Monoclonal antibody defines CA 19-9 in pancreatic juices and sera

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SUMMARY In a retrospective study pancreatic juice samples (n=213) and corresponding serum samples (n=110) were assayed for their concentration of monoclonal antibody defined CA 19-9/GICA (gastrointestinal cancer associated antigen). Serum CA 19-9 values were found to be good diagnostic and discriminating markers for pancreatic disorders and were raised (>50 u/ml) in more than 80% of the pancreatic cancer patients compared to 8.5% of the pancreatitis group and none of the control group. In contrast pancreatic juice CA 19-9 values showed a considerable overlap between groups. On the basis of recent molecular data on the monoclonal antibody 19-9 defined antigen(s) – that is, monosialoganglioside, mucin – it is proposed that the discrepancies between serum and pancreatic juice findings are due to a specific undirected endocrine release of the mucin into sera in pancreatic tumour patients while in pancreatic juices of all diagnostic groups high CA 19-9 activities are either owing to coexistence of glycolipid and mucin or that the latter is a physiologically exocrine product.

Although various tumour markers have been described for pancreatic cancer¹⁻⁷ they have so far been of limited diagnostic value. This has been either because of insufficient specificity or lack of standardised quantitation systems.

By means of hybridoma technology a radio-immunometric assay has been recently developed and first radio-immunometric quantitations of a carbohydrate determinant [(a sialylated lacto N-fucopentaose II)⁸] by monoclonal antibody 19-9⁹⁻¹¹ in sera of patients suffering from GI-tract cancer have revealed a high specificity (98.5%) and sensitivity (79%) for pancreatic cancer.¹²

In this retrospective study we have analysed pancreatic juice samples and sera of patients with pancreatic and extrapancreatic diseases by use of CA 19-9 radioimmunoassay.

Methods

PATIENTS

Pancreatic juice samples of 327 patients were

collected. On the basis of standard diagnostic methods – that is, ERCP, ultrasound, computed tomography, angiography, laparotomy, histology, follow up or necropsy findings, the data of 213 patients were further analysed: group 1 consisted of 68 patients with pancreatic cancer including cancer of the papilla of Vater. Group 2 consisted of 71 patients with various forms of chronic pancreatitis. Group 3 consisted of 74 patients in whom a disorder of the pancreas had been excluded and their symptoms could be related to non-malignant extrapancreatic diseases: chronic forms of hepatitis (n=8), liver cirrhosis (n=5), gastritis (n=15), gastric/duodenal ulcer (n=15), cholangitis/cholelithiasis (n=18), pyelonephritis (n=1), functional gastrointestinal disorders (n=12). This group is taken as the control group.

TECHNIQUES

Sterile 4 ml tubes were each filled with 2 mg trypsin inhibitor (Serva, Inc, Heidelberg, FRG), aprotinin (2000 Kallikrein inactivator units, Bayer, Inc, Leverkusen, FRG), and 5 mg epsilon-aminocaproic acid (Merck, Inc, Darmstadt, FRG). Pancreatic juices obtained by transduodenal pancreatic duct cannulation under simultaneous

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secretin stimulation (50 units intravenously/Boots, Nottingham, UK) were transferred to the lyophilised protease inhibitor containing tubes and centrifuged for 5 minutes at 3000 g, 4°. The supernatants were stored at -80°C until further analyses. Serum samples (group 1 n=46, group 2 n=37, group 3 n=27), were obtained on completion of the pancreatic juice collection.

All samples were analysed in duplicate for CA 19-9 in a solid phase radioimmunoassay according to the manufacturers recommendation (Centocor, Malvern, PA, USA). Quantities of CA 19-9 were expressed in units, which is an arbitrary activity corresponding to approximately 0.8 ng of purified antigenic material.¹²

For analysing the results the statistical analysis system, Kruskal-Wallis-Test,¹³ was used to detect differences between the groups. This is a non-parametric procedure of one-way analysis of variance on ranks.

