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SERUM TRYPSINOGEN-2 AND TRYPSIN-2-α_1-ANTITRYPSIN COMPLEX IN MALIGNANT AND BENIGN DIGESTIVE-TRACT DISEASES. PREFERENTIAL ELEVATION IN PATIENTS WITH CHOLANGIOCARCINOMAS

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Serum concentrations of trypsinogen-2 and trypsin-2-α_1-antitrypsin (trypsin-2-AAT) were determined in 145 patients with malignant and 61 with benign digestive-tract diseases. The validity of these tests for detection of cancer was compared with that of CA19-9 and CEA. Elevated levels of trypsinogen-2 (>90 μg/l) and trypsin-2-AAT (>25 μg/l) were found in 46% and 42%, respectively, of patients with malignant disease and the levels of trypsinogen-2 were significantly higher than in those with benign disease ($p < 0.005$). High trypsinogen-2 and trypsin-2-AAT concentrations were found most often in patients with biliary and pancreatic cancer, but also in benign obstructive biliary disease. Our results suggest that trypsinogen-2 and trypsin-2-AAT are new potential markers for cholangiocarcinomas.

Pancreatic trypsinogen is a major proteolytic enzyme produced by pancreatic acinar cells (Mallory and Travis, 1973). It is excreted into pancreatic juice as 2 major isoenzymes, trypsinogen-1 and trypsinogen-2 (Robinson *et al.*, 1972). Trypsinogen plays a role in the pathophysiology of pancreatitis (Ohlsson and Eddeland, 1975) and increased serum levels occur in patients with acute pancreatitis and in infants with cystic fibrosis (Elias *et al.*, 1977; Steinberg *et al.*, 1985; Crossley *et al.*, 1979; Itkonen *et al.*, 1990). Inadvertent intrapancreatic activation of trypsinogen to trypsin occurs in acute pancreatitis (Borgström and Ohlsson, 1978). When it reaches the circulation, active trypsin is inactivated by the major trypsin inhibitors in serum, α_2-macroglobulin and α_1-antitrypsin (AAT) (Ohlsson, 1988). We have developed sensitive time-resolved immunofluorometric assays (IFMA) for trypsinogen-2 and the trypsin-2-AAT complex (Itkonen *et al.*, 1990; Hedström *et al.*, 1994) and have shown that trypsinogen-2 in serum (Itkonen *et al.*, 1990) and in urine (data not shown) and trypsin-2-AAT in serum (Hedström *et al.*, 1994) are strongly elevated in acute pancreatitis.

Elevated levels of total trypsin immunoreactivity in serum have been observed in patients with pancreatic cancer (Elias *et al.*, 1977; Lake-Bakaar *et al.*, 1979; Fabris *et al.*, 1990; Moller-Petersen and Smidt-Jensen, 1983). Because trypsinogen-2 and trypsin-2-AAT are better markers for pancreatitis than trypsinogen-1 (Itkonen *et al.*, 1990; Hedström *et al.*, 1994), we wanted to evaluate their usefulness as markers for pancreatic cancer. Since tumor-associated trypsin-2 is expressed by various tumor-cell lines (Koivunen *et al.*, 1991; Miszczuk-Jamska *et al.*, 1991) we also studied patients with other digestive-tract cancers. Patients with benign digestive-tract diseases were used as controls. The validity of these markers was compared with that of the routinely used tumor markers CA 19-9 and carcinoembryonic antigen (CEA).

MATERIAL AND METHODS

Patients and serum samples

Serum samples were obtained from 145 patients with malignant and 61 with benign digestive-tract diseases from the 2nd and 4th Departments of Surgery, Helsinki University Central Hospital, from June 1990 through February 1995 (Table I). Samples from patients with malignant disease were obtained before treatment and stored at −20°C or −70°C until analyzed. The diagnosis of cancer was verified by histology or cytology. Classification according to stage was based on data from clinical examination, imaging methods and findings at operation. Fifteen patients (10%) with malignant disease had a local tumor and all other patients had either locally spread or metastasized disease (Table I). The diagnosis of benign disease was based on clinical, radiological, endoscopical or surgical findings or on histopathological examination.

