Trypsinogen activation peptides (TAP) concentrations in the peritoneal fluid of patients with acute pancreatitis and their relation to the presence of histologically confirmed pancreatic necrosis

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Abstract

This study measured the volume and colour, as well as concentrations of trypsinogen activation peptides (TAP) in the peritoneal fluid of 22 patients with acute pancreatitis and related these findings to the presence of pancreatic necrosis. Nine patients had a severe attack with histologically confirmed pancreatic necrosis, seven a severe attack without confirmed necrosis, and six a mild attack, also without confirmed necrosis. A free fluid volume >20 ml or free fluid colour >grade 5 on the Leeds chart, or both detected histologically confirmed pancreatic necrosis with a sensitivity of 100% and specificity of 31%. A total peritoneal fluid TAP concentration of ≥96 nmol detected histologically confirmed pancreatic necrosis with a sensitivity of 89% and specificity of 85%, figures comparable with contrast enhanced computed tomography. These findings suggest that the measurement of peritoneal fluid TAP concentrations can detect effectively histologically confirmed pancreatic necrosis and that such measurements may prove useful in the selection of patients for surgery.

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unproved, that pancreatic necrosis only develops where intraglandular trypsinogen activation occurs.^{9 10} If this is the case then we would expect to see a correlation between the presence and extent of pancreatic necrosis and the serum concentration of trypsin, in patients with acute pancreatitis. Failure to show such a relation in the past may result from the failure of the immunoreactive trypsin assay to differentiate between the active enzyme (trypsin) and the parent zymogen (trypsinogen).¹¹ This problem has been overcome recently by the development of an immunoassay using antibodies directed against the C terminal end of the trypsinogen activation peptides (TAP) and which reports the activation of the zymogen.¹² We have shown previously the effectiveness of urinary TAP measurements in determining the severity of acute pancreatitis.¹³ In this study we examine the ability of TAP concentrations, measured in peritoneal fluid, to detect histologically confirmed pancreatic necrosis.

Methods

PATIENTS

A consecutive series of 309 patients with acute pancreatitis were reported prospectively between February 1986 and July 1989. A diagnosis of acute pancreatitis was made on the basis of a serum amylase greater than 720 IU/l (equivalent to a value of 1200 IU/l as measured by the Phadebas method), in the presence of a compatible clinical picture of the disease. Patients, who were considered, on clinical assessment at the time of admission to hospital, to have moderate or severe acute pancreatitis were entered into a randomised trial comparing standard conservative treatment plus intraperitoneal aprotinin treatment, with standard conservative treatment alone. One hundred and four patients fulfilled the entry criteria as outlined above. Fifty four (51%) patients were randomised to receive standard conservative treatment alone, did not have peritoneal aspiration, and will not be considered further. The remaining 50 (49%) patients were randomised to the treatment group and had peritoneal aspiration. It is this group of patients that are examined in this study.

In two patients it was realised, after peritoneal aspiration, that the initial diagnosis

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One of the principal factors determining the outcome of an attack of acute pancreatitis is the presence of pancreatic necrosis.¹⁻⁴ This occurs in 10 to 15% of patients and is associated with a death rate of 30 to 40%.⁴⁻⁸ For this reason its early identification and prompt treatment remains an important priority in the treatment of acute pancreatitis. It has been suggested, although it remains

TABLE 1 Clinical details on 22 patients with acute pancreatitis who had diagnostic peritoneal aspiration and lavage. Ranges are shown in brackets

	Severe pancreatitis with confirmed necrosis	Severe pancreatitis without confirmed necrosis	Mild pancreatitis without confirmed necrosis
Median age (y)	55 (29-62)	34 (31-74)	53 (37-68)
Sex (M:F)	8:1	7:0	4:2
Aetiology			
Alcohol	5	5	4
Gall stones	2	1	2
Unknown	2	1	0
Median Glasgow score	5 (3-6)	2 (2-6)	1 (0-5)
Median stay (days)	50 (1-90)	17 (12-64)	12 (6-27)

TABLE II Complications in 22 patients with acute pancreatitis who had diagnostic peritoneal aspiration and lavage. The number of complications was greater than the number of patients as one patient may suffer more than one complication

