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Role of interleukin-6 in acute pancreatitis. Comparison with C-reactive protein and phospholipase A

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Abstract

Plasma values of immunoreactive interleukin-6, C-reactive protein and phospholipase A have been determined in serial samples from 24 patients with acute pancreatitis ('mild' pancreatitis nine, 'severe' pancreatitis 15). Median plasma concentrations of interleukin-6, C-reactive protein, and phospholipase A activity were significantly higher in patients with 'severe' illness ($p<0.001$) than those with 'mild' illness. A particularly marked increase in interleukin-6 was found in two patients with necrotising pancreatitis and fatal outcome. Significant correlations between plasma concentrations of interleukin-6 and phospholipase A ($p=0.0218$) and C-reactive protein and phospholipase A activity ($p<0.0001$) were found in patients with 'severe' disease. These findings in a limited number of patients with acute pancreatitis are promising in that raised interleukin-6 correlated with clinical severity and with two other established markers, C-reactive protein, and phospholipase A activity.

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Evidence is gathering that activated endogenous inflammatory mediators have an important role in the pathogenesis of acute pancreatitis.[1 2] In the early phases of inflammation, the chemotactic factors C5a, N-formyl-methionyl peptides, leukotriene B_4, platelet activating factor, and neutrophil activating peptides[3 4] activate polymorphonuclear neutrophils. At the site of inflammation, polymorphonuclear neutrophils release numerous active substances, such as lysosomal enzymes,[5–8] oxygen free radicals,[9 10] and vasoactive substances.[11 12] Several reports suggest that polymorphonuclear neutrophils may synthesise and release neutrophil activity peptides/interleukin-8[13] and interleukin-1β.[14 15] It has been shown that interleukin-1β is a potent inducer of interleukin-6 in peripheral blood monocytes,[16 17] fibroblasts, endothelial cells and keratinocytes.[18] A major activity of interleukin-6 is induction of acute phase protein synthesis in the liver.[19 20] Thus, increased serum concentrations of C-reactive protein in patients with acute pancreatitis is a consequence of the stimulation of hepatocytes by cytokines.[21–23] The duration and the magnitude of raised serum concentrations of C-reactive protein are related to the extent of tissue injury.[24 25]

Phospholipase A, a marker of phagocytic activity in inflammation and necrosis,[26 27] has been implicated in the development of pancreatic necrosis and pulmonary failure during the course of acute pancreatitis.[28] Inflammatory cells, such as polymorphonuclear neutrophils, monocytes, macrophages, and platelets, have been shown to contain and secrete phospholipase A_2.[29] Although the biological functions of phospholipase A of non-pancreatic origin have not yet been elucidated, it seems that this enzyme plays a role in the generation of leukotriene B_4 and platelet activating factor.[30]

We determined the value of sequential measurements of plasma concentrations of interleukin-6 in the prediction of outcome in acute pancreatitis. Furthermore, we compared the interleukin-6 concentrations with those of C-reactive protein and phospholipase A.

Methods

PATIENTS

In order to assess the value of interleukin-6 as a prognostic marker in acute pancreatitis, blood samples were taken daily from all patients with acute pancreatitis admitted consecutively to the participating hospitals. Blood was collected on the day of admission (day 1) until day 7 or until discharge from hospital if sooner. Plasma and serum were obtained by centrifugation of blood samples at 1300 *g* for 10 minutes and stored frozen at −80°C until analysis.

The diagnosis of acute pancreatitis was based on a consistent clinical picture, a two-fold increase of serum amylase (upper limit of normal, 160 IU/l) or serum lipase (upper limit of normal, 190 IU/l), and morphologic abnormalities compatible with acute pancreatitis on contrast enhanced, computed tomography study of the pancreas and/or an ultrasound scan within the first 48 hours of hospital admission. The severity of pancreatitis was assessed by the clinical outcome – that is, number of complications that occurred during the course of the disease. A 'mild' attack was uncomplicated or one with only minor complications; a 'severe' attack included at least three prognostic factors

List of complications in 15 patients with severe acute pancreatitis

Complication	*Cases (n)*
Respiratory insufficiency (PaO_2 <60 mm Hg)	8
Pancreatic necrosis	7
Sepsis	6
Shock	5
Pancreatic abscess	3
Pancreatic pseudocyst	3
Renal failure	2
Consumptive coagulopathy	2
Encephalopathy	1

Seven patients died.

