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Activation of cellular immune response in acute pancreatitis

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Abstract

***Background*—Inflammatory mediators have recently been implicated as potential markers of severity in acute pancreatitis.**

***Aims*—To determine the value of neopterin and polymorphonuclear (PMN) elastase as markers of activation of cellular immunity and as early predictors of disease severity.**

***Patients*—Fifty two non-consecutive patients classified according to their clinical outcome into mild (n=26) and severe pancreatitis (n=26).**

***Methods*—Neopterin in serum and the PMN elastase/A1PI complex in plasma were measured during the first three days of hospital stay.**

***Results*—Within three days after the onset of acute pancreatitis, PMN elastase was significantly higher in the severe pancreatitis group. Patients with severe disease also showed significantly higher values of neopterin on days 1 and 2 but not on day 3 compared with patients with mild disease. There was a significant correlation between PMN elastase and neopterin values on days 1 and 2. PMN elastase on day 1 predicted disease severity with a sensitivity of 76·7% and a specificity of 91·6%. Neopterin did not surpass PMN elastase in the probability of predicting disease severity.**

***Conclusions*—These data show that activation of cellular immunity is implicated in the pathogenesis of acute pancreatitis and may be a main contributory factor to disease severity. Neopterin was not superior to PMN elastase in the prediction of severity.**

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Keywords: acute pancreatitis, polymorphonuclear elastase, neopterin, prognosis, cellular immunity.

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The pathogenesis of acute pancreatitis is a very complex process and remains poorly understood. Recent evidence suggests that the inflammatory response plays an important role in the pathophysiology of the disease and contributes considerably to the complications.[1 2] It has been shown that the severity in acute pancreatitis is closely related to the degree of activation of the inflammatory response. Inflammatory mediators have been implicated as potential markers of the intensity of the initiating inflammatory stimulus.[3–5] The most promising early markers of disease severity, which may also reflect clinical outcome, include serum levels of polymorphonuclear (PMN) elastase,[6] C-reactive protein,[7–9] trypsinogen activation peptide,[10] and interleukin 6.[11] Severity prediction with the measurement of neopterin, a marker of macrophage activation by endotoxins or γ-interferon,[12] has scarcely been assessed in patients with acute pancreatitis.[13 14]

We have measured serum concentrations of neopterin and plasma levels of PMN elastase as markers of activation of cellular immunity and as early predictors of disease severity in acute pancreatitis.

Methods

PATIENTS

Fifty two non-consecutive patients (25 men and 27 women, mean age 58 years, range 24–89) were studied. Only patients admitted to hospital within 12 hours after the onset of abdominal pain were included. The diagnosis of acute pancreatitis was based on typical clinical symptoms, at least a twofold increase in serum concentrations of specific pancreatic enzymes (amylase or lipase), and characteristic features in a contrast enhanced computed tomography study of the pancreas and/or an ultrasound scan within 48 hours of hospital admission. The cause of acute pancreatitis was gall stones in 46%, chronic alcoholism in 23%, unknown causes in 25%, and other causes (primary hyperparathyroidism, administration of furosemide, postsurgery) in 6% of patients. Patients were classified according to criteria of severity established at the International Symposium on Acute Pancreatitis in Atlanta in 1992,[15] into two groups: mild acute pancreatitis (n= 6) (minimal organ dysfunction and uneventful recovery), and severe acute pancreatitis (n=26) (associated with organ failure and/or local complications, such as necrosis, abscess, or pseudocyst). Complications seen in the group of 26 patients with severe disease included respiratory insufficiency (Pao_2 <60 mm Hg) in 10 patients, sepsis in nine, upper gastrointestinal haemorrhage in one, renal failure in two, encephalopathy in two, consumptive coagulopathy in two, pancreatic pseudocyst in four, pancreatic abscess in two, and pancreatic necrosis without systemic complications in four. The total mortality rate was 7·7% (2/26 patients).