Methods

Trypsinogen-2 and trypsin-2-AAT were determined by time-resolved immunofluorometric assays (IFMA) (Itkonen *et al.*, 1990; Hedström *et al.*, 1994). The trypsinogen-2 IFMA uses the monoclonal antibody 14D4 as catcher and 14F10 as tracer. The trypsin-2-AAT IFMA uses the 14F10 antibody as catcher and a polyclonal rabbit antibody to AAT as tracer (Dako, Glostrup, Denmark). The catcher antibody is coated onto microtitration wells and the tracer antibody labeled with a europium-chelate. Sample and assay buffer are pipetted into the coated wells. After incubation for 1 hr, the wells are emptied, washed twice with wash solution by an automated washer, and filled with assay buffer containing tracer antibody. After further incubation for 1 hr, the wells are washed 4 times. Enhancement solution is added to each well, and after 5 min the fluorescence is measured with a 1234 Delfia Research Fluorometer (Wallac, Turku Finland). The reference range for trypsinogen-2 is 18–90 μg/l and that for trypsin-2-AAT 2.3–12 μg/l (Hedström *et al.*, 1994). To increase the specificity of trypsin-2-AAT, we analyzed sensitivity at a higher cut-off, 25 μg/l (Fig. 1*b*).

The serum concentrations of CA 19-9 and carcinoembryogenic antigen (CEA) were determined by commercially available CA 19-9 and CEA assays (Bayer, Immuno-1, Tarrytown, NY). Cut-off values of 37 kU/l and 3 μg/l, respectively, were used.

Alkaline phosphatase (AFOS), bilirubin and amylase were determined by routine methods and the values were obtained from clinical records, when available. Cut-off values of 275 U/l for AFOS, 20 μmol/l for bilirubin and 300 U/l for amylase were used.

Statistical analysis

The ability of various tests to differentiate between malignant and benign digestive-tract disease was estimated on the basis of sensitivity and specificity at various cut-off levels. The validity of the tests was further evaluated by receiver-operating

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Abbreviations: AP, acute pancreatitis; AAT, a_1-antitrypsin; ROC, receiver-operating characteristic.

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TABLE I - SURVEY OF THE DIAGNOSIS, NUMBER OF CASES AND STAGES IN BENIGN AND MALIGNANT DIGESTIVE-TRACT DISEASES

Diagnosis	n	Stage			
		I	II	III	IV
Malignant disease					
Cancer of ampulla Vateri	5	0	4	1	0
Hepatocellular carcinoma	17	1	0	11	5
Biliary-tract cancer	20	2	2	4	12
Pancreatic cancer	60	7	14	16	23
Gastric cancer	15	1	5	1	8
Colorectal cancer	28	4[1]	10[1]	6[1]	8[1]
Benign disease					
Liver cirrhosis	10				
Obstructive biliary disease	18				
Non-obstructive biliary disease	17				
Chronic pancreatitis	9				
Pancreatic pseudocysts	7				

[1]Staging according to Dukes (Dukes and Bussey, 1985).

