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Endotoxaemia and serum tumour necrosis factor as prognostic markers in severe acute pancreatitis

A R Exley, T Leese, M P Holliday, R A Swann, J Cohen

Abstract

Endotoxaemia and circulating tumour necrosis factor are important prognostic factors in severe sepsis and are implicated in the pathogenesis of septic shock. Because clinical and pathological features in acute pancreatitis are similar to septic shock this study sought to determine whether endotoxin and tumour necrosis factor were prognostic factors in 38 patients with prognostically severe acute pancreatitis. Endotoxaemia, present in 19/37 (51%) patients on day 1, was more common in non-survivors than survivors (10/11, 91% *v* 9/26, 35%, p=0·003). Day 1 serum endotoxin concentrations were higher in patients with a severe outcome (median (interquartile range) 314 (173–563) pg/ml *v* 0 (0–185) pg/ml, p<0·01) and in non-survivors (266 (173–586) pg/ml *v* 0 (0–165) pg/ml, p<0·01). Serum tumour necrosis factor was detectable in 47 of 109 samples (43%) from 38 patients (median 35 pg/ml, range 5–943 pg/ml). Day 1 serum tumour necrosis factor correlated with a worse prognostic score and a severe outcome in all patients (n=38, r=0·36, p=0·027; r=0·33, p<0·05) and with mortality in patients with gall stones (n=23, r=0·50, p=0·02). Our data suggest that endotoxin and tumour necrosis factor could be prognostic factors in severe acute pancreatitis.

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Pancreatic necrosis and local sepsis have been strongly implicated in the pathogenesis of evolving pancreatitis,[1-3] although the initiating events remain controversial. High risk groups can be selected using prognostic scores and patients with pancreatic necrosis identified using modern imaging techniques.[4-6] Current strategies for treatment include the use of early surgery or peritoneal lavage.[6 7] A novel approach follows the observation that many clinical features in acute pancreatitis are also characteristic of septic shock – namely, the systemic illness and the significant mortality from the adult respiratory distress syndrome and multi organ failure.[1 8] Accordingly, it has been suggested that severe acute pancreatitis, like septic shock, might be a consequence of excessive activation of macrophages and neutrophil polymorphs with the release of secondary mediators.[8] In severe sepsis important prognostic factors which are also implicated in the pathogenesis of septic shock include endotoxaemia and the leucocyte products tumour necrosis factor, and interleukin-1.[9] We have sought to detect the presence of endotoxaemia and serum tumour necrosis factor in patients with severe acute pancreatitis and determine whether they are prognostic factors for patient outcome.

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Methods

PATIENTS

The study cohort consisted of the first 38 consecutive patients with prognostically severe acute pancreatitis, as defined by three or more adverse criteria,[4] taken from a randomised controlled study of intravenous fresh frozen plasma *versus* colloid.[10] In our cohort there were 24 women and 14 men with a median age of 72·5 years (range 44–91). The aetiology of pancreatitis was gall stones in 23 patients, idiopathic in nine, alcohol in three, hypothermia in one, hyperlipidaemia in one, and postoperative in one. Patient outcome was defined as severe if multi organ failure or complications of acute pancreatitis developed.[11] There were no significant differences between the fresh frozen plasma and plasma protein fraction subgroups at entry nor in outcome[10]; in particular the beta error for this study was <5% (PPF *v* fresh frozen plasma where alpha=5%, beta=3·7% for severe outcome, beta=2·1% for death[12]).

Blood samples were drawn at presentation (day 1), day 3, and day 7, into sterile pyrogen free tubes, centrifuged promptly and serum stored at −70°C before analysis. Serum endotoxin concentrations were measured by a quantitative kinetic Limulus amoebocyte lysate microassay with a sensitivity of 25 pg/ml.[13] Serum tumour necrosis factor was measured using a modified tumour necrosis factor specific enzyme linked immunoadsorbent assay (ELISA) with a sensitivity of 5 pg/ml.[14] A p value <0·05 was regarded as statistically significant using Spearman rank correlation, two-tailed Mann Whitney U tests and Fisher's exact test.

Results

Fourteen of 38 patients with prognostically severe acute pancreatitis had a severe outcome and there were 11 deaths (Table I, II). Blood cultures were negative in 11/17 patients and Gram negative bacteraemia was only found in patients 6 and 9. Patient 5 (mild outcome) had Viridans streptococci and coagulase negative stapylococci isolated as probable contaminants. Patient 25 (mild outcome) had *Staphylococcus aureus* isolated from blood cultures and a central venous line.

Endotoxaemia was present in 19/37 (51%) patients at presentation and was significantly more common in non-survivors than survivors (10/11, 91% *v* 9/26, 35%, p=0·003, Figure 1). Serum endotoxin at presentation was signific-

antly higher in patients who had a severe outcome (median (interquartile range) 314 (173–563) pg/ml *v* 0 (0–185) pg/ml) and in non-survivors (266 (173–586) pg/ml *v* 0 (0–165) pg/ml, $p<0{\cdot}01$, Figure 2, Table II).