LABORATORY TESTS

Blood samples were collected under standard conditions. EDTA plasma and serum were obtained daily from all patients between 8 00

and 9 00 am during the first three days of their hospital stay. EDTA plasma samples were centrifuged at 400 *g* for 15 minutes. Serum samples were centrifuged at 1300 *g* for 10 minutes at 37°C. Plasma and serum aliquots were stored frozen at –80°C until analysis which was performed within 30 days after sampling.

Activities (30°C) of amylase (EC 3.2.1.1) and lipase (EC 3.1.1.3) were determined in serum with commercial test kits (Boehringer, Mannheim, Germany). The upper limit of normal was 190 IU/l for serum amylase and 195 IU/l for serum lipase. Neopterin in serum was measured by radioimmunoassay, with a reference value of <10 nmol/l. The PMN elastase/A1PI complex in plasma was determined by an immunoactivation method (IMAC, E Merck, Darmstadt, Germany), with a normal range of 12–32 µg/l.

In a reference group of 120 healthy subjects, mean values of neopterin and PMN elastase were 5·89 nmol/l (95% confidence interval 2·4–9·4 nmol/l) and 34·5 µg/l (95% confidence interval 10–58 µg/l).

STATISTICAL ANALYSIS

Descriptive analysis for all variables measured on days 1–3 in both groups of patients with acute pancreatitis was made. The degree of association among variables of inflammatory response was assessed using Spearman coefficients. To compare the levels of inflammatory response in relation to the prognosis of pancreatitis the Mann-Whitney U test was used. To quantify the ability of PMN elastase and neopterin (either separately or taken together) to predict severity of acute pancreatitis, sensitivity, and specificity were determined using a logistic regression model in which prognosis of pancreatitis was considered the dependent variable and markers of inflammatory response were considered independent variables. Results were considered significant when $p<0·05$.

Results

The peak value for PMN/A1PI complex in the two groups of acute pancreatitis patients was reached early on day 1 (mild pancreatitis: median 87·30 µg/l, interquartile range 23·50–167·65 µg/l; severe pancreatitis: median 398·5 µg/l, interquartile range 241·5–550 µg/l). Within three days after the onset of acute pancreatitis, the values of PMN elastase revealed highly significant differences ($p<0·0001$) between the "mild" and "severe" groups (Fig 1). Serum levels of neopterin increased in both groups. The peak value was reached on day 2 (mild pancreatitis: median 8·5 nmol/l, interquartile range 6–13·8 nmol/l; severe pancreatitis: median 12·4 nmol/l; interquartile range 9·17–17·82 nmol/l). Patients with severe pancreatitis showed significantly higher neopterin values on days 1 ($p=0·035$) and 2 ($p=0·04$) compared with patients with mild disease, although differences on day 3 were not significant (Fig 2). There was a statistically significant correlation between PMN elastase and neopterin levels on day 1 ($r=0.4$; $p<0·02$) and day 2 ($r=0·8$; $p<0·0001$) (Fig 3).

There were no statistically significant differences in neopterin and PNM elastase values between patients with mild acute pancreatitis and controls on days 1 and 2. On the other hand, statistically significant differences in serum levels of neopterin among the various aetiologies of acute pancreatitis were not found.

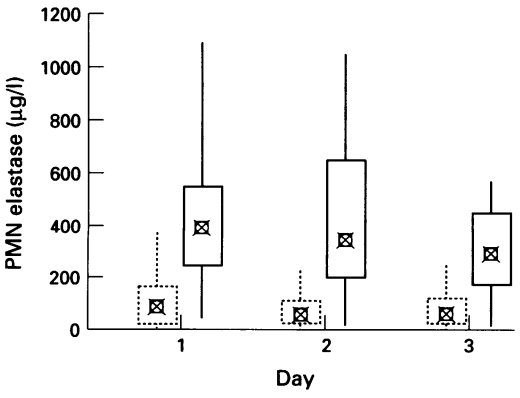

Figure 1: Multiple box plot of PMN elastase during days 1–3 (median values and interquartile range). Dotted line box, mild pancreatitis; continuous line box, severe pancreatitis.