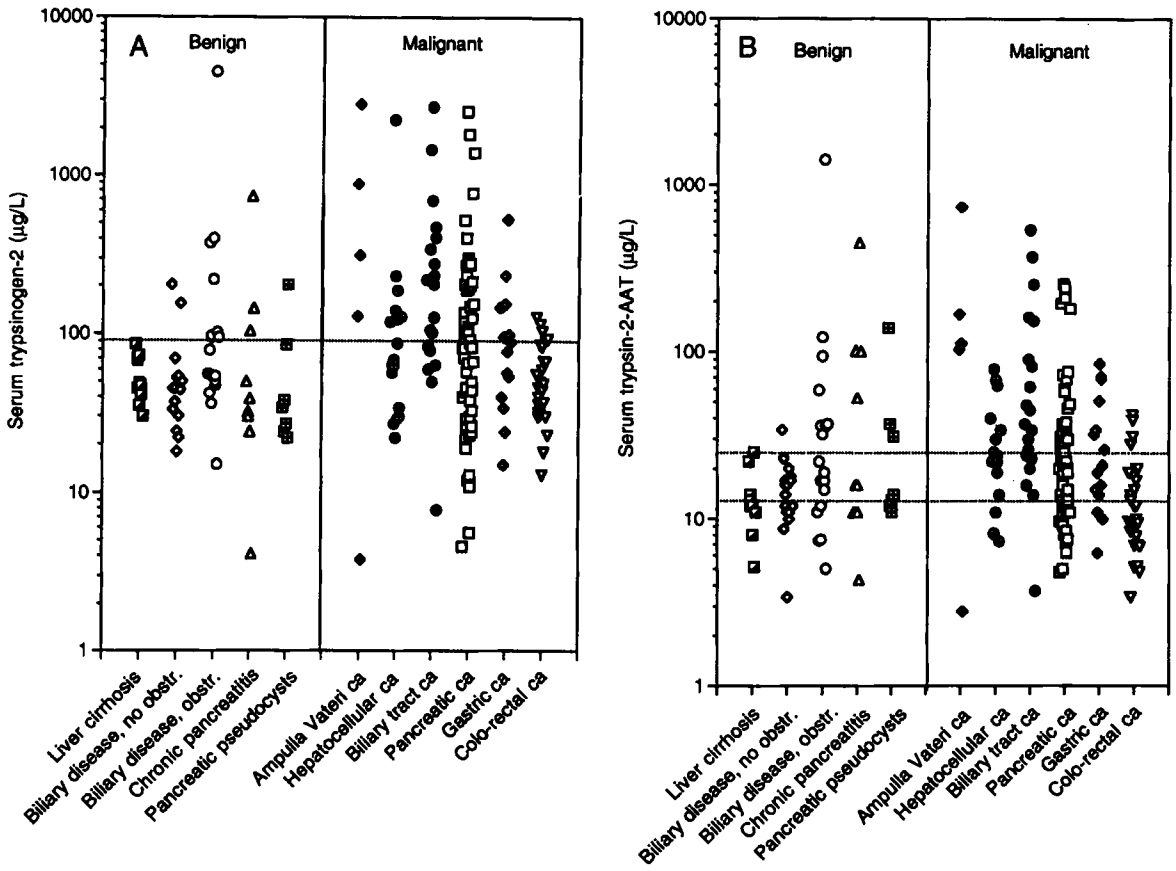


FIGURE 1 - Serum trypsinogen-2 *(a)* and trypsin-2-AAT *(b)* levels in benign and malignant digestive-tract diseases. The upper reference limit is marked with a dashed line. In *(b)* the upper dashed line indicates the cut-off used in this study. Ca = cancer.

characteristic (ROC) curve analysis. The area under the curve (AUC) of the ROC plot describes the accuracy of the test: 1 indicates 100% sensitivity and specificity and 0.5 no discriminatory power (Zweig and Campbell, 1993). A univariate z-score test was performed with the CLABROC program (C.E. Metz, Department of Radiology, University of Chicago Medical Center, Chicago, IL) to estimate the significance of the difference between the areas under the ROC curves. The correlation between the values of trypsinogen-2 and trypsin-2-AAT, CA 19-9, bilirubin, AFOS and amylase was calculated with the least square method. The significance of the differences between the values of various groups was calculated with the Mann-Whitney *U* test.

RESULTS

Trypsinogen-2 and trypsin-2-AAT in digestive-tract cancers

In all digestive-tract cancers, elevated trypsinogen-2 levels were observed in 67 patients (46%) and trypsin-2-AAT levels in 61 patients (42%) (Fig. 1*a*, *b*) and the levels of trypsinogen-2 were significantly higher than in patients with benign disease ($p < 0.005$). The highest trypsinogen-2 and trypsin-2-AAT

TABLE II - CONCENTRATIONS OF TRYPSINOGEN-2, TRYPSIN-2-AAT, CA 19-9 AND CEA IN PATIENTS WITH MALIGNANT AND BENIGN DIGESTIVE-TRACT DISEASES

Diagnosis	n	Trypsinogen-2		Trypsin-2-ATT		CA 19-9		CEA	
		Median (μg/l)	Range (μg/l)	Median (μg/l	Range (μg/l)	Median (kU/l)	Range (kU/l	Median (μg/l)	Range (μg/l)
Malignant disease									
Cancer of ampulla Vateri	5	315	3.8–2800	110	2.8–740	17	4.0–2900	1.6	0.3–2.0
Hepatocellular carcinoma	17	78	22–2300	22	7.3–79	21	1.4–8100	2.2	0.8–290
Biliary-tract cancer	20	170	7.8–2700	41	3.7–540	220	0.1–21000	1.7	0.5–22
Pancreatic cancer	60	90	4.6–2600	21	4.8–260	520	0.6–67000	4.3	0.3–890
Gastric cancer	15	89	15–530	21	6.2–85	18	0.2–8900	1.1	0.4–53
Colorectal cancer	28	50	13–130	9.9	3.4–42	12	0.1–940	4.3	0.3–250
Benign disease									
Liver cirrhosis	10	58	30–86	12	5.1–22	27	0.1–161	1.6	0.3–10
Obstructive biliary disease	18	56	15–4500	19	5.0–1400	22	0.1–1600	0.9	0.2–3.2
Non-obstructive biliary disease	17	45	18–204	14	3.4–34	4.6	0.1–44	1.0	0.1–2.3
Chronic pancreatitis	9	39	4.1–740	16	4.3–450	7.8	0.1–470	1.6	0.8–5.5
Pancreatic pseudocysts	7	34	22–200	14	11–140	4.2	2.2–300	1.0	0.5–2.6

TABLE III - FREQUENCY OF ELEVATED TRYPSINOGEN-2, TRYPSIN-2-AAT, CA 19-9 AND CEA VALUES IN PATIENTS WITH MALIGNANT AND BENIGN DIGESTIVE-TRACT DISEASES

