Inflammatory response in the early prediction of severity in human acute pancreatitis

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Abstract

The role of the inflammatory response in acute pancreatitis and its relation with the clinical course was examined. This study examined if the serial measurement of polymorphonuclear granulocyte (PMN) elastase/A1PI complex, phospholipase A catalytic activity, C reactive protein, and other acute phase proteins, and the protease inhibitor α_2 -macroglobulin, provides meaningful information for prognosis. Eighty non-consecutive patients with acute pancreatitis, classified according to their clinical outcome into mild (n=40) and severe pancreatitis (n=40), were followed up daily. Between 48 hours, median values of PMN-elastase, C reactive protein – and most of the acute phase proteins – and phospholipase A activity, were significantly higher in the severe pancreatitis group. PMN elastase shows a dynamic course and it reaches an early peak value at days 1–2, followed by C reactive protein (days 2–4) phospholipase A (day 3), and a negative peak for α_2 -macroglobulin (days 4–5). PMN elastase (day 1) and C reactive protein (day 2) were selected by discriminant analysis as the most useful variables studied to allow the early accurate prediction of severity (sensitivity 100%, specificity 95%). Little or no predictive additional value was found for all other variables studied. These results strongly suggest a close relation between inflammatory parameters and clinical course in acute pancreatitis, and discriminant analysis of these variables provides a useful method to classify severity.

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Pathophysiology of acute pancreatitis is a very complex process, which entails the action of ischaemia reperfusion injury,^{1–3} intrapancreatic enzyme activation,^{4–6} and leucocyte infiltrate byproducts.^{7–9} Recent evidence suggests that mediators produced and released by activated inflammatory cells, may contribute considerably to the complications – multiorgan failure, pancreatic necrosis, sepsis, hypermetabolism – and significant mortality in acute pancreatitis.^{10–12} This study was performed to test the hypothesis that the severity in acute pancreatitis is closely related to the intensity of local inflammatory response.¹³ We measured serially, on days 1–5, in 80 patients with acute pancreatitis (40 mild, 40 severe disease) plasma concentrations of polymorphonuclear granulocyte (PMN) elastase/A1PI complex, –

as a specific marker of granulocyte activation,¹⁴ – acute phase proteins – as markers of metabolic response from hepatocytes to circulating cytokines, secreted mainly by activated monocytes-macrophages (interleukin 6, interleukin β , tumour necrosis factor α),¹⁵ phospholipase A catalytic activity – as marker of phagocytic activity in inflammation and necrosis¹⁶ and α_2 -macroglobulin, the main plasma protease inhibitor – as marker of protease/protease inhibitor imbalance.¹⁷

Descriptive and explorative data analysis was made for all variables (days 1–5). By using the data obtained within 48 hours of admission, we applied discriminant function analysis to predict severity in acute pancreatitis.

Methods

PATIENTS

We studied a group of 80 non-consecutive patients, 43 men and 37 women with a median age of 58 years (range 23–89). The diagnosis of acute pancreatitis was based on typical clinical symptoms and at least a twofold increase of specific pancreatic serum enzymes (pancreatic amylase or lipase). Further inclusion criteria were a contrast enhanced computed tomography study of the pancreas or an ultrasound scan within 48 hours of hospital admission, or both. Patients were classified according to their clinical outcome into two groups: mild pancreatitis (n=40) (uncomplicated or with only minor complications), and severe pancreatitis (n=40) resulting in death, local pancreatic complication – abscess, pseudocyst or necrosis – or systemic complications. The cause of acute pancreatitis was gall stones in 55%, chronic alcoholism in 17.5%, and other or unknown causes in 27.5% of the patients. On admission, all patients were treated medically according to accepted methods. Necrotising pancreatitis was confirmed by laparotomy or after contrast enhanced computed tomography in 18 patients (45% of severe group).

In mild cases no life threatening complications were seen, whereas patients with severe disease (Table) frequently manifested respiratory insufficiency defined as $\text{PaO}_2 < 60$ mm Hg (24 cases), sepsis (16 cases), consumptive coagulopathy (9 cases), shock (7 cases), renal failure (7 cases), severe hypocalcaemia (2 cases), and encephalopathy (2 cases). Death occurred in 11 of 40 patients with severe pancreatitis.