Complications	Pancreatitis with confirmed necrosis (9 patients)	Severe pancreatitis without confirmed necrosis (7 patients)
Respiratory failure	2	5
Renal failure	1	1
Confirmed pancreatic necrosis	9	—
Pancreatic collection	2	—
Pancreatic pseudocyst	1	—
Pancreatic abscess	2	—
Died	5	—

of acute pancreatitis was incorrect: one had a traumatic transection of the pancreas and the other extensive mesenteric venous infarction. They, and one further patient who refused peritoneal aspiration after randomisation, were withdrawn from the study. Free peritoneal fluid was aspirated from 26 (55%) of the remaining 47 patients and a sufficient volume of fluid was available for analysis in 22 of these. There was insufficient fluid available for analysis in the four remaining patients.

Nine patients were classified retrospectively as suffering from a severe attack of acute pancreatitis with confirmed pancreatic necrosis (five from specimens obtained at postmortem examination and four from specimens obtained at the time of surgery), seven as having severe disease without confirmed necrosis, and six as having mild disease, also without confirmed necrosis (Table I).

The three patient groups were comparable in terms of age, sex, aetiology, and hospital stay (Table I). The prolonged median stay and lack of differences in the length of hospital stay between the patient groups, was because of two patients with mild disease who remained in for a cholecystectomy and another who had a delayed discharge for social reasons. Table II shows the complications suffered by patients with severe disease.

Intraperitoneal aprotinin treatment failed to influence the mortality or morbidity of acute pancreatitis¹⁴: 48 (48%) patients suffered a mild and 53 (52%) a severe outcome.

PERITONEAL ASPIRATION

Peritoneal aspiration was performed with an 8 French gauge peritoneal dialysis catheter (Kimal Scientific Products Ltd, Uxbridge, England), which was inserted into the peritoneal cavity through a small subumbilical stab incision, after the infiltration of local anaesthetic. Any free fluid was aspirated and its volume and colour recorded as described previously.¹⁵ It was centrifuged at 4°C within

TABLE III Details of the peritoneal fluids from 22 patients with acute pancreatitis who had diagnostic peritoneal aspiration and lavage. The ranges are shown in brackets

	Severe pancreatitis with confirmed necrosis	Severe pancreatitis without confirmed necrosis	Mild pancreatitis without confirmed necrosis
Median fluid volume (ml)	140 (60–1120)	120 (11–900)	21.5 (10–210)
Median fluid colour	8 (6–8)	6 (3–8)	4 (3–5)
Median TAP (nmol/l)	4.08 (0.42–21)	0.33 (0–1.68)	0.00 (0–0.4)
Median TAP × fluid volume (nmol)	442 (41–4570)	17 (0–840)	0 (0–84)

one hour of being taken, frozen, and stored at –20°C until analysis. The median (range) times between the onset of symptoms and peritoneal aspiration were 23 hours (14 to 58) for patients with confirmed necrosis, 23 hours (2 to 101) for patients with severe attacks but without confirmed necrosis, and 26 hours (15 to 39) for patients with mild attacks. These differences were not significant ($H=0.66$, df (degrees of freedom)=2, $p=0.719$, Kruskal-Wallis test).

ASSAYS

Trypsinogen activation peptide (TAP)

The TAP assay was performed in duplicate in the department of surgery, St George’s Hospital Medical School, London. Frozen peritoneal fluid samples (300 µl) were thawed, mixed with an equal volume of 25 mmol/l TRIS/HCL buffer, pH 7.4, containing 0.15 mol/l NaCl, 10 mmol/l EDTA, 2.5 mg/ml bovine serum albumin, and 20 mmol/l NaN₃, and incubated in a boiling water bath for 10 minutes. The samples were centrifuged at 1000 *g* for five minutes and the supernatants (100 µl) assayed for TAP as described by Hurley *et al.*¹²

Amylase

Measurements of serum amylase concentrations were made on a Hitachi 737 random access discrete analyser (Mannheim, Germany) using an enzymatic colorimetric assay (α-amylase PNP (Mannheim, Germany). The normal range was 0–200 IU/l and the within batch precision was 1.01% at an amylase of 1129 IU/l.