Diagnosis	Trypsinogen-2 > 90 μg/l		Trypsin-2-AAT > 25 μg/l		CA 19-9 >37 kU/l		CEA >3 μg/l	
	n	(%)	n	(%)	n	(%)	n	(%)
Malignant disease								
Cancer of ampulla Vateri	4	(80)	4	(80)	1	(20)	0	(0)
Hepatocellular carcinoma	7	(41)	6	(35)	6	(35)	3	(18)
Biliary tract cancer	13	(65)	14	(70)	14	(70)	5	(25)
Pancreatic cancer	30	(50)	26	(43)	48	(80)	43	(72)
Gastric cancer	7	(47)	7	(47)	7	(47)	5	(33)
Colorectal cancer	6	(21)	4	(14)	10	(36)	16	(57)
All malignant	67	(46)	61	(42)	86	(59)	72	(50)
Benign disease								
Liver cirrhosis	0	(0)	0	(0)	5	(50)	2	(20)
Obstructive biliary disease	8	(44)	8	(44)	7	(39)	2	(11)
Non-obstructive biliary disease	2	(12)	1	(6)	1	(6)	0	(0)
Chronic pancreatitis	3	(33)	4	(44)	3	(33)	1	(11)
Pancreatic pseudocysts	1	(14)	3	(43)	2	(29)	0	(0)
All benign	14	(23)	16	(26)	18	(30)	5	(8)

levels were seen in patients with cancer of the ampulla Vateri, cholangiocarcinoma and pancreatic cancer. Elevated trypsinogen-2 and trypsin-2-AAT values were observed in 4 patients (80%) with cancer of the ampulla Vateri, in 13 (65%) and 14 (70%) of those with biliary tract cancer and in 30 (50%) and 26 (43%) of those with pancreatic cancer, respectively (Fig. 1*a, b;* Table III). In patients with colorectal cancer, only 6 (21%) and 4 patients (14%), respectively, showed levels above the cut-off (Table II). Patients with Stage-4 disease had a significantly higher ($p < 0.05$) median concentration of trypsinogen-2 and trypsin-2-AAT than those with Stage 1–3 diseases.

Trypsinogen-2 and trypsin-2-AAT in benign digestive-tract diseases

In benign digestive-tract diseases, elevated levels of trypsinogen-2 were observed in 14 (23%) patients and trypsin-2-AAT in 16 (26%) patients (Fig. 1*a, b*). Thus, the specificity for exclusion of these conditions was 77% and 74%, respectively. If the conventional upper reference limit for trypsin-2-AAT (12 μg/l) was used, elevated levels were seen in 62% of patients, decreasing specificity to 38%. Therefore, the higher cut-off for trypsin-2-AAT was used in this study. The highest trypsinogen-2 and trypsin-2-AAT levels in benign diseases were seen in patients with obstructive biliary disease, elevated levels occuring in 8 patients (44%). Trypsinogen-2 and trypsin-2-AAT were only occasionally elevated in patients with chronic pancreatitis, pancreatis cysts and non-obstructive biliary disease (Fig. 1*a, b;* Table III).

Comparison of trypsinogen-2, trypsin-2-AAT and CA 19-9, CEA

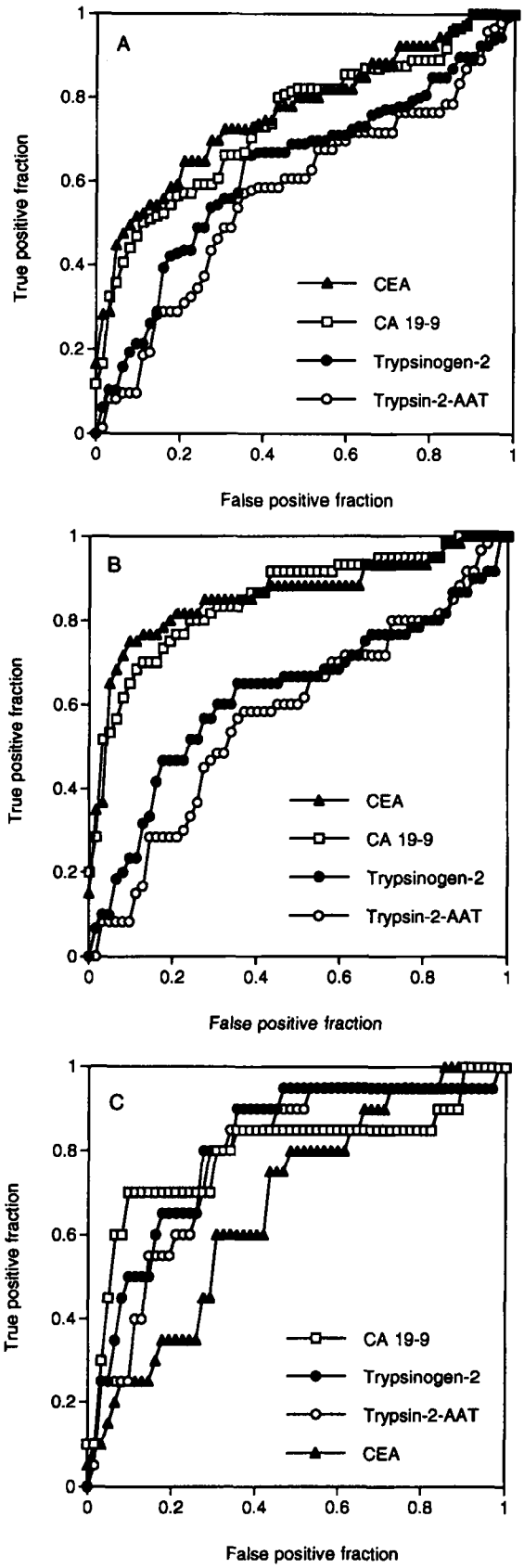
For differentiation between all malignant and benign diseases, the area under the ROC curve (AUC) was 0.738 for CEA (SD = 0.035), 0.712 for CA 19-9 (SD = 0.037), 0.607 for trypsinogen-2 (SD = 0.040) and 0.540 for trypsin-2-AAT (SD = 0.042). The AUC of CA 19-9 and CEA was larger than that of trypsinogen-2 ($p < 0.04$) and trypsin-2-AAT ($p < 0.03$), but AUC of CA 19-9 was not significantly different from that of CEA ($p > 0.05$) (Fig. 2*a*).

