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Plasma Concentrations of CA-50 in Relation to Tumour Burden in Exocrine Pancreatic Cancer

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The tumour-associated antigen CA-50 was analysed in plasma from 90 patients with cytologically verified exocrine carcinoma of the pancreas, and related to the size of the primary carcinoma, the largest metastasis of the liver and the degree of tumour-transformed liver parenchyme at 143 examinations. The median concentration of CA-50 in the patients with metastases of the liver was significantly higher than in the group lacking metastases. Spearman rank correlation test at three different levels of CA-50 (all values, exceeding 100 U/ml and exceeding 200 U/ml), showed a correlation between CA-50 and diameter of the primary pancreatic carcinoma in patients with liver metastases, but not in the group lacking liver metastases. No correlations were seen between CA-50 levels and size of liver metastasis or degree of tumour-transformed liver parenchyme. Hence, high plasma concentrations of CA-50 in patients with diagnosed or suspected exocrine pancreatic carcinoma could strongly indicate metastatic processes.
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INTRODUCTION

NOVEL MONOCLONAL ANTIBODIES (Mabs) have identified several tumour-associated antigens. Among them, the related CA-50 and CA-19-9 are of special interest in exocrine pancreatic cancer.

The Mab C-50 was derived by immunisation of mice with a human colorectal adenocarcinoma cell-line, COLO 205, and is an IgM type antibody. It reacts with antigens containing the sialylated Lewis a structure and an identical structure devoid of fucose [1, 2]. These antigens are defined as CA-50 antigens and occur on cell membranes as glycolipids (gangliosides) and in plasma as glycoprotein [3].

CA-50 has been identified in plasma and tissue extracts in patients suffering from epithelial malignant tumours, such as colorectal, gastric, pancreatic, uterine, urinary bladder and prostatic, with sensitivities varying between 65 and 90%. It is also expressed in small amounts in meconium, normal adult pancreas and gastric and gallbladder mucosa [4, 5]. For pancreatic cancer, sensitivities of about 80% and specificities of about 60% have been reported from collected series [6].

The Mab C-19-9 was also derived by the same immunisation procedure, but is an IgG-antibody reacting similarly as the C-50 Mab against the sialylated Lewis a structure, but not to the antigen devoid of the fucose residue. The CA-19-9 antigens are also present as glycolipids on cell membranes and glycoproteins in plasma, with distributions in tissues and epithelial malignancies similar to those of CA-50 [7–9].

The immunochemical staining patterns for CA-50 and CA-19-9 in pancreatic adenocarcinomas and cystadenocarcinomas differ only slightly, with CA-50 expression in some CA-19-9 negative specimen [10]. There are also high correlations (r = 0.77–0.93) between serum levels of the two antigens in cancer patients [10, 11]. Although it has been shown that moderately to well-differentiated ductal adenocarcinomas and cyst adenocarcinomas are associated with elevated serum levels of CA-19-9 and CA-50, no clear correlations between tissue levels, degree of histological expression and serum levels are apparent [10, 12, 13].

Regarding serum levels of the antigens in relation to tumour stage, a good correlation has been reported for CA-19-9, but the degree of differentiation did not appear to have any influence [14]. The diagnostic accuracy of serum CA-19-9 in patients with known pancreatic carcinomas has been reported almost comparable to that of findings with computed tomography (CT) [15]. However, another study compared the serum levels of both CA-19-9 and CA-50 and found no correlations to resectability of the carcinomas [11].

None of these studies focussed on the relation of the tumour antigen level in blood and the tumour burden. We have therefore evaluated the concentration of CA-50 in relation to clinically assessed tumour burden. Furthermore, we have examined the effect of metastases and tumour transformation in the liver on the plasma antigen concentrations.

