Clinical relevance of intracellular cytokines IL-6 and IL-12 in acute pancreatitis, and correlation with APACHE III score

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ABSTRACT

Pro-inflammatory cytokines are involved in the pathogenesis of acute pancreatitis (AP). Here, we measure and correlate clinically the percentages of peripheral blood mononuclear cells (PBMC) that contain interleukin (IL)-6 and IL-12 and compare these with acute physiology and chronic health evaluation (APACHE III) scores in 30 patients with AP. Severity of AP is determined according to the Atlanta criteria. Patients with severe AP (n=15) had significantly higher IL-6 values compared to those with mild AP (n=15). IL-12 levels correlated well with aetiological factors (alcohol and biliary pathology) in patients with AP. Correlation was seen between IL-6 value and APACHE score in severe AP. A score of 30 points was used as the cut off between mild (<30) and severe (>30) cases, with a sensitivity of 80% and specificity of 100%. Cut off percentages for IL-6- and IL-12-positive PBMCs were >25% (positive predictive value [PPV]: 100%) and >9% (PPV: 70%), respectively. Based on these results, it would seem logical to use both APACHE III score and IL-6 percentage to assess severity in patients with AP.

KEY WORDS: APACHE. Interleukin-6. Interleukin-12. Pancreatitis, acute necrotizing.

Introduction

The symptoms of acute pancreatitis (AP) range from mild discomfort to complete prostration. Nonetheless, it remains a common and potentially fatal disease in which some patients develop extensive pancreatic inflammation and necrosis, a systemic inflammatory response and multiple organ failure. Although the exact mechanisms that trigger the inflammatory process are not completely understood, it is generally accepted that pro-inflammatory cytokines released by activated leucocytes play an important role in the pathogenesis of pancreatitis.[1,2] Serum levels of pro-inflammatory cytokines, including tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IL-8, are reported to be significantly higher in severe AP than in mild pancreatitis.[3-10]

IL-6 is a multifunctional protein that has important roles in host defence, acute-phase reactions, immune responses and haemopoiesis. It affects B-cell differentiation and antibody production, cytotoxic T-cell growth, differentiation and activation.[11] It is the principal mediator of acute-phase protein response and is always observed about 48 hours before an increase in C-reactive protein.[8,12-14] The rapid response suggests that it might be valuable in assessing and monitoring the severity of pancreatitis.

Serum IL-12 concentration is also increased in AP.[15] Functionally, IL-12 has multiple effects on both natural killer (NK) cells and T cells. It promotes the growth of activated NK cells and CD_4^+ and CD_8^+ T cells, increases cell-mediated NK cell cytotoxicity and contributes indirectly to macrophage activation through lymphocyte interferon synthesis.[16]

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Several scoring scales exist that predict both mortality and morbidity in patients with AP. These systems include computed tomography (CT) grading. Glasgow coma score (GCS), Ranson criteria, the multiple organ failure score and acute physiology and chronic health evaluation (APACHE) III.[17-23] The GCS and Ranson multiple scoring systems require 48 hours of data collection; however, APACHE III can be calculated at any time and shows prognostic correlation with AP, as increasing scores are associated with poor prognosis.

CD14 antigen is a glycosyl-phosphatidyl inositol-linked single-chain surface membrane glycoprotein with a molecular weight of 55 kDa, which is found on cells of myelomonocytic lineage and is strongly expressed on monocytes and macrophages. Here, we use this property of the CD14 molecule to identify monocytes that contain intracellular cytokines.[24]

The aim of this study is to assess and correlate clinically the intracellular expression of IL-6 and IL-12 in a mononuclear cell population that expresses cell surface

Table 1. Characteristics of healthy controls and patients with acute pancreatitis

	Controls (n=30)	Patients		
		Severe (n=15)	Mild (n=15)	Total (n=30)
Male-female ratio	25:5	13:2	12:3	25:5
Mean age (yrs)	36.5	40.9	33.6	38.4
Aetiology	Non-smokers			
Gall stone		1	13	14
Alcohol		13	1	14
Others		1	1	2

CD14, obtained from peripheral blood samples from patients with inflammatory pancreatic disease, and compare this with APACHE III score in order to aid the assessment of prognosis in AP.