Results

CA 19-9 was measured in 213 pancreatic juice samples. Raised concentrations (> 37 U/ml) were found in all diagnostic groups (Table). Especially high values (>10⁴ U/ml) were obtained in pancreatic cancer patients (1) (p<0.01 Kruskal-Wallis-Test).

Percentual distribution of pancreatic juice CA 19-9 values were calculated for group 1, 2, and 3 to enable their evaluation differentially (Fig. 1). Although in pancreatic cancer patients (1) hardly any CA 19-9 values below 10² U/ml were found there was a large overlap between groups within the range 10²-10⁴ U/ml CA 19-9. On the other

Fig. 1 Percentual distribution of pancreatic juice CA 19-9 values. Group 1: pancreatic cancer, group 2: pancreatitis, group 3: controls.

hand 31% of the pancreatic cancer patients (1) compared to 12.7% of the pancreatitis group (2) and 12% of the controls (3) showed pancreatic juice CA 19-9 concentrations of more than 10⁴ U/ml.

Corresponding serum CA 19-9 results were available in 110 patients (Table). Significantly (p<0.01, Kruskal-Wallis-Test) raised levels of CA 19-9 (>37 U/ml) were found in the pancreatic cancer group (1). In contrast, the other groups and especially that of pancreatitis patients showed lower values (Fig. 2). When taking cutoff values of 37 U/ml 85% of the pancreatic cancer patients (1) but only 14% of the pancreatitis patients and 15% of the control group showed scores beyond this. By raising the cutoff value to 50 U/ml the discrimination between the pancreatic cancer group (81% >50 U/ml) and group 2 (8.5% >50 U/ml) and group 3 (0 >50 U/ml), respectively, could be improved.

Taking into account that the amount of a tumour marker secreted or shed by tumour cells will also depend on the tumour mass we tried to correlate CA 19-9 scores with pancreatic tumour staging. For this purpose CA 19-9 values obtained from pancreatic juice (n=63) or serum analyses (n=41) were related to TNM classification¹⁴ of the corresponding patients. As outlined in Fig. 3 and 4 there was a majority of patients with advanced pancreatic cancer and high values of CA 19-9 in sera (>10² U/ml) and pancreatic juices (>10³ U/ml) respectively. It is noteworthy, however, that six out of seven patients suffering from pancreatic cancer of limited extension or lacking detectable metastases showed CA 19-9 concentrations of more than 50 U/ml in their sera.

Table CA 19-9 values in pancreatic juices and sera given are range and for showing asymmetric distribution median and quartiles 1 and 3. Kruskal-Wallis-Test shows significance of differences. Group classification as in text.

CA 19-9 (U/ml) in pancreatic juices					
Group	Range	Median	Quar- tile 1	Quar- tile 3	N
1	250-400 000	5 650	2 100	18 500	68
2	17-150 000	2 650	900	6 100	71
3	5-42 000	1 100	165	3 300	74
CA 19-9 (U/ml) in sera					
1	5-59 000	900	65	3 300	46
2	5-86	7.5	5	17.7	37
3	5-45	5	5	15.0	27

p<0.01 Kruskal-Wallis-Test

Fig. 2 Percentual distribution of serum CA 19-9 values. Group 1: pancreatic cancer, group 2: pancreatitis, group 3: controls.

TNM Distribution of serum CA 19-9 values (n=41)

		CA 19-9 (U/ml)				N
		10 - 50	50 - 100	100 - 1000	>1000	
Gr. I.1.	T ₁ N ₀ M ₀	•			••	7
	T ₂ N ₀ M ₀					
	T ₃ N ₀ M ₀		•	•	••	
Gr. I.2.	T ₁ N ₁ M ₀	•				6
	T ₂ N ₁ M ₀		•			
	T ₃ N ₁ M ₀	•	••		•	
Gr. I.3.	T _x N _x M ₁	••••	••••	•••••	•••••	28

Fig. 3

Fig. 3 & 4 CA 19-9 values in sera (Fig. 3) and pancreatic juices (Fig. 4) of pancreatic cancer patients correlated to TNM-staging. T₁: tumour limited to the pancreas, T₂: transgression of the organ boundary, T₃: Infiltration of neighbouring organs, Tx: T₁=T₃, No: no lymphogenic metastases, N₁ lymphogenic metastases, Mo: no haematogenic metastases, M₁: haematogenic metastases.