Serum tumour necrosis factor was detectable in 47 of 109 samples, 43%, from 38 patients (median 35 pg/ml, range 5–943 pg/ml). At presentation 11 of 38 patients, 30%, were tumour necrosis factor positive (median 44 pg/ml, range 8–260 pg/ml, Figure 2, Table II). Serum tumour necrosis factor at presentation correlated with a worse prognostic score and a severe outcome in all patients ($r=0{\cdot}36$, $p=0{\cdot}027$, $r=0{\cdot}33$, $p<0{\cdot}05$) and prognostic score, outcome and mortality in patients with gall stones ($r=0{\cdot}58$, $p=0{\cdot}005$, $r=0{\cdot}60$, $p=0{\cdot}005$, $r=0{\cdot}50$, $p=0{\cdot}02$). Six of 26 (23%) survivors were tumour necrosis factor positive at entry *versus* five of 11 (45%) non-survivors (median tumour necrosis factor 19 pg/ml, range 8–48 pg/ml *v* 86 pg/ml, range 17–260 pg/ml, $p=0{\cdot}08$).

Discussion

The detection of circulating endotoxin and tumour necrosis factor correlates with mortality from severe sepsis, their induction in susceptible animals reproduces septic shock and their neutralisation by monoclonal antibodies significantly decreases mortality from severe sepsis implicating these factors in the pathogenesis of septic shock.[9 15–17] Severe acute pancreatitis has clinical features common to septic shock prompting a search for common prognostic factors. Our data suggest endotoxaemia and serum tumour necrosis factor are prognostic factors for severity of outcome and mortality in severe acute pancreatitis.

In septic shock detection of endotoxaemia and serum tumour necrosis factor has not been universal, nor are tumour necrosis factor or endotoxin alone invariably lethal.[9 18–20] Our data on tumour necrosis factor and endotoxin in severe acute pancreatitis and previous small studies in acute pancreatitis are reminiscent of earlier work in septic shock.[21–23] Current data from patients with severe sepsis suggest such discrepancies reflect the transient detection of mediators in blood, the importance of interacting mediators and the presence of endogenous

TABLE I *Patients with severe outcome: presenting features*

No	Age (yr)	Sex	Aetiology	Prognostic score	Blood cultures
6	67	M	Idiopathic	5	*Escherichia coli*, *Coagulase-ve Staphylococci
7	73	M	Idiopathic	3	Negative
9	81	F	Gall stones	5	Coliforms, *Streptococcus faecalis*
11	57	M	Gall stones	3	N/A
14	81	M	Gall stones	4	Negative
19	76	M	Idiopathic	3	*Coagulase-ve Staphylococci
20	58	M	Gall stones	4	N/A
21	84	M	Idiopathic	3	N/A
22	78	F	Gall stones	3	N/A
26	61	F	Idiopathic	3	Negative
30	91	F	Hypothermia	3	N/A
35	77	M	Alcohol	3	**Staphylococcus aureus*
36	60	F	Gall stones	3	Negative
39	63	F	Alcohol+gall stones	3	N/A

No: patient's trial number; prognostic score: modified Glasgow prognostic score where ≥3 adverse criteria indicates prognostically severe acute pancreatitis (ref[9]). yr: years; F: female; M: male; *probable skin contaminants; Coagulase-ve coagulase negative; N/A not available.

TABLE II *Patients with severe outcome: Day 1 tumour necrosis factor, endotoxin and complications*

No	Tumour necrosis factor	Endotoxin	Complications	Survival
6	0	188	Multi organ failure	Died day 15
7	51	314	Toxic Pancreatic necrosis+abscess	Died day 30
9	18	218	Severe cholangitis, cardiac failure, ileus pancreatic phlegmon	Home day 90
11	17	160	Multi organ failure	Died day 3
14	260	595	Toxic Pancreatic necrosis+abscess	Died day 30
19	0	359	Multi organ failure	Died day 8
20	194	173	Toxic Pancreatic necrosis+abscess	Died day 30
21	0	183	Multi organ failure	Died day 7
22	0	0	Pancreatic pseudocyst	Home day 11
26	0	588	Ileus Pancreatic phlegmon	Home day 19
30	86	530	ARDS	Died day 3
35	0	563	Multi organ failure	Died day 5
36	0	0	Toxic Pancreatic necrosis+abscess	Died day 34
39	0	4315	ARDS Pancreatic necrosis	Died day 36

No: patient's trial number; TNF: serum tumour necrosis factor pg/ml; Endotoxin pg/ml; ARDS: adult respiratory distress syndrome.

