Increased serum trypsinogen 2 and trypsin 2-α_1 antitrypsin complex values identify endoscopic retrograde cholangiopancreatography induced pancreatitis with high accuracy

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Abstract

***Aims*—To evaluate the clinical utility of two new tests for serum trypsinogen 2 and trypsin 2-α_1 antitrypsin complex (trypsin 2-AAT) in diagnosing and assessing the severity of acute pancreatitis (AP) induced by endoscopic retrograde cholangiopancreatography (ERCP).**

***Patients*—Three hundred and eight consecutive patients undergoing ERCP at Helsinki University Central Hospital in 1994 and 1995.**

***Methods*—Patients were followed prospectively for pancreatitis and clinical outcome. They were tested for serum trypsinogen 2, trypsin 2-AAT, and amylase in samples obtained before and one, six, and 24 hours after ERCP.**

***Results*—Pancreatitis developed in 31 patients (10%). Their median serum trypsinogen 2 increased 26-fold to 1401 µg/l at six hours after the procedure and trypsin 2-AAT showed an 11-fold increase to 88 µg/l at 24 hours. The increase in both markers was stronger in severe than in mild pancreatitis, and in patients without pancreatitis there was no significant increase. Baseline trypsinogen 2 and trypsin 2-AAT concentrations were elevated in 29% and 32% of patients, respectively. The diagnostic accuracy of a threefold elevation over the baseline value was therefore analysed. The sensitivity and specificity of these parameters in the diagnosis of post-ERCP pancreatitis was 93% and 91%, respectively, for serum trypsinogen 2 at six hours after the examination, and 93% and 90%, for trypsin 2-AAT at 24 hours.**

***Conclusions*—Serum trypsinogen 2 and trypsin 2-AAT reflect pancreatic injury after ERCP. High concentrations are associated with severe pancreatic damage. The delayed increase in trypsin 2-AAT compared with trypsinogen 2 appears to reflect the pathophysiology of AP. A greater than threefold increase in trypsinogen 2 six hours after ERCP is an accurate indicator of pancreatitis.**

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Keywords: trypsinogen 2; trypsin 2-α_1 antitrypsin complex; ERCP; pancreatitis

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Endoscopic retrograde cholangiopancreatography (ERCP) is an important aid in the diagnosis and treatment of biliary and pancreatic disorders.[1] It is, however, associated with potentially serious complications such as pancreatitis, bleeding, perforation, sepsis, and possible long term effects of the therapeutic manoeuvres.[2 3] The most common complication of ERCP is acute pancreatitis (AP). The incidence of clinically detectable AP after ERCP ranges from 0 to 39%.[2 4 5] The majority of episodes of post-ERCP pancreatitis have been classified as mild with rapid spontaneous resolution.[2 6] Severe pancreatitis is reported to occur in 0.5–7% of patients.[4 7] Adequate treatment of severe post-ERCP pancreatitis requires early recognition and institution of therapy—aggressive venous hydration and close monitoring. There are no reliable clinical or procedural risk factors that predict the development or severity of pancreatitis after ERCP. A laboratory test for early identification of patients at risk would therefore be of wide clinical use.

The pathophysiology and possible risk factors of ERCP induced pancreatitis are poorly understood. Factors reported to affect the risk include physicians' experience, procedure related infection, overfilling of the pancreatic duct with acinarisation of the contrast media, repeated or difficult pancreatic duct cannulations and injections, ionic contrast medium, underlying pancreatic diseases, α_1 antitrypsin or α_2 macroglobulin deficiency, young age, and sphincterotomy for sphincter of Oddi dysfunction or a non-dilated bile duct.[1 2 4 5 8–13]

An asymptomatic increase in amylase and other pancreatic enzyme levels may occur in up to 70% of patients after ERCP.[4 5 14] Therefore, measurement of serum or urine amylase often results in a groundless suspicion of pancreatitis. Hence there is a need for a more specific and accurate method for diagnosing and estimating the severity of pancreatic damage during the first few hours after ERCP. The current severity grading is only retrospective.[15]

Trypsinogen is an inactive precursor of trypsin secreted from the acinar cells into the pancreatic juice. Trypsinogen occurs as two major isoenzymes, trypsinogen 1 (cationic trypsinogen) and trypsinogen 2 (anionic trypsinogen).[16 17] In healthy subjects, the serum concentration of trypsinogen 1 is higher than