STATISTICAL ANALYSIS

Data were analysed using medians, the Mann-Whitney U test, Fisher’s exact test, and Kruskal-Wallis test as appropriate. To avoid repetition in the text the Mann-Whitney U test has been used unless stated otherwise. Confidence intervals were used in preference to *p* values in judging the relevance of differences.¹⁶

The total amount of TAP contained within the peritoneal fluid was calculated by multiplying the free fluid TAP concentration by the total volume of fluid aspirated.

Results

TAP CONCENTRATION

The distribution of TAP concentrations within the three groups was not uniform ($H=13.10$, $df=2$, $p=0.001$, Kruskal-Wallis test) (Table III). Significantly higher TAP concentrations were seen in the peritoneal fluid of patients with severe pancreatitis associated with confirmed necrosis when compared with patients with severe disease but without confirmed pancreatic necrosis (95.6% CI=0.4 to 9.0 nmol/l, $p<0.4$) or those with mild

Figure 1: Free peritoneal fluid TAP concentrations in nine patients with severe pancreatitis with histologically confirmed necrosis (SWN), seven patients with severe acute pancreatitis without histologically confirmed necrosis (SWCN), and six patients with mild disease (M).

disease (96.1% CI=0.68 to 10.4 nmol/l, $p<0.006$). Significant differences were not seen between patients with severe attacks without confirmed necrosis and mild attacks (96.2% CI=-0.07 to 1.4 nmol/l, $p<0.5$). Using a peritoneal fluid TAP concentration of ≥ 2 nmol/l (Fig 1) we could distinguish between necrotising and other forms of acute pancreatitis with a sensitivity of 67% (six of nine patients) and specificity of 100% (13 of 13 patients).

The distribution of the total TAP content of the free peritoneal fluid within the three groups was not uniform ($H=11.75$, $df=2$, $p=0.003$ (adjusted for ties), Kruskal-Wallis test) (Table III). Significantly higher total TAP concentrations were seen in the peritoneal fluids of patients with severe pancreatitis associated with confirmed necrosis when compared with patients with severe disease but without confirmed pancreatic necrosis (95.6% CI=25.8 to 2100.0 nmol, $p=0.026$) or those with mild disease (96.1% CI=96.1 to 2444.0 nmol, $p=0.0024$). Significant differences were not seen between patients with severe attacks without confirmed necrosis and mild attacks (96.2% CI=-8.6 to 630.0 nmol, $p=0.15$).

Figure 2: Total free peritoneal fluid TAP concentration in nine patients with severe pancreatitis with histologically confirmed necrosis (SWN), seven patients with severe acute pancreatitis without histologically confirmed necrosis (SWCN), and six patients with mild disease (M).

Using a cut off value of 96 nmol we could detect pancreatic necrosis with a sensitivity of 89% and specificity of 85% (Fig 2).

FLUID VOLUME

The distribution of fluid volumes within the three groups was not uniform ($H=6.47$, $df=2$, $p=0.04$, Kruskal-Wallis test) (Table III). There were no significant differences in the volume of fluid obtained from patients with severe attacks with confirmed necrosis and severe attacks without confirmed necrosis (95.6% CI=-390.1 to 189.1 ml, $p<0.75$) or between those with severe attacks without confirmed necrosis and mild attacks (96.2% CI=-4.1 to 490 ml, $p<0.22$). Significant differences were seen, however, between patients with severe attacks with confirmed necrosis and mild attacks (96.1% CI=34.9 to 225 ml, $p<0.05$). A free fluid volume of >20 ml predicted pancreatic necrosis with a sensitivity of 100% (nine of nine patients) but the specificity of only 38% (four of 13 patients).