LABORATORY TESTS

Blood samples were collected under standard conditions. EDTA plasma and serum were

Complications in the severe acute pancreatitis group (n=40)*

Complication	Cases (n) (M/F)	Cause	Median value			
			Age (y)	ELAS [†] ** (µg/l)	CRP [†] ** (µg/l)	
Pancreatic necrosis	18 (14/4)	Gall stones	8	50	448	170
		Alcohol	6			
		Idiopathic	2			
		Alcohol & gall stones	2			
Respiratory insufficiency	25 (18/7)	Gall stones	12	58	504	157
		Alcohol	7			
		Idiopathic	4			
		Alcohol & gall stones	2			
Sepsis	16 (14/2)	Gall stones	9	60	510	153
		Alcohol	5			
		Mixed	2			
Coagulopathy	9 (6/3)	Gall stones	6	67	366	151
		Alcohol	2			
		Mixed	1			
Shock	7 (4/3)	Gall stones	6	69	374	178
		Alcohol	1			
Renal failure	7 (3/4)	Gall stones	5	58	366	167
		Idiopathic	2			
Death	11 (5/6)	Gall stones	8	67	366	188
		Alcohol	1			
		Idiopathic	1			
		Mixed	1			

*A more detailed table containing a list of individual clinical features in the severe acute pancreatitis group is available upon request, **untransformed values of PMN elastase (day 1) and CRP (day 2).

obtained daily from all patients during the first five days of stay in hospital, by centrifugation at 1300 g for 10 minutes. Plasma and serum aliquots were stored frozen at -80°C until analysis.

Enzymatic activities (30°C) of α amylase (EC 3.2.1.1) and lipase (EC 3.1.1.3) were determined in serum with commercial test kits (Boehringer, Mannheim, Germany).

PMN elastase/A1PI complex in plasma was determined by an immunoactivation method (IMAC, E Merck, Darmstadt, Germany).¹⁸ Phospholipase A catalytic activity (37°C) (EC 3.1.1.4) was determined by a fully automated photometric assay based on a commercially available phosphatidylcholine substrate (Boehringer Mannheim, Germany), and the measurement of free fatty acid released (NEFA enzymatic kit, Wako, Japan).¹⁹ Serum concentrations of C reactive protein were measured by an automated particle enhanced nephelometric immunoassay (Behringwerke AG, Marburg, Germany). Antichymotrypsin and inter α trypsin inhibitor were measured by turbidimetry by using commercially available specific antibodies (Dakopatts, Denmark). α₂-Macroglobulin, α₁ protease inhibitor, and α₁ acid glycoprotein were measured by nephelometry (Technicon reagents, Tarrytown NY, USA).

Blood samples from 150 healthy adults of both sexes, aged between 20 and 80 years, were used to establish a reference interval for the laboratory parameters tested.

STATISTICAL ANALYSIS

Preprocessing of data and exploratory analysis

Descriptive and exploratory analysis for all variables measured on days 1–5 in both groups of patients with acute pancreatitis was made. Multiple box and whisker plots were used. Plots synthesised information on central values (median), dispersion of variables (quartiles), and asymmetry with respect to normal

distribution. The application of square root transformation (SQR) of variables was used to make dispersions more homogeneous and to ensure the normal distribution of data. Data are given as medians and quartiles. To determine statistically differences (p<0.05) between the median values, the two tailed Mann-Whitney U test was used.

Predictive analysis

Stepwise discriminant function analysis was applied to the data obtained within 48 hours of admission to predict the severity of acute pancreatitis. Furthermore age, sex, cause of acute pancreatitis, and the increment value of all variables assayed (value at day 2 minus value at day 1) were considered. The analysis was carried out using the BMDP statistical package (BMDP P7, 1988). If x₁, x₂,..., x_k are discriminating variables for mild and severe acute pancreatitis, the procedure establishes a linear function (discriminant function)

$$z=c_1x_1+c_2x_2+...+c_kx_k$$

in which z is the score of the discriminant function for each case and c the weighing coefficient. The method used to select the discriminating variables and to determine the coefficients c₁, c₂,...,c_k develops in successive steps. With each step, a variable that is shown to have the greatest discriminating power, is included in the discriminant function. Variables that do not contribute significantly (at the 5% probability value) to the final equation are subsequently excluded from the model.

The discriminant function transforms the data on each patient into a score (z value) that can be plotted as a point on a straight line. To classify optimally a new patient as a case of severe or mild acute pancreatitis, a cut off value of z is established by considering the prevalence of severe (0.1) and mild (0.9) acute pancreatitis and the cost of erroneous classification (classification rule).

Validation

The jackknife procedure was used to validate the discriminant function. One patient at a time was removed from the sample, and the discriminant functions are recalculated for the remaining patients. The procedure was repeated for each patient in the group, and the efficacy of classification (sensitivity, specificity) was assessed. 'Jackknifed' values are a better estimate of the performance of classification in future patients than those obtained using primary discriminant analysis.