AUC for differentiation between pancreatic cancer and benign diseases for CA19-9 (0.834, SD = 0.037) and CEA (0.828, SD = 0.038) were significantly larger than those for trypsinogen-2 (0.601, SD = 0.051, $p < 0.001$) and trypsin-2-AAT (0.537, SD = 0.052, $p < 0.001$). Thus, CA 19-9 and CEA were clearly superior to trypsinogen-2 and trypsin-2-AAT (Fig. 2*b*).

When differentiating biliary tract cancer from benign disease, AUC was 0.796 for trypsinogen-2 (SD = 0.056), 0.761 for CA 19-9 (SD = 0.068), 0.760 for trypsin-2-AAT (SD = 0.058) and 0.643 for CEA (SD = 0.069). Thus, trypsinogen-2 had slightly larger AUC than CA 19-9, trypsin-2-AAT and CEA, but the differences were not statistically significant ($p > 0.05$) (Fig. 2*c*).

CEA had the highest AUC value for differentiation of colorectal cancer from benign diseases (0.759, SD = 0.062) followed by CA 19-9 (0.587, SD = 0.064), trypsinogen-2 (0.453, SD = 0.063) and trypsin-2-AAT (0.343, SD = 0.062). Only AUC for CEA differed significantly ($p < 0.01$) from that of the other markers.

The trypsinogen-2 and trypsin-2-AAT values correlated strongly in both benign (r = 0.98, $p < 0.0001$) and malignant diseases (r = 0.77, $p < 0.0001$). CA 19-9 did not correlate with either trypsinogen-2 (r = 0.14) or trypsin-2-AAT (r = 0.12), nor did CEA correlate with trypsinogen-2 (r = 0.01) or trypsin-2-AAT (r = 0.07).



Correlation between trypsinogen-2 and AFOS, bilirubin and amylase

To clarify the effect of biliary obstruction and pancreatic irritation on serum trypsinogen-2, the values were correlated with serum AFOS, bilirubin and amylase in patients with benign and malignant (cholangiocarcinoma) biliary diseases (Fig. 3*a–f*). In benign biliary diseases, AFOS and bilirubin were available from all patients and amylase from 21 (60%) patients. In biliary tract cancers AFOS was available from 18 patients (90%), bilirubin from 17 (85%) and amylase from 15 (75%).

There was a significant correlation between trypsinogen-2 and bilirubin ($r = 0.49$, $p < 0.05$) in patients with benign biliary diseases, but not in patients with cholangiocarcinoma ($r = 0.19$, $p = 0.44$). The correlation with AFOS was significant in patients with cholangiocarcinoma ($r = -0.55, p < 0.05$) but not in patients with benign biliary diseases ($r = 0.01$, $p = 0.93$). There was no significant correlation with amylase in patients with benign biliary diseases ($r = 0.07, p = 0.76$) nor in patients with cholangiocarcinoma ($r = 0.41, p = 0.12$).