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PATIENTS AND METHODS

90 consecutive, unselected patients with cytologically verified exocrine pancreatic cancer were studied. Age ranged between

Table 1. Size of primary pancreatic carcinoma and frequency distribution at examinations of patients with and without liver metastasis

	Frequency	
Pancreatic carcinoma (cm)	Metastasis	No metastasis
0–2	6	9
>2–4	37	35
>4–6	38	11
>6–8	4	2
>8–10	1	—
Total	86	57

Table 2. Size of largest metastasis in the liver and amount of tumour-transformed liver parenchyme at examination

Liver metastasis (cm)	Frequency	Tumour transformation (%)	Frequency
0–2	45	5	24
>2–4	24	10	30
>4–6	16	15	8
>6	1	20	11
		25	10
		30	1
		>30	2
Total	86		86

38 and 93 years; there were 47 men (average age 66.7 years) and 43 women (67.5 years).

One or a combination of the following methods was used to measure the diameter of the primary carcinoma as well as the diameter of the largest metastasis of the liver and the amount of metastatic transformed liver parenchyme: (1) laparotomy and/or autopsy (about 70%); (2) CT (about 20%); (3) ultrasonography; (4) nuclear magnetic resonance (NMR); (5) endoscopic retrograde cholangiopancreatography (ERCP); and (6) percutaneous transhepatic cholangiography (PTC). Due to the uncertainties inherent in each method, the diameters were estimated in intervals of 2 cm (also according to the TNM classification) and the extent of tumour-transformed liver parenchyme in intervals of 5%. Depending on their survivals, patients were examined a total of 143 times at intervals of 3–6 months (58 patients once, 17 patients twice, 10 patients 3 times, 4 patients 4 times and 1 patient 5 times).

Plasma collected during each examination was frozen at −20°C until analysed for CA-50. An immunoradiometric method [16] (CanAg CA-50 IRMA, Pharmacia CanAg, Gothenburg, Sweden) was used according to the manufacturer's instructions, and the upper reference limit was set at 20 U/ml. Spearman rank correlation and Mann–Whitney test were used for non-parametric statistical evaluations.

RESULTS

83 of the 90 patients had the tumour located in the caput of the pancreas, 3 in the corpus and 3 in the cauda. 1 patient had multiple tumours in the pancreas.

Of the total 143 examinations, detectable metastasis was present on 86 occasions (51 patients at the initial examination and 7 cases who subsequently developed metastases). Table 1 shows the frequency distributions of the size of the primary tumour in the patients with and without liver metastases at the time of examination. In each group, the majority of the tumours had diameters within the interval 2–6 cm.

For the group with liver metastases, frequency distributions of the size of the largest liver metastasis and percentage metastatic transformed liver parenchyme are shown in Table 2. More than half of the patients had a diameter of the liver metastasis and percentage tumour-transformed liver parenchyme of less than 2 cm and 10%, respectively.

Although CA-50 was analysed at each examination (n = 143), the statistical analyses were confined to the initial value for each patient (n = 90), unless a change in the diameter of the primary pancreatic carcinoma was recorded (n = 17). Thus, 107 observations were included, 41 derived from the group lacking detectable metastases (30 patients with unaltered diameter of the primary tumour, 2 patients with increased diameter and the 7 patients with metastasis evident at later examinations), and the remaining 66 from the group with liver metastases (43 patients without increased diameter of the primary tumour, 8 patients with increases and the 7 patients who subsequently developed metastasis). 11 of the observations in the latter group were derived from the 7 patients, who were diagnosed to be free from detectable metastasis of the liver during their first visit, but later developed it. Hence these patients were represented in both the groups but did not jeopardise the statistical evaluation, since the CA-50 concentrations were from different sampling times and diagnostic outcomes.

The concentrations of CA-50 in plasma varied between 10 and 4800 U/ml in patients without detectable liver metastases (mean 520 U/ml, median 190 U/ml). In patients who had liver metastases, the antigen concentration varied between 10 and 622 000 U/ml (mean 12 276 U/ml, median 743 U/ml). Statistical evaluation using the Mann–Whitney test showed that the difference in the median values in these two groups was significant (P = 0.0001), despite the exclusion of the sample with the extreme CA-50 level of 622 000 U/ml (P = 0.0002).