TABLE 1 *Indications for ERCP*

Indication	*No. of examinations*	*No. of elevated trypsinogen 2 values before ERCP*	*No. of elevated trypsin 2-AAT values before ERCP*
Suspicion of common bile duct stones	114	27	32
Pancreaticobiliary tumour/stent placement	60	26	25
Obstructive jaundice	31	9	7
Chronic pancreatitis	29	10	12
Chronic abdominal pain	21	1	2
History of acute pancreatitis	13	7	6
Pancreatic pseudocyst	11	5	8
Common bile duct obstruction/stricture	9	1	
Pancreatic fistula	5	1	3
Dysfunction of sphincter of Oddi	3	1	1
Biliary fistula	2	1	1
Miscellaneous	10	1	1

Upper reference limit for trypsinogen 2 is 90 µg/l and for trypsin 2-AAT is 12 µg/l.

TABLE 2 *Technical factors of ERCP and the risk for postprocedural pancreatitis*

	Pancreatitis		
	+	−	*p Value*
Duration of ERCP (min)	33	26	0.027*
Endoscopic papillotomy (EP)			
Precut	4	17	0.12
EP	20	132	0.089
EP to pancreatic duct	4	11	0.042*
Mean total volume of dye injected (ml)			
Into CBD	37	35	0.42
Into pancreatic duct	4.5	6.5	0.97
Mean total number of injections			
Into CBD	4.0	4.5	0.27
Into pancreatic duct	2.0	2.2	0.70
Contrast medium concentration			
Omnipaque 140 mg/ml	25	195	0.083
Omnipaque 240 mg/ml	4	53.	0.62
Extraction of calculi	4	35	0.77
Stent placement and EP	10	44	0.012*
Common bile duct dilatation	1	5	0.45
Diagnostic procedure	2	49	0.19
Total no. of patients	31	277	

* Significance difference, tested with Fisher's exact test or with the Mann-Whitney U test.

that of trypsinogen 2, whereas in AP the trypsinogen 2 levels are higher.[18 19] We have previously shown that trypsinogen 2 and trypsin 2-α_1 antitrypsin complex (trypsin 2-AAT) are better diagnostic and prognostic markers of AP than amylase or C reactive protein.[19–21] Earlier methods for determining immunoreactive trypsin have mainly measured trypsinogen 1. The clinical utility of trypsinogen 2 and trypsin 2-AAT in detecting ERCP induced pancreatitis has not previously been studied.

The aim of this study was to evaluate the clinical usefulness of the serum trypsinogen 2 and trypsin 2-AAT measurements in diagnosing and predicting the severity of post-ERCP pancreatitis.

Patients and Methods

PATIENTS

All 308 patients who underwent ERCP between September 1994 and December 1995 in our department were included in this prospective, consecutive study. There were 168 females and 140 males, with a mean age of 64 years (range 22–94 years). Table 1 shows the indications for ERCP examinations.

ENDOSCOPIC PROCEDURES

ERCPs with or without therapeutic procedures were performed by a single experienced physician (JH) in a standard fashion. After an overnight fast, patients were premedicated with diazepam and atropine, the dose being adjusted for age and tolerance. Antibiotic prophylaxis (ceftriaxone 2.0 g) was given intravenously to all patients one hour before the procedure. Duodenal relaxation was achieved with glucagon administered intravenously after duodenal intubation. Olympus JF IT 10 or TJF 10 side viewing duodenoscopes were used. Contrast medium (iohexol 140 or 240 mg/l, Omnipaque, Nycomed, Norway) was slowly injected manually under fluoroscopic control to avoid pancreatic acinarisation. Selective cannulation of the biliary and/or pancreatic duct was attempted in all patients. When indicated, endoscopic papillotomy or sphincterotomy, stone extraction, and stent placement were performed in the same session.

METHODS

The diagnosis of ERCP pancreatitis was based on a rise in serum or urine amylase values of at least threefold above the upper reference limit within 24 hours and persistent abdominal pain for at least 24 hours after the procedure. Contrast enhanced computed tomography was performed to detect severe AP (n=8).