DISCUSSION

Elevated serum concentrations of trypsinogen-2 and trypsin-2-AAT were found in almost half of the patients with malignant digestive-tract disease and in 1 out of 4 of those with benign disease. Very strongly elevated values were observed in patients with pancreatic cancer, cholangiocarcinoma and cancer of the ampulla Vateri. In gastric cancer, the elevation was moderate and in colorectal cancer it was only marginal. The values of trypsinogen-2 and trypsin-2-AAT correlated strongly in patients with both benign and malignant disease, but trypsinogen-2 was generally the more accurate marker for cancer. This appears to be related to the fact that trypsin-2-AAT is the better marker for pancreatitis (Hedström *et al.*, 1994). It also suggests that the mechanisms causing elevation of trypsinogen immunoreactivity are different in patients with cancer and in those with pancreatitis.

In patients with cholangiocarcinoma, clearly elevated levels of trypsinogen-2 (65%) and trypsin-2-AAT (70%) were present in a majority of cases. The strong elevation of these markers in patients with cholangiocarcinoma was unexpected. We therefore studied whether the increase was due to disturbed liver function, which is a common non-specific cause of elevated serum levels of many tumor markers (Collazos *et al.*, 1993). Interestingly, all 10 patients with liver cirrhosis had normal trypsinogen-2 and trypsin-2-AAT levels. Thus, hepatic dysfunction does not appear to be the mechanism causing elevation of trypsinogen-2 and trypsin-2-AAT. Although many patients with high trypsinogen-2 and trypsin-2-AAT values had biliary obstruction, the correlation with AFOS, bilirubin or amylase was only low to moderate. As evidenced by low amylase values (*i.e.* 73% were below the cut-off) the high levels were not due to pancreatic irritation either.

The high trypsinogen-2 and trypsin-2-AAT levels in cholangiocarcinomas may be explained by the expression of trypsinogen in malignant biliary epithelial cells, which has recently been demonstrated by immunohistochemistry (Terada *et al.*, 1995). Trypsinogen is also expressed by the epithelium of intrahepatic bile ducts and peribiliary glands (Terada and Nakaruma, 1991) and of extrahepatic peribiliary glands (Terada *et al.*, 1993). We observed elevated trypsinogen-2 and trypsin-2-AAT levels in some patients with benign biliary diseases. Therefore, it is likely that trypsinogen from damaged biliary

FIGURE 2 – ROC curves showing the accuracy of the various tests in differentiating between malignant and benign diseases *(a)*, pancreatic cancer and benign disease *(b)*, and cholangiocarcinoma and benign disease *(c)*.