The Spearman rank correlation test was used to compare the relationship between the tumour size of the primary cancer and the plasma concentration of CA-50. The test was performed at three different plasma levels of the antigen: including all the values of CA-50 for the group; excluding all values below 100 U/ml; and excluding all values below 200 U/ml. As seen in Table 3, the correlation between CA-50 and the tumour size is significant at all levels, improving in the cases when CA-50 exceeded 200 U/ml. Similar results were obtained for the correlation test on patients who had the cancer located in the caput (n = 83).

When the patients were subanalysed and the Spearman rank correlation test performed at the three different levels of CA-50 for the group lacking liver metastasis, there was no correlation between the antigen level and the diameter of the pancreatic

Table 3. Correlation of CA-50 and size of pancreatic carcinoma

CA-50	All	>100 U/ml	>200 U/ml
n*	107	88	70
r (Spearman)	0.395	0.382	0.478
P	0.0000	0.0004	0.0001

*No. of observations.

Table 4. Correlation of CA-50 and size of pancreatic carcinoma and effect of liver metastasis

CA-50	All	>100 U/ml	>200 U/ml
No metastasis			
n*	41	25	17
r (Spearman)	0.198	0.261	0.308
P	0.204	0.200	0.218
Liver metastasis			
n*	66	63	53
r (Spearman)	0.403	0.366	0.451
P	0.001	0.005	0.002

*No. of observations.

carcinoma, as shown in Table 4. However, in the patients with detectable liver metastasis, there was a significant correlation between these two parameters, improving at higher antigen concentrations. The rank correlation test was not affected when one sample with an extreme CA-50 concentration of 622 000 U/ml was excluded (r = 0.44 for the group with CA-50 exceeding 200 U/ml).

The CA-50 concentrations were affected in the 17 patients with tumour progress as follows. In the 2 patients without metastasis but with an increase in diameter of the pancreatic cancer, CA-50 was unaltered in 1 case and increased 8-fold in the other. In the 7 cases where the patients subsequently developed detectable liver metastasis, the concentration of CA-50 was unaltered in 1 case and increased around 2-fold in 5 cases and 19-fold in 1 case. The diameter of the primary carcinoma was also increased in these cases. Of the 8 patients with liver metastasis, an increase in the size of the metastasis was observed in 7 patients and the diameter of the primary tumour increased in all cases. The CA-50 concentration was unaltered in 1 case, and increased around 2-fold in 3 patients and around 8-fold in 4 cases.

DISCUSSION

The correlation between serum concentrations of the tumour-associated antigens CA-19-9 and CA-50 and the absence or presence of pancreatic carcinoma in symptomatic patients has been shown to be good, with high sensitivity and specificity [11]. However, only a few studies concerning the relationship between tumour burden and plasma levels of the antigens have been published. Even though the prognosis is still poor, knowledge of the tumour burden is very helpful for the management of a patient suffering from exocrine pancreatic carcinoma. Small tumours can be resected [17]; there are even exceptional reports on disappearance of cancers after cytotoxic treatment [18]; and in incurable cases the mode of palliation is very important.

The methods commonly used to estimate the size of the primary carcinoma, as well as the extent of metastasis in the liver, are based on ultrasonography, CT, laparotomy and, to a lesser extent, ERCP, PTC, angiography and NMR. However, these morphological methods are not optimal, since they do not reflect the total number of tumour cells, but give an estimation that includes oedema, necrotic areas, inflammatory cells, collagenous tissue etc. The synthesis of the tumour-related antigens is instead most likely related to the number of tumour cells. Hence, these methods do not give any cytological or histological information, whereas an immunological assay would have certain theoretical advantages.

The level of the tumour-associated antigen in the plasma depends on several factors, such as mode of release into the circulation (continuous or discontinuous), elimination rate, metabolism and, probably, the locality of the tumour affecting release either into blood circulation or into the gastrointestinal tract. Hence, the plasma level should be regarded as representing a "momentary picture".