Pancreatitis was graded as mild if an outpatient had to be hospitalised or a planned admission was prolonged to three days or less after the procedure and as severe if hospital stay lasted more than three days or if complications occurred.[15] Blood samples for assay of trypsinogen 2, trypsin 2-AAT, and amylase were collected before and at one, six, and 24 hours after ERCP.

Recorded technical factors associated with ERCP included age, sex, indication for ERCP, duration of the procedure, laboratory data (liver function tests, coagulation status, white blood cell count, C reactive protein), technical details of the procedure (table 2), success or failure rate, gall bladder status, medical history, and complications. Patients were followed prospectively for evidence of pancreatitis, bleeding, perforation, and other complications.

LABORATORY METHODS

The concentrations of trypsinogen 2 and trypsin 2-AAT in serum were measured by time resolved immunofluorometric assays.[18] The assay for trypsinogen 2 utilises two monoclonal antibodies against trypsinogen 2 produced by immunisation with trypsinogen isolated from mucinous ovarian cyst fluid. The trypsin 2-AAT assay uses a monoclonal capture antibody and a commercial polyclonal antibody against AAT (Dako, Glostrup, Denmark) as the tracer. Results of both assays are obtained within one hour, and are available on a stat basis (analyser: AutoDelfia, Wallac, Turku, Finland). The reference range of 18–90 µg/l for trypsinogen 2 in serum is based on measurements in 264 healthy adults.[18 20] For trypsin 2-AAT the reference range is 2.3–12 µg/l, as determined by measurements in 120 healthy adults.[19 22]

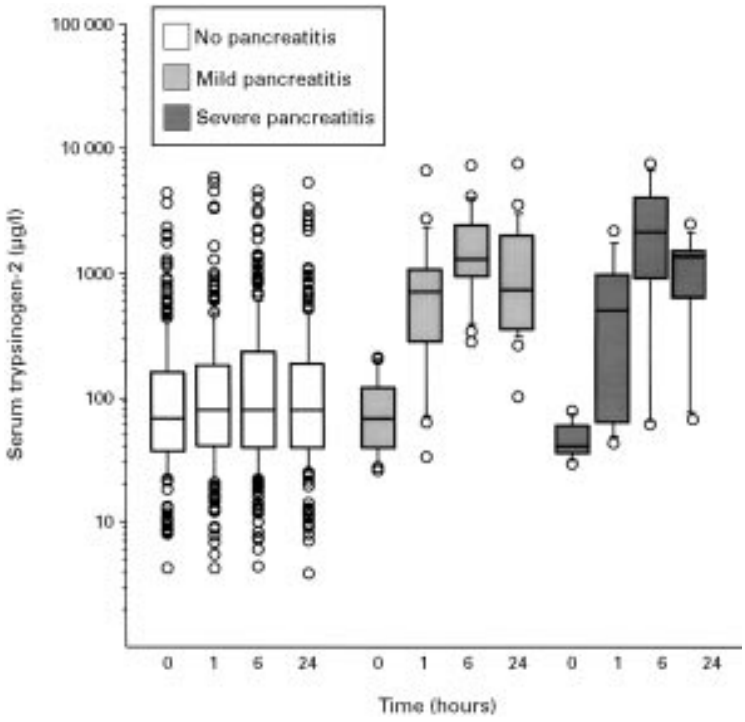

Figure 1: Box plots showing the concentration of serum trypsinogen 2 (25–75% interquartile range, mean, 95% range, and outliners) in patients without pancreatitis, with mild pancreatitis, and with severe pancreatitis before, and at one, six, and 24 hours after ERCP.