TNM Distribution of pancreatic juice CA 19-9 values (n=63)

		CA 19-9 (U/ml)				N
		10 - 100	100 - 1000	1000 - 10000	>10000	
Gr. I.1.	T ₁ N ₀ M ₀		•	•	••	12
	T ₂ N ₀ M ₀				•	
	T ₃ N ₀ M ₀		••	••	•••	
Gr. I.2.	T ₁ N ₁ M ₀				•	7
	T ₂ N ₁ M ₀			•		
	T ₃ N ₁ M ₀			•••	••	
Gr. I.3.	T _x N _x M ₁	•	•••	•••••	•••••	44

Fig. 4

Discussion

This study has confirmed first results^{10-12 15} that serum CA 19-9 is a good marker for pancreatic disease. Thus in pancreatic cancer more than 80% of the patients had CA 19-9 values greater than 37 U/ml in contrast with 14% of the pancreatitis group and 15% of the patients without demonstrable pancreatic disorder. Raising the cutoff value to 50 U/ml the diagnostic accuracy could be improved, thus only 8.5% of the pancreatitis patients and none of the patients with non-malignant extrapancreatic disorders showed higher scores in contrast with 81% of the pancreatic carcinoma patients.

Regarding the diagnostic potential of the serum CA 19-9 for pancreatic cancer it has to be considered, however, that the majority of pancreatic tumour patients in this retrospective study were suffering from advanced cancer. This is of particular interest because recent analyses of CA 19-9 in colorectal cancer patients showed that the number of positive results correlated well with the tumour extension thus declining in Duke's A patients when compared with Duke's C patients.¹⁶ In view of this a more restricted diagnostic value of serum CA 19-9 has to be expected in patients suffering from pancreatic tumours with limited extension. Preliminary results obtained by correlating tumour staging with CA 19-9 scores presented in this study do not exclusively rule out that patients with lower tumour extension could be detected by high CA 19-9 scores, a matter which requires further investigation. For diagnostic purposes – that is, tumour localisation in patients with high serum CA 19-9 values and to distinguish between benign and malignant pancreatic lesions CA 19-9 concentrations were additionally determined in pancreatic juices. Despite the fact that the pancreatic cancer group showed no values below 10² U/ml and that more than 30% of this group had CA 19-9 values greater than 10⁴ U/ml there was a considerable overlap of results between the pancreatic cancer group and the other diagnostic groups. In regard to the apparent discrepancy between serum CA 19-9 and pancreatic juice CA 19-9 values, as shown in this study, recent intriguing data on the chemical nature of monoclonal antibody 19-9 reactive antigen(s) may be crucial. First antigen analyses revealed that the antigen defined by monoclonal antibody 19-9 was a monosialoganglioside.¹⁷ Immunoperoxidase studies of various malignant (carcinomas of the lung, thyroid, urinary bladder, ovary, prostate) and normal adult tissues (lung, biliary ducts, pancreas) as well as of fetal organs (gastrointestinal tract,

biliary ducts, pancreas) showed the presence of monoclonal antibody 19-9 defined antigen(s).¹⁸⁻¹⁹ Taking the results of enzymatic studies in combination with immunohistological data it was suggested that the antigenic moieties defined by monoclonal antibody 19-9 were predominantly glycoproteins.¹⁹ This is supported by previous data²⁰ which show that the 19-9 antigen extracted from the sera of patients with pancreatic or gastrointestinal tumours is a mucin (Mr > 5 × 10⁶) which carries the same epitope as the monosialoganglioside. Thus it may be concluded that the overall high concentrations of CA 19-9 in pancreatic juices in this study may be either due to the simultaneous determination of the glycolipid and mucin antigen or that the 19-9 defined mucin is a physiologically exocrine secreted antigen which is secreted in an endocrine fashion in the malignant state.

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