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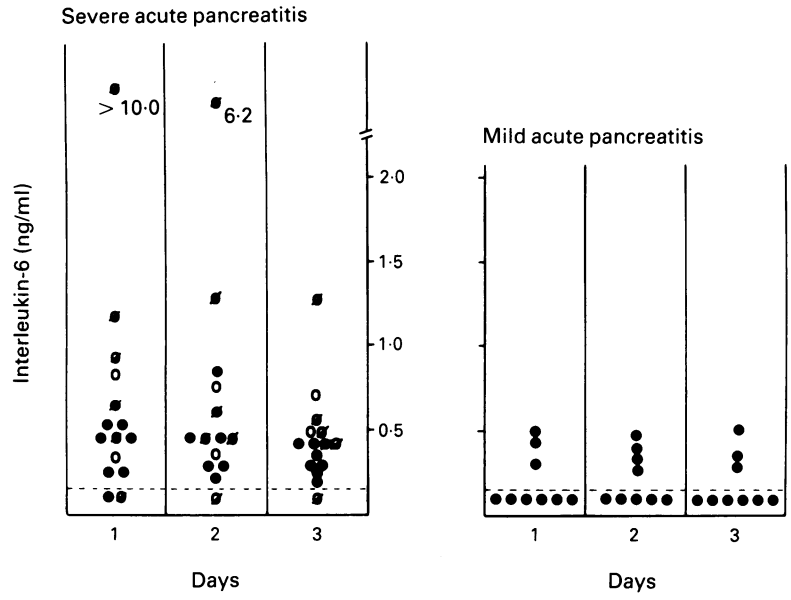

Figure 1: Individual values of interleukin-6 concentration in patients with 'mild' and 'severe' acute pancreatitis on days 1–3 (broken line, lower limit of detection (0·15 ng/ml); ○, necrosis; ◑, necrosis and sepsis; ●, sepsis, no necrosis; ●, no necrosis nor sepsis).