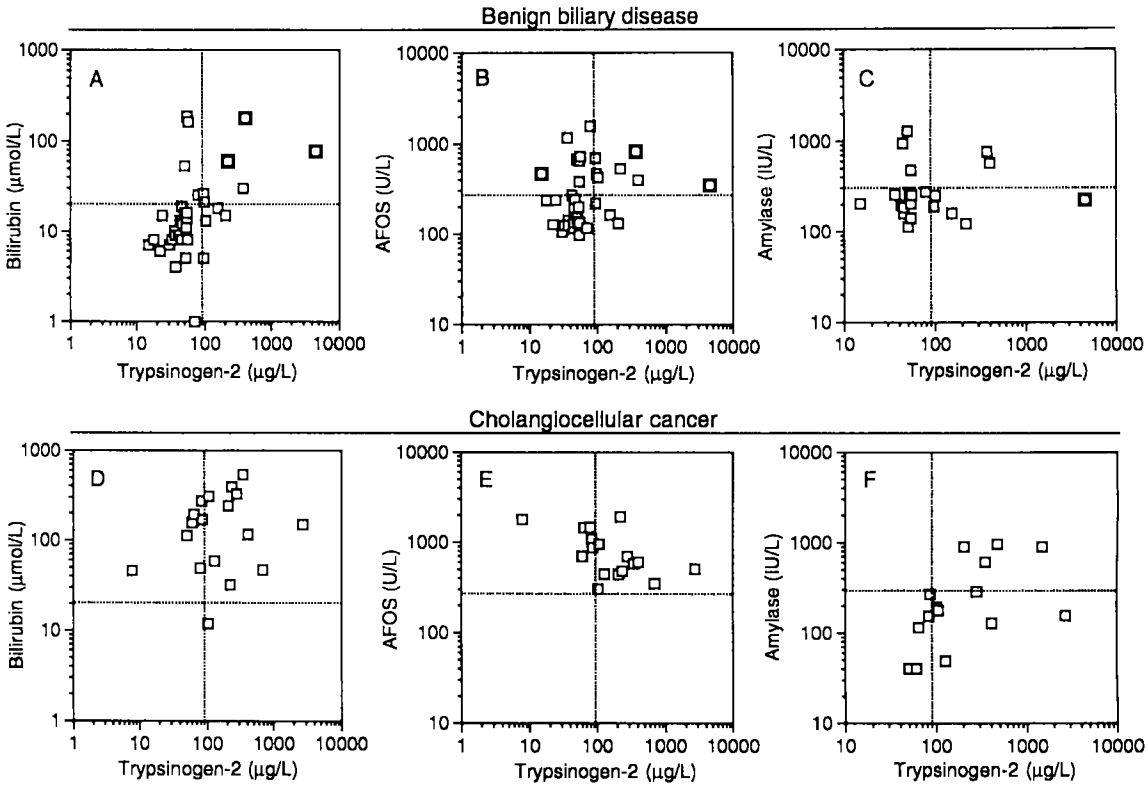


FIGURE 3 – Comparison of the serum concentrations of trypsinogen-2 and bilirubin, AFOS and amylase in benign biliary disease (*a–c*) and in cholangiocarcinoma (*d–f*). The cut-off values are marked with dashed lines.

ducts might contribute to the elevated serum trypsinogen levels in patients with both benign and malignant biliary tract diseases. In patients with benign biliary disease, the trypsinogen-2 and trypsin-2-AAT values were moderately correlated with elevated bilirubin and AFOS levels and poorly correlated with amylase values. This suggests that the trypsin immunoreactivity in serum was of biliary rather than of pancreatic origin.

Half of the patients with pancreatic cancer had elevated levels of trypsinogen-2 and trypsin-2-AAT. Based on the low amylase values in these patients (*i.e.* 81% were below the cut-off) the elevated levels of trypsinogen and trypsin-2-AAT immunoreactivity are mainly derived from the tumor rather than being due to pancreatic irritation. The accuracy of trypsinogen-2 and trypsin-2-AAT was clearly inferior to that of CEA or CA 19-9, which are the most commonly used markers for pancreatic cancer. Contrary to results from earlier studies showing a better accuracy of CA 19-9 (Haglund *et al.*, 1986), the accuracy of CEA was similar to that of CA 19-9 in the present study.

We have previously shown that 2 trypsinogen isoenzymes are produced by ovarian tumors. These are similar to pancreatic trypsinogen-1 and -2 with respect to amino-terminal sequence, molecular weight and immunoreactivity, but they differ from these with respect to isoelectric point and stability. Hence, they were called tumor-associated trypsinogen-1 and -2 (TAT-1 and TAT-2), respectively (Koivunen *et al.*, 1989). Very high levels of TAT-2 were observed in cyst fluid from mucinous ovarian tumors (Koivunen *et al.*, 1990). It will be interesting to determine whether the trypsinogen-2 produced by biliary epithelium and cancer is of the tumor-associated or the pancreatic type.

Recent studies have suggested that tumor-associated trypsinogen plays an essential role in cancer invasion and metastasis by degrading trypsin-sensitive extracellular matrix proteins (Koivunen *et al.*, 1991). It has been suggested that pancreatic trypsinogen plays a role in tumor invasion of pancreatic cancer (Ohta *et al.*, 1994). Thus, the expression of trypsinogen in biliary-tract cancers could be a factor contributing to their aggressive behavior.

In conclusion, our study suggests that the high serum levels of trypsinogen-2 and trypsin-2-AAT occur in malignant biliary-tract disease. This trypsinogen appears to be derived from the biliary epithelium. Our results suggest that trypsinogen-2 and trypsin-2-AAT are new potential markers for cholangiocarcinomas.

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REFERENCES

BORGSTRÖM, A. and OHLSSON, K., Studies on the turnover of endogenous cathodal trypsinogen in man. *Europ. J. clin. Invest.*, **8**, 379–382 (1978).

COLLAZOS, J., GENOLLA, J. and RUIBAL, A., Serum levels of CA 50 in nonmalignant liver diseases: a clinical and biochemical study. *Amer. J. Gastroenterol.*, **88**, 409–412 (1993).

CROSSLEY, J.R., ELLIOTT, R.B. and SMITH, P.A., Dried-blood spot screening for cystic fibrosis in the newborn. *Lancet*, **I**, 472–474 (1979).

ELIAS, E., REDSHAW, M. and WOOD, T., Diagnostic importance of changes in circulating concentrations of immunoreactive trypsin. *Lancet,* **II,** 66–68 (1977).

FABRIS, C., BASSO, D., DEL-FAVERO, G., MEGGIATO, T., PICCOLI, A., ANGONESE, C., PLEBANI, M., BONVICINI, P., BRULINA, A. and NACCARATO, R., Renal handling of amylase and immunoreactive trypsin in pancreatic cancer and chronic pancreatitis. *Clin. physiol. Biochem.,* **8,** 30–37 (1990).

HAGLUND, C., ROBERTS, P.J., KUUSELA, P., SCHEININ, T.M., MÄKELÄ, O. and JALANKO, H., Evaluation of CA 19-9 as a serum tumour marker in pancreatic cancer. *Brit. J. Cancer,* **53,** 197–202 (1986).

