Serum complex of trypsin 2 and α_1 antitrypsin as diagnostic and prognostic marker of acute pancreatitis: clinical study in consecutive patients

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Abstract

Objective—To estimate the usefulness of serum concentrations of the complex of trypsin 2 and α_1 antitrypsin in diagnosing and assessing the severity of acute pancreatitis in comparison with serum C reactive protein, amylase, and trypsinogen 2 concentrations (reference markers).

Design—Markers were measured in consecutive patients admitted with acute abdominal pain that was either due to pancreatitis or to other disease unrelated to the pancreas (controls).

Setting—Department of surgery of a teaching hospital in Helsinki.

Subjects—110 patients with acute pancreatitis and 66 with acute abdominal diseases of extrapancreatic origin. On the basis of the clinical course, acute pancreatitis was classified as mild (82 patients) or severe (28 patients).

Main outcome measures—Clinical diagnosis of acute pancreatitis and severity of the disease.

Results—At admission all patients with acute pancreatitis had clearly raised concentrations of trypsin 2- α_1 antitrypsin complex (32 $\mu\text{g/l}$), whereas only three of the controls had such values. Of the markers studied, trypsin 2- α_1 antitrypsin complex had the largest area under the receiver operating curve, both in differentiating acute pancreatitis from extrapancreatic disease and in differentiating mild from severe disease.

Conclusions—Of the markers studied, trypsin 2- α_1 antitrypsin complex was the most accurate in differentiating between acute pancreatitis and extrapancreatic disease and in predicting a severe course for acute pancreatitis.

antitrypsin.¹⁴ If the inhibitory capacity is exceeded activation of other pancreatic enzymes may be triggered, leading to severe complications.¹⁷

The complex of trypsin and α_1 antitrypsin can be specifically measured with an assay based on a capture antibody specific for trypsin 2 and a detector antibody recognising α_1 antitrypsin. Concentrations of trypsinogen 2 reflect leakage of enzymes from the pancreas and those of trypsin 2- α_1 antitrypsin complex the release of active trypsin 2.¹⁸ Thus concentrations of the complex may reflect the severity of the disease. Activation of trypsinogen can be estimated by measurement of trypsinogen activation peptides in urine, and this method has been successfully used to assess the severity of acute pancreatitis.¹⁹

We investigated the accuracy of serum concentrations of trypsin 2- α_1 antitrypsin complex in diagnosing and assessing the severity of acute pancreatitis in comparison with those of the reference markers trypsinogen 2, C reactive protein, and amylase.

Patients and methods

We studied a consecutive series of 110 patients with acute pancreatitis and 66 with acute gastrointestinal extrapancreatic disease admitted to this department of surgery from March 1992 to November 1993. The final diagnosis of pancreatitis was based on findings of upper abdominal pain accompanied by the typical appearance of acute pancreatitis on ultrasonography or computed tomography. If severe pancreatitis was suspected, contrast enhanced computed tomography was used to detect necrosis of the pancreas. Ultrasonography or computed tomography was performed in 103 (94%) patients with acute pancreatitis. In seven patients the diagnosis was based on clinical data alone (table 1).

The median age for all patients with acute pancreatitis was 43 years (range 21–91); 88 were men. The cause of the disease was regarded as alcohol misuse in 82 patients (table 1). A biliary origin was deduced when gall stones were seen on ultrasonography, endoscopic retrograde pancreatography, or laparotomy or at necropsy. The patients were classified according to clinical outcome as having mild (82 patients) or severe disease (28 patients).

All patients with mild acute pancreatitis improved spontaneously with conservative treatment, and none developed local pancreatic or systemic complications. Their median stay in hospital was six days (table 1).