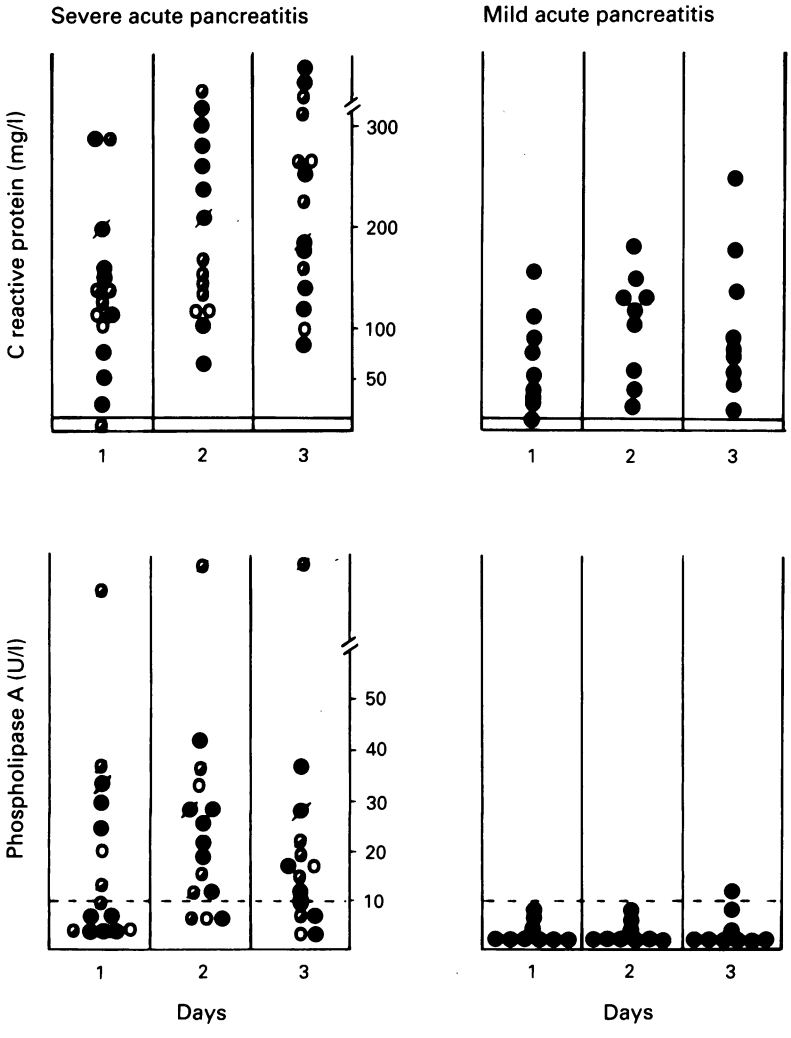

Figure 2: Individual values of C-reactive protein concentration and phospholipase A levels in patients with 'mild' and 'severe' disease on days 1–3; (○, necrosis; ◑, necrosis and sepsis; ●, sepsis, no necrosis; ●, no necrosis nor sepsis).

of severity according to the criteria of Blamey *et al*,[31] major organ failure, a pancreatic complication (pseudocyst, abscess, or necrosis), or death.

A group of 24 non-consecutive patients, 12 men and 12 women with a median age of 58 years (range 30–83) was studied. Patients were selected retrospectively on the basis of the clinical outcome: 'mild' pancreatic attack (nine patients) and 'severe' pancreatic attack (15 patients). A larger number of patients with 'severe' disease was included, because it was assumed that abnormalities of interleukin-6, C-reactive protein, and phospholipase A would be presumably more marked.

The aetiology of acute pancreatitis was gall stones in 58%, chronic alcoholism in 17%, and other or unknown causes in 25% of the patients. On admission, all patients were treated medically according to general accepted methods. Complications that occurred during the course of the disease are shown in the Table. Operation was undertaken in 12 patients with severe pancreatitis because of signs of abscess, necrosis, or pseudocyst. Necrotising pancreatitis was confirmed by laparotomy or computed tomography in seven patients. Death occurred in seven of the 15 patients with severe pancreatitis. The total mortality rate was 29·2% (seven of 24 patients).

Plasma immunoreactive interleukin-6 was measured by a solid phase immunoassay with the multiple sandwich principle (Intertest-6^{TM} enzyme linked immunoadsorbent assay (ELISA) kit, Genzyme Corp, Boston, MA, USA). The lower limit of detection is about 0·15 ng/ml and no detectable cross reaction with other human cytokines (interleukin-1α and β, interleukin-2, interleukin-3, interleukin-4, or TNFα occurred. Results were expressed as ng/ml (mean of duplicates). Phospholipase A serum catalytic activity was determined by a fully automated photometric method based on a phosphatidylcholine substrate and the production of free fatty acids with phospholipase A_2 and phospholipase A_1.[32] The lower limit of detection is about 2 IU/l (37°C). Results were expressed as IU/l (37°C). Serum concentrations of C-reactive protein were measured by an automated particle enhanced nephelometric immunoassay (Behring-Werke AG, Marburg, Germany). The lower limit of detection is about 0·6 mg/l. Results were expressed as mg/l.

Statistical analysis was performed using the two tailed Mann-Whitney U test and regression analysis. Statistical significance was set at $p<0·05$.

Results

Individual interleukin-6 concentrations in plasma occurring between the first and third day of illness are shown in Figure 1. Most patients with 'mild' acute pancreatitis had values below the lower limit of detection of 0·15 ng/ml, whereas interleukin-6 concentrations were persistently raised in most patients with 'severe' disease. Interleukin-6 concentrations tended to be higher in patients with necrotising pancreatitis and/or sepsis. Two patients with necrotising pancreatitis had particularly increased