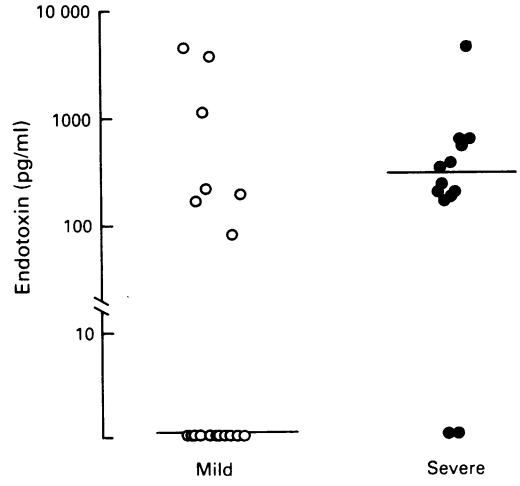

Fig 1A

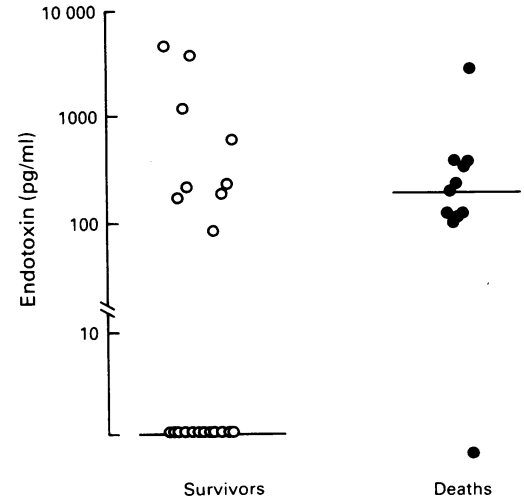

Fig 1B

Figure 1: A Serum endotoxin v outcome. Serum endotoxin in pg/ml, median values shown by horizontal bars. Serum endotoxin concentrations at entry were significantly higher in patients with a severe outcome v mild outcome, median 314 pg/ml interquartile range 173–563 pg/ml v median 0 pg/ml range 0–185 pg/ml, $p<0{\cdot}01$. B Serum endotoxin v mortality. Serum endotoxin concentrations at entry were significantly higher in non-survivors than survivors, median 266 pg/ml interquartile range 173–586 pg/ml v median 0 pg/ml range 0–165 pg/ml, $p<0{\cdot}01$.

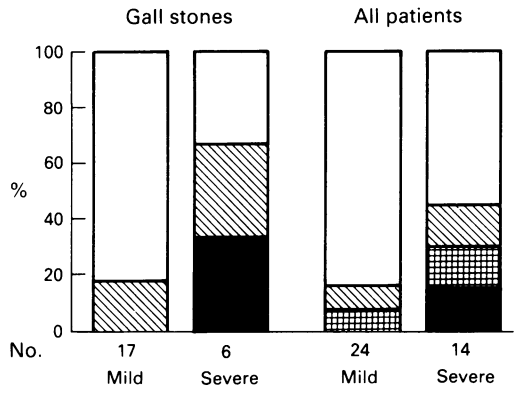

Fig 2A

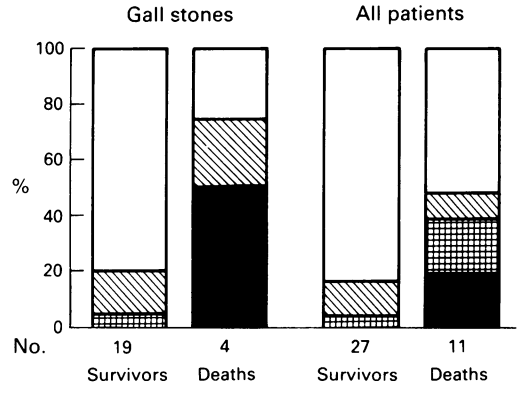

Fig 2B

Figure 2: Serum tumour necrosis factor in pg/ml □ <10 ▨ <20 ▦ <100 ■ <500. A Serum tumour necrosis factor v outcome. Serum tumour necrosis factor correlated with a severe outcome – that is, multi organ failure or complications of acute pancreatitis, r=0·33 p<0·05 all patients, r=0·60 p=0·005 for patients with gall stones. Serum tumour necrosis factor concentrations were significantly higher in patients with a severe outcome p=0·023. B Serum tumour necrosis factor v mortality. In patients with gall stones serum tumour necrosis factor correlated with mortality and serum tumour necrosis factor concentrations were significantly higher in non-survivors than survivors, n=23, r=0·50, p=0·022 and n=23, p=0·022.

inhibitors.[9 19 24] Endotoxaemia without bacteraemia is a well recognised entity and in severe acute pancreatitis it might result from local sepsis, pancreatic and intraabdominal necrosis causing absorption of gut derived endotoxin.[21 25 26]

The identification of endotoxin, phospholipase A2, tumour necrosis factor, and granulocyte-elastase as prognostic factors in acute pancreatitis is insufficient to imply pathogenesis, despite persuasive analogies.[8 27 28] Intervention with specific antagonists to endotoxin, tumour necrosis factor and interleukin-1 should enable us to test the hypothesis that the progression of acute pancreatitis is driven by excessive leucocyte stimulation with the release of secondary mediators.[8 16 17 24]

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