Patients with severe acute pancreatitis had one or more major local or systemic complications of the disease—that is, respiratory failure (17 patients), renal failure (eight patients), septicæmia (seven patients), abscess (two patients), and necrosis of pancreas (17 patients). Four patients died of haemorrhagic pancreatitis, which was verified at necropsy. Twenty six patients required intensive care, and seven patients underwent surgery, either necrosectomy or distal pancreatic resection. Necrosis of the pancreas was confirmed by contrast enhanced computed tomography, by laparotomy, or at necropsy. The median stay in hospital was 23 days (table 1).

Introduction

Typical clinical features with raised serum amylase concentrations are still the main diagnostic criteria in acute pancreatitis, but they are helpful in only 80% of cases.¹ In the remainder, the diagnosis is possible only on the basis of the medical history, clinical course, and findings on radiology (after ultrasonography and computed tomography) and laparotomy.^{2–6} Concentrations of amylase may not be high initially, and high concentrations may occur in abdominal disorders that can be confused with acute pancreatitis.^{7–8}

Acute pancreatitis has an unpredictable outcome, with serious complications in 20–30% of cases^{9–10} and a mortality of 10–20%.^{10–11} Rapid assessment of the severity of disease facilitates prompt institution of supportive treatment and improves prognosis.¹

Proteolytic enzymes may have a major role in the pathophysiology of pancreatitis,^{12–14} and serum trypsinogen concentration seems to reflect pancreatic damage.¹⁵ The ratio of the two major trypsinogen isoenzymes, trypsinogen 1 and trypsinogen 2, in normal plasma is 3.8,¹⁶ but in acute pancreatitis the serum concentrations of trypsinogen 2 are greatly increased.¹⁵

Pathological intrapancreatic activation of trypsinogen to trypsin occurs in acute pancreatitis.¹⁷ In blood, active trypsin is inactivated by α_2 macroglobulin and α_1

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Table 1—Clinical features of patients with acute pancreatitis according to severity of disease. Values are numbers (percentages) of patients unless stated otherwise

	Mild disease (n = 82)	Severe disease (n = 28)	Total (n = 110)
Median age (years)	45	40	43
Sex ratio (M:F)	64:18	24:4	88:22
No of episodes of pancreatitis:			
1	32 (39)	24 (86)	56 (51)
≥2	50 (61)	4 (14)	54 (49)
Cause:			
Alcohol misuse	57 (70)	25 (89)	82 (75)
Biliary disease	13 (16)	1 (4)	14 (13)
After trauma	0	1 (4)	1 (1)
Unknown	12 (15)	1 (4)	13 (12)
Method of diagnosis:			
Ultrasonography	70 (85)	25 (89)	95 (86)
Computed tomography	28 (34)	26 (93)	54 (49)
Contrast enhanced computed tomography	24 (29)	22 (79)	46 (42)
Anamnesis, clinical findings, and exclusion of other causes alone	7 (9)	0	7 (6)
Clinical course:			
Treatment in intensive care unit	0	26 (93)	26 (24)
Conservative treatment	82 (100)	21 (75)	103 (94)
Surgical treatment	0	7 (25)	7 (6)
Median stay in hospital (days)	6	23	8
Surgical procedure:			
Necrosectomy	0	4 (14)	4 (4)
Distal pancreatic resection	0	3 (11)	3 (3)

Patients with acute abdominal disease of extrapancreatic origin were categorised into the control group on the basis of clinical, radiographic, endoscopic, or surgical findings. The diagnoses were acute appendicitis (11 patients), acute biliary disease (10 patients), intestinal obstruction (six patients), peptic ulcer or oesophagitis (seven patients), diverticular disease (five patients), gastroenteritis (five patients), genitourinary disease (three patients), and other gastrointestinal disease (19 patients). Their median age was 45 years (range 20–90). Thirty eight patients were women.

SAMPLES

First samples were collected within 12 hours of admission in all control patients and in 35 patients with acute pancreatitis. In 64 patients the initial sample was

collected 12–24 hours after admission and in 11, 24–48 hours after admission. Later blood samples were collected daily between 0700 and 0800. Consecutive samples were obtained from patients with acute pancreatitis during the first three days after admission. Serum samples were stored at -20°C until analysed.