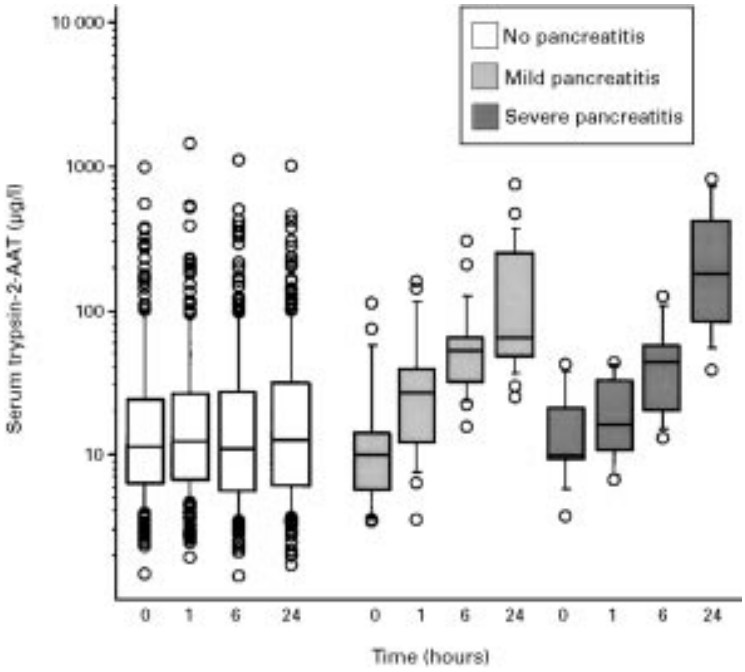

Figure 2: Concentrations of trypsin 2-AAT before, and at one, six, and 24 hours after ERCP.

STATISTICAL ANALYSIS

Comparison of continuous data was performed with the Mann-Whitney U test. For discrete variables, Fisher's exact test was used. A p value of less than 0.05 was considered statistically significant.

Results

Detectable acute pancreatitis developed in 31 of the 308 patients studied. Twenty three of them were classified as mild with rapid spontaneous recovery. Eight patients (26%) had a severe episode of pancreatitis with prolonged hospital stay or complications.

The median baseline level of serum trypsinogen 2 in the patients with ERCP induced AP was 53 µg/l (range 17–189 µg/l); one hour after ERCP it was 485 µg/l (range 35–6988 µg/l), at six hours 1401 µg/l (range 73–8990 µg/l), and at 24 hours 1008 µg/l (range 90–7311 µg/l) (fig 1). The median concentration at six hours was 26 times the baseline value. The differences between patients with and without ERCP induced pancreatitis were statistically significant at all time points ($p<0.001$).

The median baseline level of serum trypsin 2-AAT in patients with postprocedural pancreatitis was 8 µg/l (range 3–114 µg/l) and an 11-fold elevation to 88 µg/l (range 32–944 µg/l) was observed at 24 hours after ERCP (fig 2). This difference was statistically significant ($p<0.001$).

Six hours after ERCP, the trypsinogen 2 concentrations were higher in severe pancreatitis (median 2440 µg/l, range 93–8990 µg/l) than in mild pancreatitis (median 1090 µg/l, range 278–7400 µg/l) ($p=0.19$). The ability of trypsinogen 2, trypsin 2-AAT, and amylase to differentiate mild from severe ERCP induced pancreatitis was also evaluated by receiver operating characteristic (ROC) curve analysis. The area under the ROC curve (AUC) was 0.67 for trypsinogen 2 and only 0.60 for trypsin 2-AAT because of the considerable overlapping between the groups. However, amylase had an AUC of only 0.54, which approaches 0.5, and so fails to differentiate between the patients. The median trypsin 2-AAT concentration 24 hours after ERCP in patients with severe pancreatitis was 162 µg/l (range 36–770 µg/l), and in mild postprocedural pancreatitis was 68 µg/l (range 31–940 µg/l) ($p=0.38$) (fig 2).

A substantial proportion of the patients (29%) had elevated trypsinogen 2 levels (>90 µg/l) and 32% had elevated trypsin 2-AAT levels (>12 µg/l) before ERCP (table 1). The frequency of elevated amylase values before the examination was 21%. We therefore analysed whether an ERCP induced increase above the baseline value would provide better accuracy. With a threefold increase as a cut off value, the sensitivity of trypsinogen 2 one hour after the procedure was 74%, after six hours 93%, and after 24 hours 90%, the specificity being 87%, 91%, and 95%, respectively. For trypsin 2-AAT the sensitivity at 24 hours was 93% and the specificity 90%. Because the diagnosis of pancreatitis was based on amylase, the sensitivity of a threefold increase in amylase activity was, by definition, 100% while the specificity was 81%. The differences in specificity between amylase (53 false positives out of 277) and trypsinogen 2 (15–35 false positives out of 277) as well as trypsin 2-AAT (27 false positives out of 277) were statistically significant ($p<0.001$). The correlations of trypsinogen 2 and trypsin 2-AAT with amylase activity at different time points were fairly low (correlation coefficients 0.21–0.45) (fig 3). The correlation between trypsinogen 2 at six hours