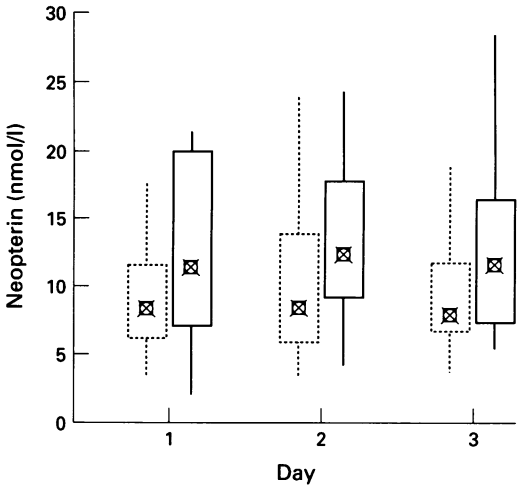

Figure 2: Multiple box plot of neopterin during days 1–3 (median values and interquartile range). Dotted line box, mild pancreatitis; continuous line box, severe pancreatitis.

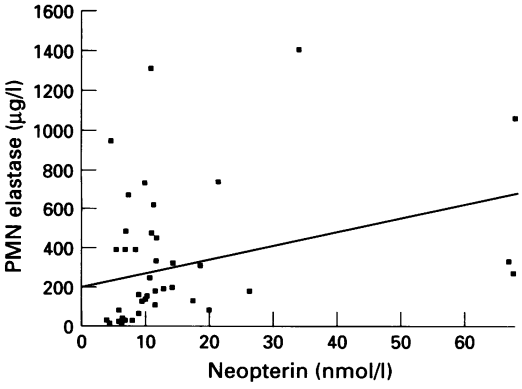

Figure 3: Correlation between PMN elastase and neopterin on day 1.

In the logistic regression models, setting a cutoff point of ≥0·50 for the probability of developing severe acute pancreatitis resulted in a sensitivity of 76·7% and 79%, specificity of 91·6% and 88·5%, and overall efficacy of 86% and 84% for PNM elastase on days 1 and 2, respectively; and a sensitivity of 21% and 32%, specificity of 92·6% and 81% and overall efficacy of 66·6% and 62% for neopterin values on days 1 and 2, respectively. These percentages were not significantly improved using an "optimal" cutoff point as indicated by receiver operating characteristics (ROC) curves.

Discussion

It has been shown that intensity of the inflammatory response closely correlates with disease severity in acute pancreatitis. Assessment of the degree of pancreatic inflammation by measuring cellular mediators or molecular markers of activation of humoral systems has therefore been used to predict disease severity.[3–5]

Results of this study strongly suggest that activation of cellular immunity is implicated in the pathogenesis of acute pancreatitis and that a more intense activation occurs in patients with severe disease. In agreement with previous studies,[4 6] the plasma concentration of PMN elastase on day 1 was able to predict disease severity with a high sensitivity and specificity. In patients with a variety of pathological conditions, Pacher *et al*[16] found a significant association between neopterin and clinical signs of sepsis, severity of adult respiratory distress syndrome, and multiple organ failure, and a statistically significant correlation between neopterin levels and elastase complex values. Our results confirm these findings in acute pancreatitis.

Data on the usefulness of neopterin levels in acute pancreatitis are limited and results of studies are controversial. Fuchs *et al*[13] have shown increased concentrations of neopterin in 9/24 patients with acute pancreatitis and a significant correlation with serum levels of sTNF-R. From these results, the authors concluded that activation of cellular immunity and consequently of macrophages, was implicated in the pathophysiology in a subgroup of patients with acute pancreatitis. By contrast, Chaloner *et al*[14] measured urine levels of neopterin and creatinine in 38 patients with acute pancreatitis (severe 27, mild 11). Although a higher neopterin/creatinine ratio was found in patients with acute pancreatitis than in controls, there were no differences between the mild and severe groups, the ratio being even higher in patients with severe disease. Decreased neopterin levels in severe acute pancreatitis were related by these authors to an inappropriate response of T lymphocytes and to "frustrated phagocytosis" that under these circumstances would contribute to the production of oxygen free radicals exceeding tissue antioxidant capacity. Recently, Uomo *et al*[17] investigated serum neopterin values in patients with mild (n=24) and severe acute pancreatitis (n=17) on the 1st and 7th day of hospitalisation. Neopterin was significantly higher in patients with severe pancreatitis than in those with mild acute pancreatitis and normal controls on days 1 and 7. Interestingly, neopterin serum levels were significantly higher on day 7 than on day 1 in severe but not in mild acute pancreatitis. Although, in our opinion, it is not difficult, a week after the onset of acute pancreatitis to differentiate severe from mild episodes on the basis of the patient's clinical condition, the findings of Uomo *et al*[17] may be important for major pancreatological centres to which patients are transferred from other hospitals at a variable period of time after the onset of the acute attack.