LABORATORY TESTS

Amylase was measured by an enzymatic method with a Hitachi 705E analyser using Amylase EPS reagents (Boehringer Mannheim, Mannheim, Germany). The reference range was 70–300 U/l (median 180 U/l).

Trypsinogen 2 and trypsin 2- α_1 antitrypsin complex were determined by time resolved immunofluorometric assays by a technician who was unaware of the clinical diagnosis. The reference range for trypsinogen 2 was 18–90 $\mu\text{g/l}$ and that for trypsin 2- α_1 antitrypsin complex 2.3–12 $\mu\text{g/l}$.¹⁶

Serum C reactive protein was assayed by an immunoturbidometric method with antiserum and standards from Orion Diagnostica (Espoo, Finland). The upper reference limit was 10 mg/l .²⁰

STATISTICAL ANALYSIS

The ability of various tests to differentiate between mild and severe acute pancreatitis and non-pancreatic disease was estimated on the basis of sensitivity and specificity at clinically relevant cut off points. The validity of the tests was further evaluated by analysis of receiver operating characteristic curves. The area under the curve of the receiver operating curve describes the accuracy of the test, 1 indicating 100% sensitivity and specificity and 0.5 no discriminatory power.²¹ Specificity and odds ratios were calculated by Fisher's test with cut off points that gave 90% and 95% sensitivity. The specificities for different markers were compared using McNemar's test. High sensitivity values were selected because of the importance of not missing severe pancreatitis.

Results

There were no significant differences in concentrations of trypsin 2- α_1 antitrypsin complex or trypsinogen 2 between men and women in either group.

Fig 1—Concentrations of trypsin 2- α_1 antitrypsin complex, trypsinogen 2, C reactive protein, and amylase in serum samples obtained within 48 hours after admission from 82 patients with mild acute pancreatitis and 28 patients with severe acute pancreatitis. Sixty six patients with acute abdominal disorders of extrapancreatic origin served as controls. Dashed horizontal line indicates upper reference limit

Table 2—Accuracy (area under curve) of different markers in differentiating acute pancreatitis from extrapancreatic disease and severe from mild pancreatitis at different times after admission

Marker	Time after admission (hours)		
	0-12	0-24	0-48
Acute pancreatitis v extrapancreatic disease			
Trypsin 2- α_1 antitrypsin complex	0.995	0.994	0.994
Trypsinogen 2	0.963	0.994	0.991
C reactive protein	0.749	0.887	0.880
Amylase	0.987	0.950	0.950
Severe pancreatitis v mild pancreatitis			
Trypsin 2- α_1 antitrypsin complex	0.819	0.854	0.860
Trypsinogen 2	0.756	0.744	0.760
C reactive protein	0.726	0.837	0.850
Amylase	0.477	0.515	0.570

Table 3—Serum marker concentrations in extrapancreatic disease and mild and severe acute pancreatitis within 48 hours after admission.* Values are medians (ranges)

	Extrapancreatic disease (n = 66)	Mild (n = 82)	Severe (n = 28)
Trypsin 2- α_1 antitrypsin complex ($\mu\text{g/l}$)	9.2 (2.4-179)	196 (32-1617)	753 (200-2598)
Trypsinogen 2 ($\mu\text{g/l}$)	27 (7.2-373)	992 (41-6871)	2251 (405-15 898)
C reactive protein (mg/l)	14 (3.0-165)	116 (5.0-361)	308 (20-496)
Amylase (U/l)	182 (28-581)	1043 (67-8028)	1392 (393-14 301)

*In 99 out of 110 cases the first value was obtained within 24 hours after admission and in 35 cases within 12 hours after admission.