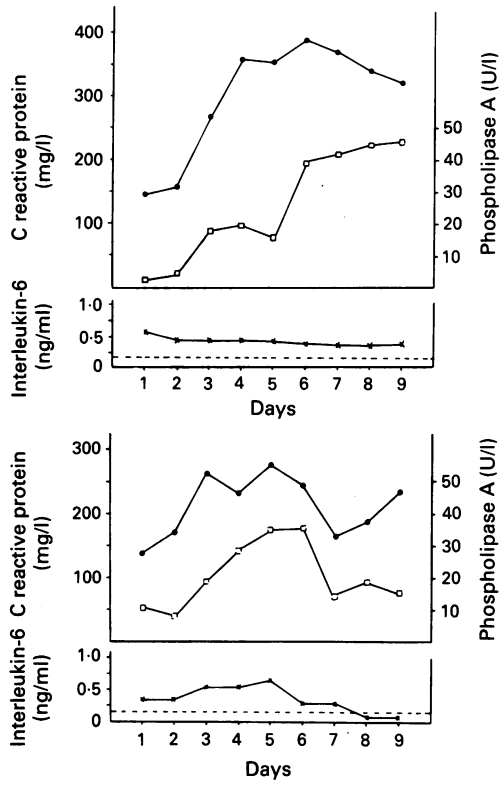

Figure 3: Time course of interleukin-6, C-reactive protein, and phospholipase A in two patients with acute necrotising pancreatitis (top, alcoholic pancreatitis; bottom, biliary pancreatitis with fatal outcome) (solid circles, C-reactive protein; open squares, phospholipase A; asterisk, interleukin-6.

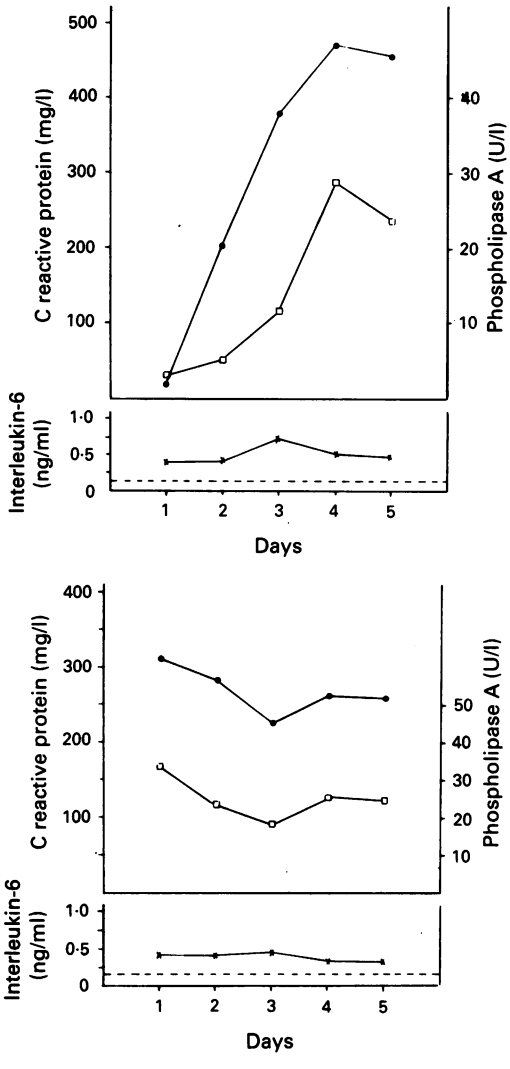

Figure 4: Time course of interleukin-6, C-reactive protein and phospholipase A in two patients with 'severe' pancreatitis with sepsis (solid circles, C-reactive protein; open squares, phospholipase A; asterisk, interleukin-6).

interleukin-6 values. The median value of interleukin-6 was significantly different between patients with 'mild' and 'severe' acute pancreatitis ($p<0·001$).

Individual serum concentrations of C-reactive protein and phospholipase A activity during the first three days of illness are shown in Figure 2. Within three days after the onset of acute pancreatitis, the median values of serum C-reactive protein and phospholipase A, respectively, revealed highly significant differences between the 'mild' and 'severe' groups ($p<0·001$). In patients with 'severe' disease, a relationship was found between increased serum concentrations of biochemical markers and the presence of pancreatic necrosis, respiratory insufficiency (PaO_2 <60 mm Hg), and sepsis.

The time course of interleukin-6, C-reactive protein, and phospholipase A in four individual patients are shown in Figures 3 and 4. In most patients of the 'severe' group, a dynamic parallel course was found between concentrations of C-reactive protein and phospholipase A, together with persistently raised concentrations of interleukin-6. A large interindividual variation was also noticed with less marked changes among patients with 'mild' disease.