FLUID COLOUR

The distribution of fluid colour between the three groups was not uniform ($H=13.62$, $df=2$, $p=0.001$, Kruskal-Wallis test) (Table III). Significant differences were noted in the colour of fluid samples between those patients with severe attacks with and without confirmed necrosis (95.6% CI=0 to 5, $p<0.03$) and severe attacks with confirmed necrosis and mild attacks of acute pancreatitis (96.1% CI=3 to 5, $p<0.003$). Significant differences were not seen, however, in the fluid colour between patients with severe attacks but without confirmed necrosis and mild attacks of acute pancreatitis (96.2% CI=-1.0 to 3, $p=0.3$). A free fluid colour of greater than grade 5 selected patients with pancreatic necrosis with a sensitivity of 100% (nine of nine patients) and a specificity of only 46% (six of 13 patients).

Where a patient had >20 ml of free fluid aspirated or free fluid colour darker than grade 5, or both pancreatic necrosis was detected with a sensitivity of 100% and specificity of 31%.

Table IV summarises the sensitivity, specificity, predictive values, and percentage correct for the above parameters.

Discussion

While the Ranson and Glasgow criteria provide effective separation between mild and severe attacks of acute pancreatitis, they cannot identify accurately patients with necrotising disease.^{17 18} Clinical assessment^{5 19 20} and visual inspection of the gland at the time of surgery have also proved ineffective.⁵ Nordback *et al*⁵ failed to show any correlation between the extent of histologically confirmed pancreatic necrosis and blood glucose, serum sodium, potassium, creatinine, calcium, triglycerides, bilirubin or transaminases, packed cell volume, white cell or platelet

TABLE IV Summary of the sensitivity, specificity, predictive values, and percentage correct of peritoneal free fluid volume, colour, TAP concentrations, and total TAP content in determining the presence of pancreatic necrosis in 22 patients with acute pancreatitis

	Sensitivity (%)	Specificity (%)	+ve PV (%)	-ve PV (%)	% Correct
TAP ≥2 nmol/l	67	100	100	81	86
Free fluid volume >20 ml	100	38	50	100	59
Fluid colour >grade 5	100	62	64	100	77
>20 ml of free fluid or colour >grade 5	100	31	50	100	59
Median TAP x fluid volume	89	85	80	92	86

+ve PV=positive predictive value, -ve PV=negative predictive value.

counts. While a number of studies have suggested that RNase,^{21 22} α₁-antiprotease, α₂-macroglobulin,²³ calcium,^{24 25} and C reactive protein^{23 26} are effective in detecting pancreatic necrosis, others have failed to confirm these findings.^{5 10 15 26-28} Ultrasound examination of the pancreas has also proved ineffective.^{20 29}

At present, contrast enhanced computed tomography provides the best method of detecting pancreatic necrosis, an area of hypoperfusion being equated with the presence of necrotic tissue.^{20 30-32} Using these criteria Buchler *et al*²³ have claimed that contrast enhanced computed tomography detects pancreatic necrosis with an accuracy of 85-90% and more recently Larvin *et al*³² have identified correctly 11 patients with histologically confirmed necrosis. Forty patients with equivocal or normal enhancement recovered without the need for surgery. Computed tomography is, however, not universally available, entails moving a sick patient away from intensive care, and is comparatively expensive. For these reasons alternative methods of detecting pancreatic necrosis have been sought. If, as has been suggested, pancreatic necrosis only develops where intraglandular trypsinogen activation occurs, then the measurement of urinary or peritoneal fluid TAP concentrations may detect effectively its presence.^{9 10} While we have shown previously the presence of TAP in the urine of patients with severe acute pancreatitis,¹³ this is the first time that TAP has been measured in the peritoneal fluid of patients.

The closest similarity to the selection criteria used in this study was provided by a multi-centre trial of peritoneal aspiration reported by Corfield *et al*¹⁹ in which patients with predicted severe disease had peritoneal aspiration. Twenty seven per cent of patients reported had either >20 ml of free fluid or fluid darker than grade 5 compared with 38% in this series. The presence of >20 ml of free peritoneal fluid, fluid of colour >grade five or a combination of the two could not detect histologically confirmed pancreatic necrosis with a specificity high enough to make them of clinical value. This failure agrees with the findings of a study by Larvin and McMahon³³ who noted that a free fluid volume >40 ml or fluid the colour of 'prune juice' could only detect pancreatic necrosis with a sensitivity of 72% and specificity of 84%.