Results

SINGLE VARIABLE ANALYSIS

PMN elastase

The median peak value for PMN elastase/A1PI complex in the two groups of acute pancreatitis was reached early on days 1–2 (Fig 1). On day 1, PMN elastase values were significantly

Figure 1: Multiple box and whisker plot of PMN elastase during the days 1–5 after the stay in hospital. Broken lines represent the upper and lower limit (interval 95%) of the reference group. M=mild acute pancreatitis; S=severe acute pancreatitis.

higher ($p < 0.00001$) in patients with severe disease (380, 304.5–499.5 $\mu\text{g/l}$, median and quartiles) compared with patients with mild disease (79.5, 34.5–117 $\mu\text{g/l}$, median and quartiles). In all patients, elastase concentrations decreased continuously during the following days. Median plasma concentrations on days 1–5 were significantly higher ($p < 0.00001$) in patients with severe disease than in those with mild pancreatitis. Values for PMN elastase $> 200 \mu\text{g/l}$, on day 1 were exclusively found in the group of severe pancreatitis.

Acute phase proteins

In individual patients, C reactive protein reaches the peak within days 2–4, with values considerably higher and persisting for longer in the group of severe pancreatitis.

The median peak value of C reactive protein was reached on day 3 in patients with severe pancreatitis (222, 141.5–303 mg/l , median and quartiles) and on day 2 in patients with mild disease (81, 34–130 mg/l , median and quartiles) (Fig 2). Median concentrations on days 1–5 were significantly higher ($p < 0.0001$) in patients with severe disease than those with

Figure 2: Time course of C reactive protein.

Figure 3: Time course of antichymotrypsin.

mild pancreatitis. Values for C reactive protein $> 300 \text{ mg/l}$ were found exclusively in the group of severe pancreatitis.

The median peak value of antichymotrypsin was reached on day 3. Antichymotrypsin concentration shows a time course slower than those showed by C reactive protein, on days 1–5 (Fig 3). Median concentrations on days 1–5 were significantly higher ($p < 0.00001$) in patients with severe disease than in those with mild pancreatitis. Within 48 hours, values for antichymotrypsin $> 140 \text{ mg/dl}$ were found in 80% of patients with severe acute pancreatitis.

The concentration of α protease inhibitor increased during the pancreatic attack in both groups, but peaked earlier in patients with mild disease (at day 2) than in those with severe disease (at day 4) (Fig 4). Median values at day 4 were significantly higher ($p < 0.0001$) in patients with severe disease compared with those with mild pancreatitis. The median peak value of α_1 acid glycoprotein was reached late on day 4.

In contrast with other acute phase proteins that increased during inflammatory response, the concentration of inter α trypsin inhibitor – a single polypeptide chain molecule very sensitive against proteolytic enzymes – tended to decrease, particularly in patients with severe pancreatitis. In patients with mild pancreatitis,

Figure 4: Time course of α_1 protease inhibitor.

Figure 5: Time course of phospholipase A catalytic activity.

median inter α trypsin inhibitor concentrations remained constant during the study period, while patients with severe disease showed a continuous decrease with a minimum on day 4. As expected, the values of several of the acute phase proteins were highly correlated. On day 1, serum concentration of C reactive protein was correlated with antichymotrypsin ($r=0.708$), α_1 acid glycoprotein ($r=0.492$), or α_1 protease inhibitor ($r=0.568$) concentration. Furthermore, a highly significant correlation between serum concentrations of C reactive protein and phospholipase A values on day 1 ($r=0.582$; $p<0.0001$) was found.

Phospholipase A activity

The serum phospholipase A concentrations rose to high values in patients with severe disease, especially in those with necrotising pancreatitis, respiratory insufficiency, shock or sepsis. In the group of patients with severe disease, the median peak value was reached on day 3 (Fig 5). The difference in serum phospholipase A activities between patients with mild and severe pancreatitis was significant ($p<0.00001$) on days 1–5. Using a phospholipase A cut off value of 15 U/l (day 3) to classify pancreatitis as severe or mild, sensitivity was 62% and specificity 94.8%.

α_2 -Macroglobulin

In patients with mild pancreatitis, median α_2 -macroglobulin concentrations remained constant, while patients with severe disease showed a continuous fall with a minimum at day 5. Significant differences ($p<0.00001$) between median α_2 -macroglobulin values in both groups were seen on days 3–5 (Fig 6).

DISCRIMINANT MULTIVARIATE ANALYSIS

Only those cases with complete data sets for all variables were entered in the model. Eight cases with extremely atypical data, most of whom presented very high values of PMN elastase and no available or missing values were excluded. Thus, 32 cases were included in the severe group and 40 in the mild group.