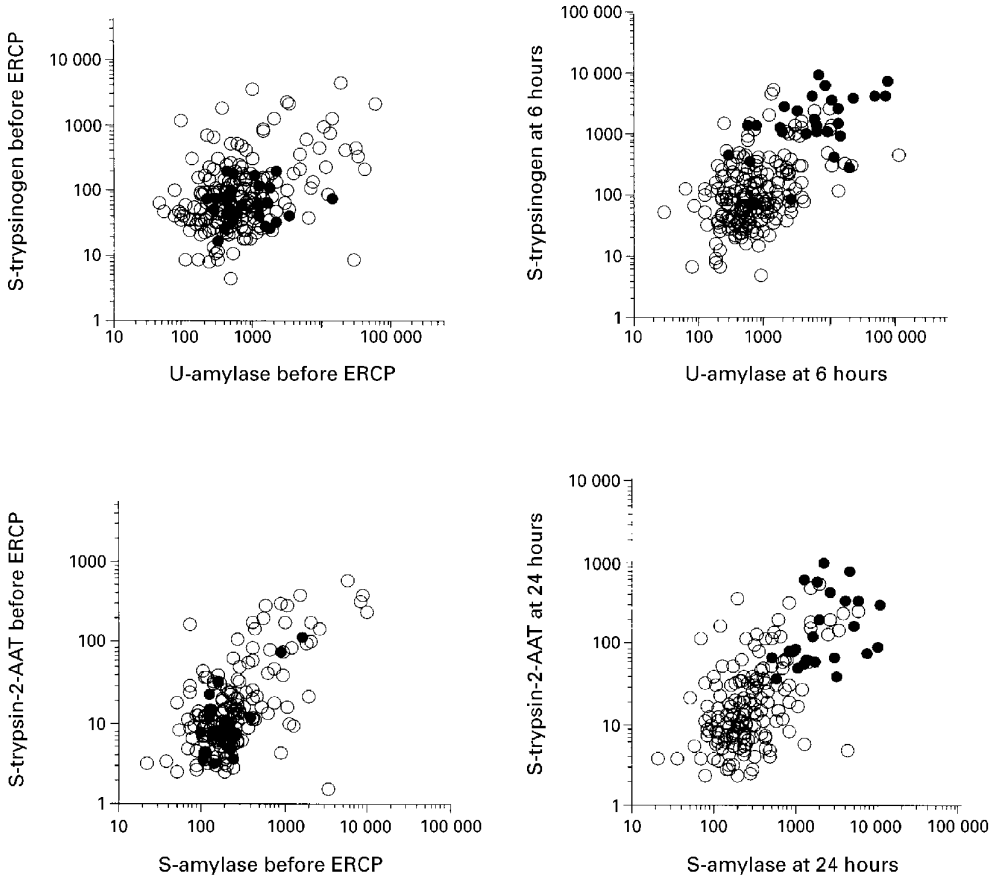

Figure 3: Correlations of trypsinogen 2 and amylase before and six hours after ERCP, and trypsin 2-AAT and amylase before and 24 hours after the procedure. Filled circles, ERCP induced pancreatitis; open circles, no pancreatitis.

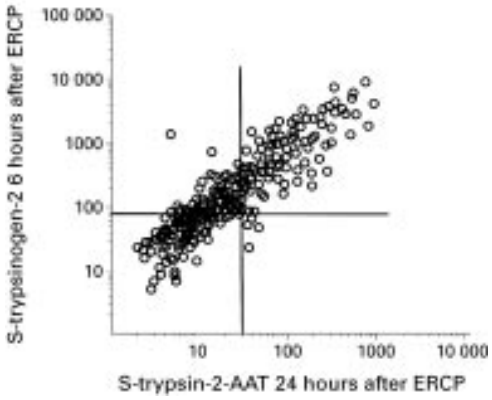

Figure 4: Scattergram of the correlation between trypsinogen 2 at six hours and serum trypsin 2-AAT at 24 hours after the examination (n=308).

and trypsin 2-AAT concentrations at 24 hours was high (r=0.70) (fig 4).