HEDSTRÖM, J., LEINONEN, J., SAINIO, V. and STENMAN, U.-H., Time-resolved immunofluorometric assay of trypsin-2 complexed with α_1-antitrypsin in serum. *Clin. Chem.,* **40,** 1761–1765 (1994).

ITKONEN, O., KOIVUNEN, E., HURME, M., ALFTHAN, H., SCHRÖDER, T. and STENMAN, U.-H., Time-resolved immunofluorometric assays for trypsinogen-1 and 2 in serum reveal preferential elevation of trypsinogen-2 in pancreatitis. *J. Lab. clin. Med.,* **115,** 712–718 (1990).

KOIVUNEN, E., HUHTALA, M.-L. and STENMAN, U.-H., Human ovarian tumor-associated trypsin. Its purification and characterization from mucinous cyst fluid and identification as an activator of prourokinase. *J. biol. Chem.,* **264,** 14095–14099 (1989).

KOIVUNEN, E., ITKONEN, O., HALILA, H. and STENMAN, U.-H., Cyst fluid of ovarian cancer patients contains high concentrations of trypsinogen-2. *Cancer Res.,* **50,** 2375–2378 (1990).

KOIVUNEN, E., RISTIMÄKI, A., ITKONEN, O., OSMAN, S., VUENTO, M. and STENMAN, U.-H., Tumor-associated trypsin participates in cancer cell-mediated degradation of extracellular matrix. *Cancer Res.,* **51,** 2107–2112 (1991).

LAKE-BAKAAR, G., MCKAVANAGH, S. and SUMMERFIELD, J.A., Urinary immunoreactive trypsin excretion: a non-invasive screening test for pancreatic cancer. *Lancet,* **II,** 878–880 (1979).

MALLORY, P.A. and TRAVIS, J., Human pancreatic enzymes: characterization of anionic human trypsin. *Biochemistry,* **12,** 2847–2851 (1973).

MISZCZUK-JAMSKA, B., MERTEN, M., GUY-CROTTE, O., AMOURIC, M., CLEMENTE, F., SCHOUMACHER, R.A. and FIGARELLA, C., Characterization of trypsinogens 1 and 2 in two human pancreatic adenocancer cell lines; CFPAC-1 and CAPAN-1. *FEBS Lett.,* **294,** 175–178 (1991).

MOLLER-PETERSEN, J. and SMIDT-JENSEN, S., The use and mechanism of urinary clearance of cathodic trypsin-like immunoreactivity to creatinine clearance ratio in the diagnosis of pancreatic cancer. *Clin. chim. Acta,* **130,** 163–170 (1983).

OHLSSON, K., Acute pancreatitis. Biochemical, pathophysiological and therapeutical aspects. *Acta gastroenterol. belg.,* **51,** 3–12 (1988).

OHLSSON, K. and EDDELAND, A., Release of proteolytic enzymes in bile-induced pancreatitis in dogs. *Gastroenterology,* **69,** 668–675 (1975).

OHTA, T., TERADA, T., NAGAKAWA, T., TAJIMA, T., ITOH, H., FONSECA, L. and MIYAZAKI, I., Pancreatic trypsinogen and cathepsin B in human pancreatic cancers and associated metastatic lesions. *Brit. J. Cancer,* **69,** 152–156 (1994).

ROBINSON, L.A., KIM, W.J., WHITE, T.T. and HADORN, B., Trypsins in human pancreatic juice—their distribution as found in 34 specimens. *Scand. J. Gastroenterol.,* **7,** 43–45 (1972).

STEINBERG, W.M., GOLDSTEIN, S.S., DAVIS, N.D., SHAMMA'A, J. and ANDERSON, K., Diagnostic assays in acute pancreatitis. A study of sensitivity and specificity. *Ann. intern. Med.,* **102,** 576–580 (1985).

TERADA, T., KIDA, T. and NAKANUMA, Y., Extrahepatic peribiliary glands express α-amylase isozymes, trypsin and pancreatic lipase: an immunohistochemical analysis. *Hepatology,* **18,** 803–808 (1993).

TERADA, T. and NAKANUMA, Y., Immunohistochemical demonstration of pancreatic α-amylase and trypsin in intrahepatic bile ducts and peribiliary glands. *Hepatology,* **14,** 1129–1135 (1991).

TERADA, T., OHTA, T., MINATO, H. and NAKANUMA, Y., Expression of pancreatic trypsinogen/trypsin and cathepsin B in human cholangiocancers and hepatocellular cancers. *Human Pathol.,* **26,** 746–752 (1995).

ZWEIG, M.H. and CAMPBELL, G., Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine (Review). *Clin. Chem.,* **39,** 561–577 (1993).