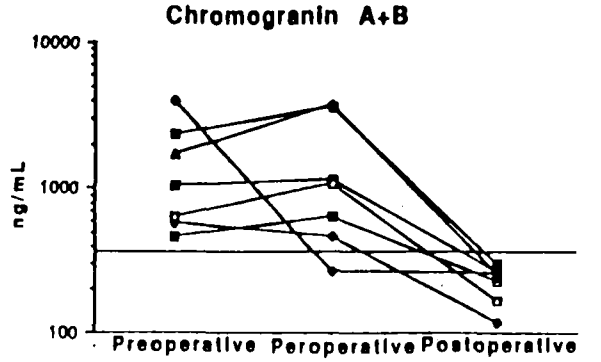

Fig. 4. Plasma chromogranin A + B levels pre-, per- and postoperatively in seven pheochromocytoma patients.

between benign and malignant disease in a variety of cancer, in particular those of the gastrointestinal tract, ovary, lung and breast (28). It can be regarded as a clinical reference marker for gastrointestinal cancer. However, a wide variety of benign conditions may also cause moderate increase of the serum CEA concentration. The sensitivity and specificity of this marker vary between different tumors and also between different studies. A positive correlation between low initial levels of CEA and survival of patients with pancreatic cancer has been demonstrated (29).

Tumor marker tests for CA 19-9, also named gastrointestinal cancer-associated (GICA), and CA-50 are based on monoclonal antibodies to colonic carcinoma cell lines. Immunohistochemical studies have shown that CA 19-9 and CA-50 are expressed in various carcinomas, including pancreatic carcinomas. However, they are also normal constituents of many tissues, e.g. pancreatic ductal epithelium. The CA 19-9 and CA-50 antigens are shed or released into the circulation and are found in increased concentrations in 70–80% of patients with pancreatic cancer (30). Also 50–60% of patients with small resectable carcinomas have elevated CA 19-9 and CA-50 levels, although very high serum concentrations usually indicate advanced disease. Slightly elevated levels can be seen in patients with benign pancreatic disease, especially in acute pancreatitis and in patients with benign obstruction of the common bile duct. CA 19-9 and CA-50 appear to have better diagnostic accuracy for pancreatic cancer than CEA (30).

We have done some preliminary studies of CEA, CA-50 and CA 19-9 in 44 patients with endocrine pancreatic tumors. Half of the patients, 22, had malignant disease, i.e. metastatic spread outside the pancreas as demonstrated by surgery and/or radiography. The remaining 22 patients had benign tumors, i.e. no evident tumor spread outside the pancreas. When serum levels for these three markers

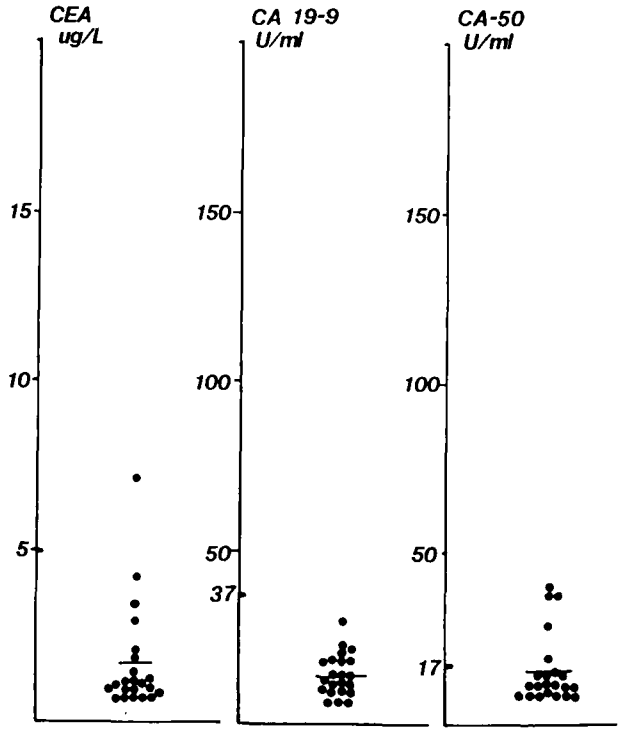

Fig. 5. Serum levels of carcinoembryonic antigen (CEA), CA 19-9 and CA-50 in 22 patients with benign endocrine pancreatic tumors. The upper reference level for CEA was 5 μg/l, for CA 19-9 37 U/ml and for CA-50 17 U/ml.