Table 4—Comparison of ability of trypsin-2- α_1 antitrypsin and C reactive protein to differentiate between severe and mild acute pancreatitis at different times after admission*

	Sensitivity (%)	Specificity (%)	Cut off point†	Likelihood ratio (95% confidence interval)
Trypsin 2-α_1 antitrypsin				
0-12 h	95	65	288	22 (2.5 to 206)
	90	65	288	22 (2.5 to 206)
0-24 h	95	64	267	39 (5.0 to 303)
	90	64	288	13 (3.6 to 48)
0-48 h	95	54	205	29 (3.8 to 226)
	90	65	288	14 (4.0 to 53)
C reactive protein				
0-12 h	95	13	9	0.14 (0.03 to 0.58)
	90	13	9	2.9 (0.80 to 11)
0-24 h	95	11	9	1.3 (0.25 to 6.5)
	90	27	20	4.0 (0.85 to 18)
0-48 h	95	10	9	2.9 (0.34 to 24)
	90	25	20	2.6 (0.71 to 9.8)

*Trypsin 2- α_1 antitrypsin complex and C reactive protein values were available in 35 cases 12 hours after admission, in 99 cases after 24 hours, and in 110 cases after 48 hours.

†Concentration: $\mu\text{g/l}$ for trypsin 2- α_1 antitrypsin complex and mg/l for C reactive protein.

Therefore men and women were considered together. In acute pancreatitis concentrations of trypsinogen 2 and trypsin 2- α_1 antitrypsin complex increased much more than did those of C reactive protein and amylase. There was also less overlap in concentrations of the complex than in those of the other markers between patients and controls. In all patients with acute pancreatitis the concentrations of the complex during the first two days were above the upper reference limit (12 $\mu\text{g/l}$) (fig 1). All patients with acute pancreatitis, but only three of the controls, had a trypsin 2- α_1 antitrypsin value $\geq 32 \mu\text{g/l}$. When the patients were compared with the controls, trypsin 2- α_1 antitrypsin had the largest area under the curve of the markers studied (table 2, fig 2). When patients with severe pancreatitis were compared with those with mild disease in the 35 samples obtained within 12 hours of admission, trypsin

Fig 2—Receiver operating curve showing accuracy of various tests in differentiating between acute pancreatitis and extrapancreatic gastrointestinal disease in samples obtained within 48 hours after admission

2- α_1 antitrypsin had a larger area under the curve than did C reactive protein (table 2). When all samples taken within 24 hours (n = 99) and 48 hours (n = 110) of admission were considered, the accuracy of trypsin 2- α_1 antitrypsin remained high, while that of C reactive protein increased and approached the accuracy of the complex (table 2). The median values and range of values for the various markers are shown in table 3.

Specificity and odds ratios were calculated at cut off values giving 95% and 90% sensitivity. At high sensitivity trypsin 2- α_1 antitrypsin complex was more specific than C reactive protein in differentiating between severe and mild acute pancreatitis in samples taken 12-48 hours after admission ($P < 0.01$ with McNemar's test) (table 4). Thus, trypsin 2- α_1 antitrypsin complex was the best in differentiating acute pancreatitis from extrapancreatic disease and mild from severe acute pancreatitis. Amylase was poor in differentiating severe from mild acute pancreatitis, whereas C reactive protein, as expected, did not differentiate acute pancreatitis from extrapancreatic disease (figs 1 and 2).

A larger proportion of patients with their first attack had severe disease than those with a recurrent attack ($P < 0.0001$).

TIME COURSE PROFILE OF THE MARKERS

Time course profiles of trypsin 2- α_1 antitrypsin complex (10 patients) and trypsinogen 2 (10 patients) showed similar patterns (fig 3). In severe acute pancreatitis concentrations of trypsin 2- α_1 antitrypsin complex and trypsinogen 2 mostly peaked in the initial sample and slowly decreased but remained high during the following days. In two out of three patients with mild acute pancreatitis the peak was observed on the second day. One patient with increasing concentrations of trypsin 2- α_1 antitrypsin complex and trypsinogen 2 died of necrotising acute pancreatitis confirmed at necropsy. Eight patients out of 10 showed decreasing concentrations of trypsin 2- α_1 antitrypsin complex and trypsinogen 2 when the initial sample was compared with that on day 3.