Peritoneal fluid TAP concentrations were noted to be significantly higher in patients with severe acute pancreatitis who had

confirmed necrosis than in either of the other two groups. The sensitivity of peritoneal fluid TAP concentrations in detecting histologically confirmed pancreatic necrosis was too low to be clinically useful. A positive predictive value of 100%, however, meant that it did identify correctly all patients with histologically confirmed necrosis. When the peritoneal fluid TAP concentration was multiplied by the free peritoneal fluid volume, a TAP concentration >96 nmol detected pancreatic necrosis with a sensitivity and specificity comparable with the gold standard: contrast enhanced computed tomography.^{8 20 32} Confirmation that peritoneal fluid TAP concentrations do correlate with disease severity comes from animal experiments.^{34 35} In a study of rats with caerulein induced pancreatitis, the greatest pool of TAP was found within the free peritoneal fluid, concentrations here being significantly higher in non-survivors than in survivors.³⁵ A total peritoneal fluid TAP concentration of 70 pmol/l predicting the outcome in 94.1% of animals. Furthermore, peritoneal fluid TAP concentrations proved to be an indicator of the histopathological severity of the disease, TAP concentrations correlating with the presence of intrapancreatic haemorrhage ($r=0.57$) and acinar cell necrosis ($r=0.72$).

The reason why raised concentrations of TAP were found in those patients without confirmed necrosis is unclear. It is possible that a degree of trypsinogen activation occurs in oedematous acute pancreatitis. Recently Yamaguchi³⁶ has described, for the first time, trypsinogen activation in rats with caerulein induced pancreatitis put under stress. Fernandez del Castillo *et al*³⁴ have confirmed these findings by detecting TAP in the urine of rats with caerulein induced pancreatitis. It may be that a similar situation arises in humans. It is also possible that a number of those patients without histologically confirmed necrosis had undetected pancreatic necrosis. Contrast enhanced computed tomography was unavailable during the study period and so it is not possible to exclude pancreatic necrosis in these patients. Despite this it should be remembered that the presence of pancreatic necrosis showed on contrast enhanced computed tomography is not an absolute indication for surgical intervention. A number of authors have shown that even patients with extensive areas of pancreatic hypoperfusion can recover with conservative treatment alone.^{17 31 37 38} In addition it is generally agreed that the decision to operate should be based upon a combination of computed tomography findings and assessment of the patients general condition; those with computed tomography confirmed necrosis and a deteriorating clinical condition, or evidence of pancreatic or peripancreatic sepsis being considered suitable for debridement.³⁹ Therefore, we can see that we need to identify correctly those patients with pancreatic necrosis who will require surgery rather than those simply with pancreatic necrosis. In this study a total peritoneal fluid

TAP concentration >96 nmol/l identified correctly eight of nine patients who either had or might have benefited from surgery.

In common with other authors' experience^{15 19 40 41} we failed to identify correctly, using clinical evaluation at the time of admission to hospital, a number of patients who were subsequently shown to have suffered a severe attack of acute pancreatitis. This failure will reduce the sensitivity of peritoneal fluid TAP measurements in detecting histologically confirmed pancreatic necrosis. This may, however, not be an important drawback as very few of the patients coming to surgery have an operation during the first week after admission to hospital. If peritoneal fluid TAP concentrations were being used as a means of selecting those patients requiring surgery, then a delay of 48 hours or longer before prediction became available would be acceptable. Such a strategy would also have the advantage of restricting the number of mild cases undergoing peritoneal aspiration. Peritoneal aspiration, when used as a means of severity assessment, has never been accepted widely into clinical practice, perhaps because of its interventional nature and the risk of visceral perforation.¹⁵ If it were possible, however, to identify pancreatic/peripancreatic necrosis, or patients who required surgery, then the balance of risks and benefits would shift in favour of peritoneal aspiration.

While this preliminary study suggests that the measurement of peritoneal fluid TAP concentrations can identify patients with confirmed pancreatic necrosis and help in selecting those patients requiring surgery, it should be noted that these findings are retrospective and will require confirmation in a prospective manner before being adopted into clinical practice.

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