We have observed increased serum levels of neopterin from day 1. The peak serum level was reached on day 2, and patients with severe disease also showed significantly higher values on days 1 and 2 compared with patients with mild disease. In our study, as in that by Chaloner *et al*,[14] there was a statistically significant correlation between PMN elastase and neopterin levels on days 1 and 2. These results indicate a more intense activation of macrophages in severe acute pancreatitis and an earlier activation of polymorphonuclear granulocytes at the inflammatory focus than of macrophages. However, measurement of neopterin levels for predicting severity in acute pancreatitis is of little value in clinical practice, since increased PMN elastase levels are more sensitive and elevation of PMN concentrations above the normal range occurs early in the course of the disease.

In summary, there is an activation of cellular immunity (polymorphonuclear granulocytes and macrophages) in the course of acute pancreatitis as shown by increased concentrations of PMN elastase and neopterin. Although disease severity correlated with levels of both inflammatory mediators, neopterin did not surpass PMN elastase in the probability of predicting disease severity.

However, the evaluation of a larger number of patients could contribute to determination of the role of neopterin in the prognostic assessment of acute pancreatitis.

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Erratum

The incorrect abstract was printed as TU1 in the 1997 BSG abstract book (*Gut* 1997; **40** (suppl 1): A1. The correct abstract is reproduced below.

OMEPRAZOLE VS MISOPROSTOL: DIFFERENT EFFECTIVENESS IN HEALING GASTRIC AND DUODENAL ULCERS VS EROSIONS IN NSAID USERS – THE OMNIUM STUDY

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INTRODUCTION: Laboratory studies suggest that gastric mucosal damage by NSAIDs is biphasic. Inhibition of prostaglandin synthesis causes superficial gastric and duodenal erosions, followed by a more acid-dependent progression to ulceration. We, therefore, investigated the hypothesis that acid suppression with omeprazole would preferentially heal and prevent ulcers whilst misoprostol would have a greater impact on erosions.
METHODS: 935 patients on continuous NSAID treatment who had gastroduodenal ulcers and/or >10 erosions at endoscopy were randomised to receive omeprazole 20mg om, 40mg om or misoprostol 200μg qid under blinded conditions for 4/8 weeks until healing (no ulcer, <5 erosions). Of those, 732 patients were re-randomised to maintenance treatment with omeprazole 20mg om, misoprostol 200μg bid or placebo for 6 months to assess relapse (ulcer or >10 erosions).
RESULTS: Both doses of omeprazole were more effective than misoprostol in healing duodenal (DU) and gastric ulcers (GU). Conversely, misoprostol was more effective in patients with erosions.

	Healing (%)					
	DU (n=184)		GU (n=374)		Erosions (n=324)	
	4w	8w	4w	8w	4w	8w
Omeprazole 20mg om	80	93	70	87	55	77
Omeprazole 40mg om	88	89	67	80	62	79
Misoprostol 200μg qid	60	77	62	73	75	87

These results were replicated during the maintenance phase. Fewer patients relapsed with ulcers (with or without erosions) on omeprazole than on misoprostol or placebo (15% vs 20% and 43% respectively). In contrast, fewer patients relapsed with erosions only on misoprostol than on omeprazole or placebo (7% vs 12% and 14%, respectively).
CONCLUSIONS: These results support the concept that NSAID-associated ulcers are more acid-dependent than erosions and hence, more easily healed and prevented with omeprazole than with misoprostol.

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