Before ERCP, the patients who developed pancreatitis actually had lower trypsinogen 2 values than those who did not. A pre-ERCP trypsinogen 2 concentration higher than 300 µg/l was inversely correlated with the risk of pancreatitis (p=0.02, Fisher's exact test). An elevated baseline concentration over 32 µg/l for trypsin 2-AAT was also associated with a lower risk, but this was not significant (p=0.27).

The frequency of high baseline values of trypsinogen 2 and trypsin 2-AAT varied between the diagnostic groups. Elevated levels were more common in patients with biliary tract cancer, in whom the median pre-ERCP concentration of trypsinogen 2 was 92 µg/l (range 23–592 µg/l), than in the rest (p=0.07). In patients with pancreatic cancer, the median baseline concentration of trypsinogen 2 was 81 µg/l (range 13–880 µg/l). A smaller rise in trypsin 2-AAT concentrations was observed in these patients. In chronic pancreatitis, the baseline values of the two markers did not differ from those of the rest of the study population.

Table 2 summarises the technical factors of the ERCP procedure and the risk of pancreatitis. The risk of pancreatitis increased with long duration of ERCP, endoscopic papillotomy (EP) to the Wirsungian duct, and stent placement with EP. However, precut papillotomy, sphincterotomy, and the number or volume of contrast media injections into the pancreatic duct had no influence on the risk. No significant differences were seen in liver function tests, infection markers, number of patients with postcholecystectomy state, or medical history. Acinar opacification was not seen in this series. The success rate of the procedure was 92%. One patient died of septic complications giving a 30 day mortality rate of 0.3%. Bleeding requiring endoscopic treatment was seen in three patients and duodenal perforation occurred in two patients.

Discussion

Our results show that trypsinogen 2 and trypsin 2-AAT reflect pancreatic injury after ERCP and support our previous findings on these markers in non-ERCP pancreatitis.[18–22] In patients developing pancreatitis raised trypsinogen 2 concentrations were already evident one hour after ERCP and peaked at six hours, whereas the trypsin 2-AAT complex did not show a clear rise until 24 hours, at which time the trend was still increasing. This suggests that the inactive proenzyme is released into the circulation in the initial phase, while activation of trypsinogen and release of active trypsin into the circulation with complex formation are later events. This interesting finding is probably explained by the sequential interactions of trypsin with various protease inhibitors within and outside the pancreas.[23] The main intrapancreatic trypsin inhibitor, pancreatic secretory trypsin inhibitor (PSTI), represents a "first-line" defence, whereas formation of the trypsin 2-AAT complex apparently starts later, when the inhibitory capacity of the intrapancreatic inhibitors is exceeded.[24] The relatively late formation of trypsin 2-AAT has not been reported before, but is in keeping with the results of earlier studies on the dynamics of trypsin α_1 protease in non-ERCP pancreatitis.[23]

The patients with severe pancreatitis had the highest concentrations of trypsinogen 2 and trypsin 2-AAT, as was also seen in earlier studies,[19–21] but this difference did not reach statistical significance, probably due to the limited number of patients in the severe pancreatitis group. These markers appear to indicate the degree of the primary pancreatic proteolytic insult during the induction of AP. In contrast to our earlier results in non-ERCP pancreatitis, trypsin 2-AAT was not superior to trypsinogen 2 in reflecting the severity of the disease. This is explained by the different time courses of these markers. Trypsinogen 2 peaked during the activation period, whereas trypsin 2-AAT was probably still increasing when the last sample was taken 24 hours after ERCP.

We suggest that a trypsinogen 2 concentration of over 3000 μg/l at six hours after ERCP should prompt the clinician to institute intensive monitoring, aggressive fluid transfusion, and antibiotic therapy.[3 25 26] Whether this assay can be utilised to improve the prognosis of patients with ERCP induced pancreatitis remains to be evaluated in a further trial. A trypsin 2-AAT concentration of over 250 μg/l at 24 hours after ERCP could also be used, but trypsinogen 2 has the advantage of increasing earlier.