C reactive protein concentrations (18 patients) were often low initially and tended to increase in mild acute pancreatitis. C reactive protein increased rapidly in eight out of nine patients, the peak being observed on day 3 in seven of the nine patients (fig 3). Five patients with severe pancreatitis had had a very high concentration of C reactive protein ($> 200 \text{ mg/l}$) at presentation.

Peak amylase ($n = 13$) values were observed in the initial sample in all cases of severe acute pancreatitis and in five out of eight cases of mild acute pancreatitis. Eleven of the 13 patients had decreasing concentrations of amylase. Interestingly, some patients with severe disease had low amylase concentrations (fig 3).

Discussion

Clearly raised serum concentrations of trypsin 2- α_1 antitrypsin complex were found at presentation in all cases of acute pancreatitis, and a normal serum concentration of this complex excluded the disease. The lowest concentration of trypsin 2- α_1 antitrypsin complex in acute pancreatitis ($32 \mu\text{g/l}$) was about three times the upper reference limit in healthy controls. Among the other markers, trypsinogen 2 and C reactive protein concentrations were normal in three cases each, and amylase values were normal in six. Thus trypsin 2- α_1 antitrypsin complex differentiated better between pancreatic and extrapancreatic disease than did trypsinogen 2, C reactive protein, and amylase concentrations. We assumed that trypsin 2- α_1 antitrypsin complex would reflect pancreatitis induced release of active trypsin 2 into the circulation. Our results suggest that this is the case. All patients with severe acute pancreatitis requiring intensive care had a trypsin 2- α_1 antitrypsin complex concentration above $200 \mu\text{g/l}$ at presentation. A single clearly false increase in this concentration was observed (fig 1). Interestingly, this patient had ovarian cancer, a tumour which may produce trypsin.²²

PREDICTION OF DISEASE SEVERITY

At presentation trypsin 2- α_1 antitrypsin complex concentration predicted the severity of acute pancreatitis much better than trypsinogen 2 and amylase concentrations. The serum concentration of C reactive protein increased with time, and when all the samples taken within 48 hours of admission were considered C reactive protein and trypsin 2- α_1 antitrypsin complex had similar accuracy. However, during the first 12 hours after admission trypsin 2- α_1 antitrypsin was more accurate than C reactive protein (area under the curve 0.82 *v* 0.73). Amylase concentration did not reflect the severity of acute pancreatitis, and in many patients the values were lower in severe than in mild disease. The area under the curve for amylase for differentiation

between mild and severe acute pancreatitis was 0.57 —that is, close to 0.5 , which indicates no discriminative power.

Because early identification of patients with severe acute pancreatitis is desirable, we have emphasised sensitivity in our analysis. Especially at cut off values giving high sensitivity trypsin 2- α_1 antitrypsin complex was more accurate than C reactive protein. Time course profiles showed that the concentrations of trypsin 2- α_1 antitrypsin complex and trypsinogen 2 were already high at presentation and mostly decreased during the first three days of admission, although they were still greatly raised on the third day. The concentrations of C reactive protein were often low at presentation but increased after one to two days. Therefore the accuracy of C reactive protein was low during the first 12–24 hours after admission. The concentrations tended to be low, with many values within the normal range at admission. Follow up samples were not available for all patients. Therefore the time course studies should be interpreted with caution.

There was a higher proportion of men among patients with acute pancreatitis whereas there was an excess of women in the control group. However, there were no significant differences in the marker concentrations between the sexes. In fact trypsinogen values in female controls tended to be higher than those in the male controls ($P = 0.15$), whereas the opposite was true in the patients with acute pancreatitis ($P = 0.45$ – 0.50). Therefore the difference in sex distribution did not improve the accuracy of trypsin 2- α_1 antitrypsin complex as a marker.