When all daily measurements from patients with 'severe' pancreatitis were taken together, highly significant correlations between serum levels of C-reactive protein and phospholipase A ($r=0·5162$; $p<0·0001$) and interleukin-6 and phospholipase A ($r=0·3089$; $p=0·0218$) were found. The correlation between daily increases of C-reactive protein and phospholipase A was also significant ($r=0·5464$; $p<0·0001$) (Fig 5).

Discussion

Interleukin-6, a multifunctional cytokine secreted by normal cells, cell lines and tumour cells, is an important mediator of the inflammatory response. It regulates immune responses, haematopoiesis and acute phase reactions, indicating that it plays a central role in host defence mechanisms.[19 33] At least six forms of differentially modified interleukin-6 phosphoglycoproteins are secreted by induced fibroblasts and monocytes.[34] $Alpha_2$-macroglobulin binds interleukin-6 and prevents proteolysis; interleukin-6 complexed to α_2-macroglobulin remains partially active in various cell culture bioassays.[35]

The availability of very sensitive bioassays for interleukin-6 allows the determination of interleukin-6 activity in clinical samples from patients with acute pancreatitis.[36] Biological assays for interleukin-6 using hybridoma cell lines, however, could be influenced by synergistically acting cytokines (interleukin-1β, tumour necrosis factor α) or inhibitory substances present in human serum and/or added to the culture media. In addition, different glycosylated forms of interleukin-6 may show different activities in the bioassay.[37] Assays based on the detection of immunoreactive interleukin-6 are preferable to biological assays when serum samples are studied. A very sensitive ELISA test, almost as sensitive as the biological assay, for the detection of interleukin-6 in biological

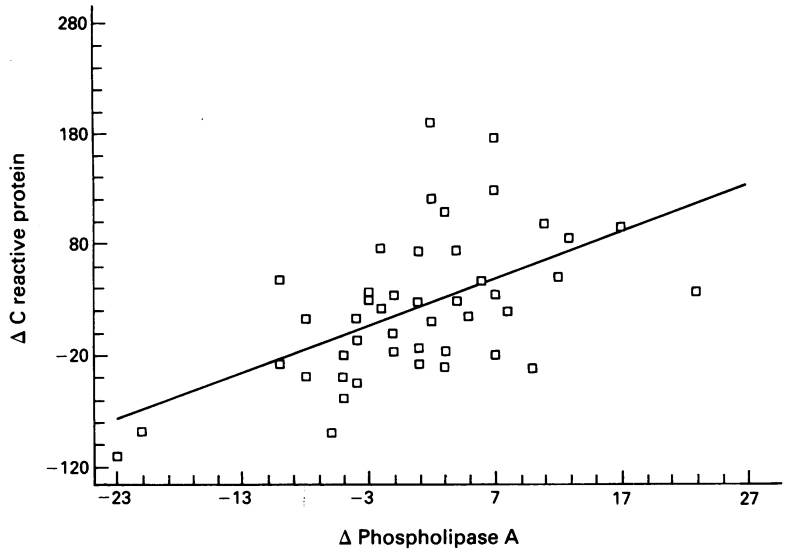

Figure 5: Correlation between daily increases of C-reactive protein (INCR (CRP)) and phospholipase A (INCR (PLA)).

fluids has recently been developed.[38] This new immunoassay could be useful for measuring low plasma concentrations of interleukin-6 in patients with acute pancreatitis.

In the present study, rises in interleukin-6 correlated both with clinical severity of pancreatitis and with two other established markers, C-reactive protein, and phospholipase A activity. In most patients with 'mild' disease no detectable concentration of interleukin-6 (<0·15 ng/ml) was shown, whereas significantly higher interleukin-6 concentration was detected in patients with 'severe' disease. In patients with 'severe' disease, the correlations observed between plasma concentrations of interleukin-6 and phospholipase A and C-reactive protein and phospholipase A activity may suggest a causal role for interleukin-6 in the acute phase response[19 33] and are consistent with the possibility of the monocyte-macrophage system as a common source for plasma concentrations of interleukin-6 and phospholipase A in severe acute pancreatitis. This should be supported, however, by measurements of the release of interleukin-6 and phospholipase A from peripheral blood mononuclear cells of patients with acute pancreatitis. Further investigations are necessary to clarify the role of inflammatory cytokines (interleukin-6, interleukin-1β, neutrophil activity peptides/interleukin-8, and TNFα) in the pathogenesis of acute pancreatitis.

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