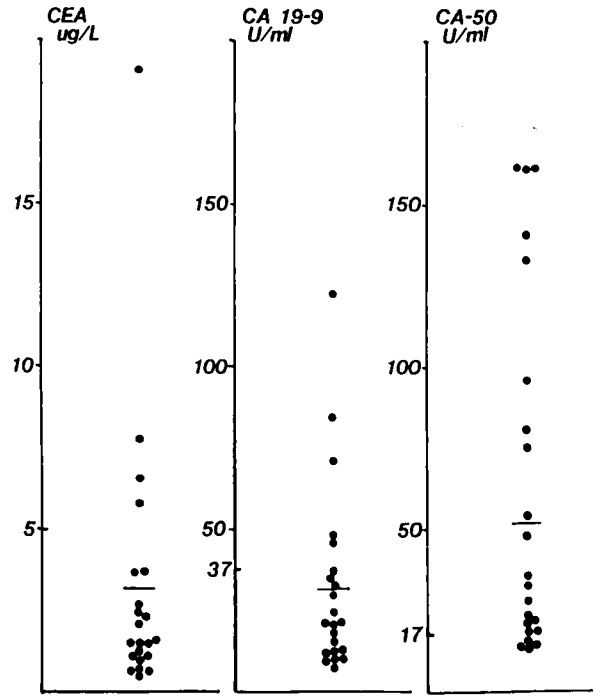

Fig. 6. Serum levels of CEA, CA 19-9 and CA-50 in 22 patients with malignant endocrine pancreatic tumors. The upper reference level for CEA was 5 μg/l, for CA 19-9 37 U/ml and for CA-50 17 U/ml.

were analyzed in the two groups of patients, a quite interesting pattern was disclosed. As is shown in Figs 5 and 6, very few (n = 6) patients (27%) with so-called benign disease had elevations of these markers. Whether these 5 patients have potentially malignant tumors remains to be elucidated. In contrast, 19/22 patients (86%) with malignant tumors had increased concentrations of at least one of the markers. CA-50 was elevated in 18 of the 22 patients (82%) with malignant disease and appears to be the most interesting among the three markers in our patients. The sensitivity and specificity for CA-50 were 81% and 77% respectively.

Survival analyses (Kaplan-Meier) could demonstrate no statistically significant difference between patients with normal and those with increased CA-50 values. However, the p-value was 0.07, and in a larger patient material CA-50 levels might show a correlation with survival. Our results are interesting, since the above mentioned tumor markers have not been investigated in endocrine pancreatic tumors before. Furthermore, our findings could indicate a common origin of exocrine and endocrine pancreatic tumors, a hypothesis that has been postulated by several authors.

Conclusion

When a patient presents symptoms that lead to the suspicion of a gastrointestinal neuroendocrine tumor, determination of serum or plasma levels of peptide markers are employed. Recent data indicate that plasma levels of chromogranins can be used as 'screening markers' for the diagnosis also in patients with a relatively small tumor burden. In the further investigation, however, other tumor markers, including both specific and general markers should be determined in order to assess what type of tumor and syndrome one is dealing with. As a rule, the whole battery of plausible tumor markers should be analysed, once the presence of the tumor has been verified, since most of the tumors produce multiple peptides and later in the course a change of hormone profile might occur. Stimulatory tests still have a value because of a higher diagnostic accuracy than the determination of basal peptide levels in many cases. The search for tumor markers that could indicate a malignant potential and possibly also give prognostic information should continue. The finding of a high frequency of increased levels of CA-50 in malignant endocrine pancreatic tumor should be further explored for the possible use as prognostic markers.

Corresponding author: Dr Barbro Eriksson, Medical Department, University Hospital, S-751 85 Uppsala, Sweden.

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