We conclude that of the markers studied, trypsin 2- α_1 antitrypsin complex is the most accurate in differentiating between acute pancreatitis and extrapancreatic disease and in predicting a severe course of the disease at presentation. Trypsin 2- α_1 antitrypsin complex and trypsinogen 2 were determined by double antibody sandwich assay using time resolved fluorescence for detection. However, because the typical concentrations are 100 to 1000 times the detection limit, the method can easily be adopted to other assay formats. The assay time is routinely two hours, but with automation the assay could easily be performed in minutes. If available on an automatic analyser on a 24 hour basis, the assay for trypsin 2- α_1 antitrypsin complex could greatly improve the diagnosis of this common and potentially lethal disease.

Fig 3—Serum concentrations of trypsin 2- α_1 antitrypsin, trypsinogen-2, C reactive protein, and amylase during first three days after admission in patients with acute pancreatitis. Dashed line denotes severe disease and solid line mild disease

Key messages

- Intrapancreatic activation of trypsinogen to trypsin is a key feature of acute pancreatitis; in blood trypsin forms a complex with α_1 antitrypsin
- This complex can be accurately measured in a sensitive immunoassay
- In this study the diagnostic and prognostic accuracy of serum concentrations of trypsin 2- α_1 antitrypsin complex was determined in acute pancreatitis
- The complex was more accurate than trypsinogen 2, C reactive protein, and amylase in differentiating between acute pancreatitis and extrapancreatic disease and in predicting a severe course for the disease
- If the immunoassay could be automated determination of concentrations of trypsin 2- α_1 antitrypsin complex could greatly improve the diagnosis of this common and potentially lethal disease

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Perinatal characteristics in relation to incidence of and mortality from prostate cancer

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Abstract

Objective—To test the hypothesis that factors causing morbidity and mortality from prostate cancer may operate in utero.

Design—Matched case-control study of singleton men born between 1874 and 1946 at one hospital.

Setting—Uppsala University Hospital.

Subjects—250 patients with prostate cancer and 691 controls, including 80 patients who died from prostate cancer and their 196 matched controls.

Main outcome measures—Mother's age at menarche, parity, pre-eclampsia or eclampsia before delivery, age at delivery and socioeconomic status; case or control's birth length and weight, placental weight, prematurity derived from gestational age, and presence of jaundice.

Results—Both pre-eclampsia (odds ratio 0, 95% confidence interval 0 to 0.71) and prematurity (0.31, 0.09 to 1.04) were inversely associated with incidence of prostate cancer. Among subjects born full term, placental weight, birth weight, and ponderal index (weight/height³) showed non-significant positive associations with prostate cancer incidence, and stronger associations with mortality.

Conclusion—Prenatal exposures that are likely correlates of pregnancy hormones and other

growth factors are important in prostate carcinogenesis and influence the natural course as well as the occurrence of this cancer.

Introduction

Prostate cancer comprises about 8% of all new cancers among men globally,¹ and the incidence seems to be increasing in many countries and ethnic groups.² Although this increase may be partly due to increased detection rates,³ a genuine increase in the incidence of clinically aggressive cancers is likely since mortality from the disease is also increasing.² The highest incidences have been reported in North America and northern Europe and the lowest in south east Asia.¹⁻⁴ In the United States, incidence and mortality are substantially higher among African-Americans at all ages.⁴

Evidence exists that early life events have a role in prostate cancer, although it is not as strong as for breast cancer.⁵ The incidence of prostate cancer increases in the first generation after migration to areas where it is more common⁶ and recent epidemiological studies also suggest a link with early life.⁷⁻⁸ Intrauterine factors have already been associated with testicular cancer⁹ and breast cancer.¹⁰⁻¹¹ Some recent reports have addressed the possible role of the intrauterine environment in prostate cancer. Ross and Henderson suggested that the intrauterine period may be aetiologically relevant to

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