In this study we have related the tumour burden, investigated by the common methods to establish tumour morphology, in a group of consecutive patients with exocrine pancreatic carcinoma, to the plasma levels of the tumour associated antigen CA-50. A concentration of 20 U/ml for CA-50 represents the upper reference limit for the method and could be defined as the cut-off limit [19]. Hence values of 100 and 200 U/ml represent 5 and 10 cut-off limits, respectively.

Our study shows that the concentrations of CA-50 do not correlate to the tumour burden of pancreatic cancer without metastases, even though the Spearman rank correlation improved when samples with CA-50 concentrations below 200 U/ml were excluded. However, there is a significant correlation to the tumour burden in patients with liver metastasis. Moreover, since the CA-50 concentrations in the samples from these patients are generally much higher compared to patients without liver metastasis, they affect the correlation to a significant level even when all patients, both without and with liver metastases, are evaluated.

Judging from the poor correlation between CA-50 and size of the primary pancreatic carcinoma in the patients without metastasis, it is apparent that the tumour antigens are not uniformly released into circulation. In our results, increases in CA-50 levels were observed in 14 of the 17 patients where the size of the primary tumour increased. In these 17 patients, 7 developed metastases in the liver subsequently and in 7 cases there was a change in the diameter of the largest metastasis, while 1 remained unchanged and in 2 cases metastases were not evident at any time. Hence, it is plausible that the process of metastasis facilitates release of the antigen into circulation.

In conclusion, the presence of high concentrations of CA-50 in the plasma of patients with clinical suspicion of or known pancreatic carcinoma, may give strong indications of on-going metastatic processes.

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Ornithine Decarboxylase Activity in Mouse Tumour Tissue in Response to Refeeding and Diet Components

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Orthinine decarboxylase (ODC) and S-adenosylmethionine decarboxylase activity (SAMD) were measured in tumour tissue in mice during periods of starvation (24 h) and refeeding. Starvation led to a 60% reduction in tumour ODC activity. Refeeding normalised the activity within 4 h. Restitution in ODC activity, representing *de novo* enzyme synthesis, preceded DNA resynthesis. SAMD activity continued to fall along the increase in ODC activity during refeeding, while difluoro-methyl-ornithine (DFMO) caused a compensatory increase in SAMD activity as expected. A fall and regain in ODC activity was associated with inhibition and regrowth of the tumour. Starvation–refeeding was not related to any decrease in tumour polyamine concentrations, while systemic DFMO blockade was. Glucose stimulated ODC when refed orally, but not when given systemically. Tumour ODC activity was not decreased in refed mice by anti-insulin, a procedure that antagonised insulin's bioactivity. Exogenous insulin did not stimulate tumour ODC activity. Our results suggest that gastrointestinal metabolism of carbohydrates stimulates the release of a factor, which initiates both ODC activity and DNA synthesis in tumour cells. This factor was not insulin.
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INTRODUCTION

POLYAMINES AND ornithine decarboxylase (ODC) activity have received interest as potential markers and regulators in malignancy and cell proliferation [1]. We recently reported the presence of high activity of ornithine decarboxylase in human head and neck tumours, and high enzyme activity correlated to short survival in these patients [2]. It is well established that ODC is controlled by either dietary factors [3, 4] or hormones and growth factors [5–7] in both transformed [8] and non-transformed [9] cells. Therefore, it is possible that refeeding of cancer patients may stimulate polyamine synthesis and thereby initiate cell proliferation [2, 10], although it has been difficult to demonstrate this effect [10–12]. Tumour ODC activity and polyamine levels may be simple and sensitive parameters to monitor initiation of RNA and DNA synthesis in biopsy specimens from human tumours in response to nutrition and tumour treatment [13]. Therefore, the objectives of this study were to evaluate whether starvation–refeeding activates ODC activity