In a recent study of Gottlieb *et al*,[27] amylase and lipase levels were analysed two hours after the ERCP. The authors reported that the magnitude of the rise which occurred early correlated with the risk of post-ERCP pancreatitis. Since most outpatients undergoing ERCP are discharged within a few hours after the procedure, early detection or firm exclusion of the complication is highly desirable. The use of two hour values of amylase and lipase was recommended for identification of patients at risk and to indicate the need for longer follow up as inpatients. In the present study, the sensitivity of a threefold rise in trypsinogen 2 at one hour was 74% and the specificity 87%; the accuracy increased until six hours. The figures at one hour are comparable with the two hour amylase and lipase levels (sensitivity 82–92% and specificity 52–76%) reported in the study of Gottlieb *et al*.[27] According to the results of this study, the optimal time lapse for the identification of patients at risk with trypsinogen 2 cannot be exactly determined; it is probably between one and six hours.

The high sensitivity and specificity of trypsinogen 2 and trypsin 2-AAT for post-ERCP pancreatitis was not unexpected, because pancreatic trypsinogen activation plays an essential role in the pathophysiology of pancreatitis.[17 23] It can activate other pancreatic proteins and protease cascades, including the kallikrein-kinin system.[28] Hereditary pancreatitis is reported to be caused by a mutation in the trypsinogen gene, which renders cationic trypsin resistant to proteolysis.[29] In earlier studies, the sensitivity and specificity of trypsinogen 2 and trypsin 2-AAT concentrations to non-ERCP induced AP approached 100%.[19 21]

In a substantial proportion of patients, the baseline values of all the markers studied were elevated. Trypsinogen 2 and trypsin 2-AAT are not completely specific for acute pancreatitis. In earlier studies on 222 patients with abdominal pain of aetiologies other than acute pancreatitis, elevated levels of trypsinogen 2 over the upper reference limit were detected in about 5%.[19–22] Trypsin 2-AAT concentrations were elevated in up to 10%,[19 22] respectively. However, naturally, the specificity of the markers is largely dependent on the cut off level used. Elevated levels have been detected in pancreatic, biliary tract, hepatocellular, and colorectal cancers as well as in chronic pancreatitis, pancreatic pseudocysts, and purulent cholangitis.[22 30]

In 60 patients in our series the indication for ERCP was an abdominal malignancy (table 1). Furthermore, almost all the patients in whom the indication for ERCP was acute biliary pancreatitis had high baseline concentrations of the markers. Thus the more frequent occurrence of high baseline concentrations of trypsinogen 2 and trypsin 2-AAT than of amylase (30% versus 21%) reduces the diagnostic utility of the two former markers for pancreatitis following ERCP. The diagnostic criterion to be used is therefore not the absolute value but the increase in concentration that is induced by ERCP. Consequently, the baseline value must be considered, when the risk of post-ERCP pancreatitis is analysed. With a threefold increase at six hours, the specificity was significantly better than that of amylase. There were seven patients in the post-ERCP pancreatitis group with an elevated baseline level, and all were detected using the threefold elevation as diagnostic method. Even with this method the accuracy of trypsinogen 2 might have been underestimated, since a few patients classified as having mild AP might have had a

non-specific rise in amylase and abdominal pain for reasons other than AP.

The negative correlation between a high baseline concentration of trypsinogen 2 and the risk of postprocedural pancreatitis is interesting. It is probably explained by selection of a series with a high rate of underlying pancreatic pathology, especially fibrosis of the gland, which has been reported to reduce the risk of inflammation.[31]

The 10% incidence of pancreatitis following ERCP in our series is in accordance with previous prospective reports.[2 10 12] The highly variable incidence of post-ERCP pancreatitis in the literature reflects differences in inclusion criteria and definitions of pancreatitis. The methods of data collection are also highly variable—prospective studies with frequent enzyme determinations and clinical follow up or retrospective studies without them.[2 6 32] The relatively high incidence in the present study results from the prospective nature of the trial and the liberal criteria of AP. Furthermore, the proportion of therapeutic ERCPs in our series was somewhat higher (83%) than in most previous reports.[10 33] Two patients developed late pancreatitis, which was not manifested by increasing trypsinogen concentrations within the short follow up period. Late development of pancreatitis after ERCP has been reported to occur in up to 15% of patients.[34]

In conclusion, our study indicates that rises in serum trypsinogen 2 and trypsin 2-AAT complex following ERCP accurately reflect the development of pancreatitis, the rise correlating with the severity of the disease. The clinical utility of high serum trypsinogen 2 as an early indicator of severe post-ERCP pancreatitis deserves to be evaluated in a larger prospective controlled study.

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