PMN elastase on day 1 was the most discriminating variable selected in the first step. This assay predicted disease severity with a sensitivity of 84%. The outcome (mild or severe disease) was correctly predicted by PMN elastase assay in 93% of cases. In the second step, serum concentration of C reactive protein on day 2 was selected. The discriminant function gave a sensitivity of 96.8%, while correctly categorising 98.6% of cases. No more useful additional information was obtained using other variables. The classification function obtained was: $z=0.46477$ (SQR) (C reactive protein (day 2)) + 0.78009 (SQR) (PMN elastase (day 1)) – 16.31885 (classification rule, $z \geq 0$: severe acute pancreatitis; $z < 0$: mild acute pancreatitis).

We set a cut off to maximise sensitivity (that is, detection of severe cases) and specificity (optimisation of the classification function) by considering the cost of erroneous classification for severe acute pancreatitis and the prevalence for each group (mild 0.9, severe 0.1). Therefore, the new function was: $z=0.46477$ (SQR) (C reactive protein (day 2)) + 0.78009 (SQR) (PMN elastase (day 1)) – 15.57958 (classification rule, $z \geq 0$: severe acute pancreatitis; $z < 0$: mild acute pancreatitis).

For this function, sensitivity was 100%, specificity 95%, and the outcome was correctly predicted in 97.2% of cases. Both variables selected by the model were not correlated ($r=-0.0262$) and their courses are fully independent.

Discussion

Recent experimental and clinical data have shown that excessive activation and systemic release of endogenous humoral mediators produced by inflammatory cells – polymorphonuclear neutrophils monocytes, and macrophages – may themselves be responsible for severe complications seen in acute pancreatitis, such as adult respiratory distress syndrome, multiorgan failure, and clinical sepsis syndrome.^{20–22} In early phases of local inflammation, chemotactic factors activate polymorphonuclear neutrophils (the first line

Figure 6: Time course of α_2 macroglobulin.

of cellular response) and monocytes. At the site of inflammation, these cells release biologically active products, such as proteolytic enzymes, reactive oxygen metabolites, vasoactive substances, and cytokines (tumour necrosis factor α , interleukin 1, interleukin 6, interleukin 8,^{7-9 13 23 24}

In this study, blood concentrations of different inflammatory markers were determined to assess whether or not the intensity of inflammatory response was correlated with the severity of pancreatitis, and to obtain a reliable model for an early and accurate prediction of prognosis.

Granulocyte elastase has been shown to be a sensitive and specific marker for the early identification of inflammation, activation of granulocytes, and the prediction of inflammatory complications.^{14 18} In this study, granulocyte elastase was the most useful marker of severity and our results coincide with those of other authors who found higher values in patients with necrotising acute pancreatitis²⁵ or severe disease^{11 24 26} than in patients with mild disease. The time course of granulocyte elastase showed an early peak on days 1–2 showing that polymorphonuclear neutrophils become highly activated in the early stages of acute pancreatitis. This assay predicted disease severity with a sensitivity of 84%. Increased concentrations of PMN elastase were clearly associated with a poor prognosis. The PMN elastase was measured in this study by using the IMAC elastase assay (E Merck) (automated version) in plasma EDTA samples. This method correlates well with the previous sandwich enzyme linked immunosorbent assay (ELISA), and it shows satisfactory precision at the clinical decision value. The IMAC elastase performed automatically on clinical chemistry analysers, provides a fast (about 10 minutes) routine method for measurement of PMN elastase concentrations in human plasma. A negative aspect of the kit is their excessive large presentation, particularly if the elastase determination is exclusively used as an aid to clinical evaluation of acute pancreatitis. In this respect, the development by the manufacturer of several kits with different presentations is welcome. Another important concern is related to the nature of the analyte – plasma must be separated carefully from blood within 60 minutes after specimen collection to prevent interference by leakage of elastase from leucocytes.

The increased serum concentrations of C reactive protein in patients with acute pancreatitis is a consequence of activation of the monocyte-macrophage system.^{15 27 29} The median peak value of C reactive protein was reached later (on days 2–4) reflecting the synthesis of acute phase proteins in the liver, secondary to the release of cytokines by activated monocyte-macrophages.

Stepwise discriminant function analysis selected PMN elastase value on day 1 and C reactive protein on day 2 as the optimal combination for predicting the severity in acute pancreatitis with 100% sensitivity and 95% specificity. Both variables were fully

independent ($r = -0.0262$) and no useful additional information was obtained from all other parameters studied. The time course of acute phase proteins, phospholipase A catalytic activity, and α_2 -macroglobulin was similar to that described by others.^{11 17 25 27–31.}

In summary, two biochemical assays easily performed routinely in most hospitals – that is, PMN elastase and C reactive protein – permit the establishment at an early stage of actual inflammatory response and predict accurately the clinical course of acute pancreatitis.

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