Material and methods

Thirty consecutive patients admitted to our hospital with AP were recruited. Diagnosis was based on the presence of at least two of the following: (i) abdominal pain, vomiting, fever and respiratory distress; (ii) hyperamylasaemia >200 SU/L (normal: 90-160 SU/L); (iii) abdominal ultrasound showing enlargement of pancreas and collection of peripancreatic fluid; (iv) contrast-enhanced CT showing enlargement of pancreas, loss of peripancreatic fat planes, hypoechogenicity (suggesting necrosis), and collections of pancreatic and peripancreatic fluid; and (v) presence of saponification, pancreatic necrosis and/or abscess at laparotomy or post-mortem examination.

Appropriate laboratory and physiological data were recorded on admission, to permit calculation of APACHE III score.[23] Criteria for severity included the presence of one or more local complications such as pancreatic necrosis, abscess or pseudocyst and/or organ failure[25] – defined as shock (systolic blood pressure <90 mm Hg), pulmonary insufficiency (PaO_2 <60 mmHg), renal failure (creatinine level >2 mg/dL after rehydration) or gastrointestinal bleeding (>500 mL/24 h). The patient was considered to have suffered a mild attack when none of the above were present.[25]

Healthy controls comprising 25 males and five females within the age group of the patients were included in the study, based on the absence of abdominal pain, vomiting and fever. All controls had a normal serum amylase level.

Cell preparation and labelling

Heparinised blood samples were collected on admission. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient centrifugation. The harvested cells were resuspended in RPMI/FCS (10% solution) at $2x10^6$ cells/well, using a 12-well plate. The intracellular protein transport inhibitor monensin (3 μmol/L) was added to the cells 4 h prior to staining, during *in vitro* activation for cytokine staining.

The cells were cultured for 24 h at 37°C in a humidified incubator with 5% CO_2. This resulted in the accumulation of cytokine proteins in the Golgi complex and thus an enhanced staining signal. Phycoerythrin (PE)-labelled anti-human IL-6/IL-12 and fluorescein isothiocyanate (FITC)-labelled anti-human CD14 (Pharmingen, San Diego, CA) were used in the study.

The lipopolysaccharide-activated (100 ng/mL) cells were stained for the cell surface marker CD14 for 15 min at 4°C, using FITC-labelled anti-human CD14 antibody. The cells were then washed, fixed and permeabilised using a Cytofix/Cytoperm kit, according to the manufacturer's instructions (Pharmingen). Intracellular cytokines (IL-6 and IL-12) were stained with PE-labelled monoclonal antibodies and the cells were analysed by flow cytometry.

Negative controls were processed and comprised cell suspensions treated with PE-labelled monoclonal antibody to an irrelevant cytokine isotype. The Cell Quest Program was used on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) to analysis the cells.[24]

Statistical analysis

Data was expressed using descriptive statistics. Quantitative variables were analysed by unpaired *t*-test and qualitative variables by χ^2 test. P <0.05 was considered statistically significant. Pearson's correlation coefficient was used. Sensitivity, specificity and positive predictive value (PPV) for AP were defined as follows: sensitivity was the proportion of patients with severe attacks correctly predicted; specificity was the proportion of patients with mild disease correctly predicted; and PPV was the proportion of patients with a positive test who had severe disease.[26] Patients were observed prospectively until discharge or death.

Results

A group of 30 consecutive patients (25 men, 5 women; mean age: 38.4 years [range: 20-65]) were studied. Fifteen developed severe AP (three died) and 15 were classified as mild. Clinical and aetiological details are summarised in Table 1.

FACScan analysis (Figure 1) showed the presence (as a percentage) of single positive or double positive cells (IL-6, IL-12, or both). There was a significant rise in IL-6-expressing

Table 2. APACHE III scores and percentage of IL-6/IL-12-positive cells in patients with acute pancreatitis and in controls

	Control	Severe	Mild	P value severe:mild	P value control:patients
APACHE III	–	34.2±8.01	14.8±5.11	<0.05	–
IL-6 (%)	5±1.2	29±5.32	21±3.14	–	<0.01
IL-12 (%)	3±1.02	9±1.36	9±1.08	–	>0.05

Fig. 1. FACScan analysis of IL-6/IL-12 expression by activated $CD14^+$ human monocytes.

The data reflects gated monocytes, based on forward and side scattered light signals.

A, D) isotype controls
B, F) unlabelled antibody
C) $CD14^+$ cells with PE isotype control
E) $CD14^+/IL\text{-}6^+$
G) $CD14^+/IL\text{-}12^+$

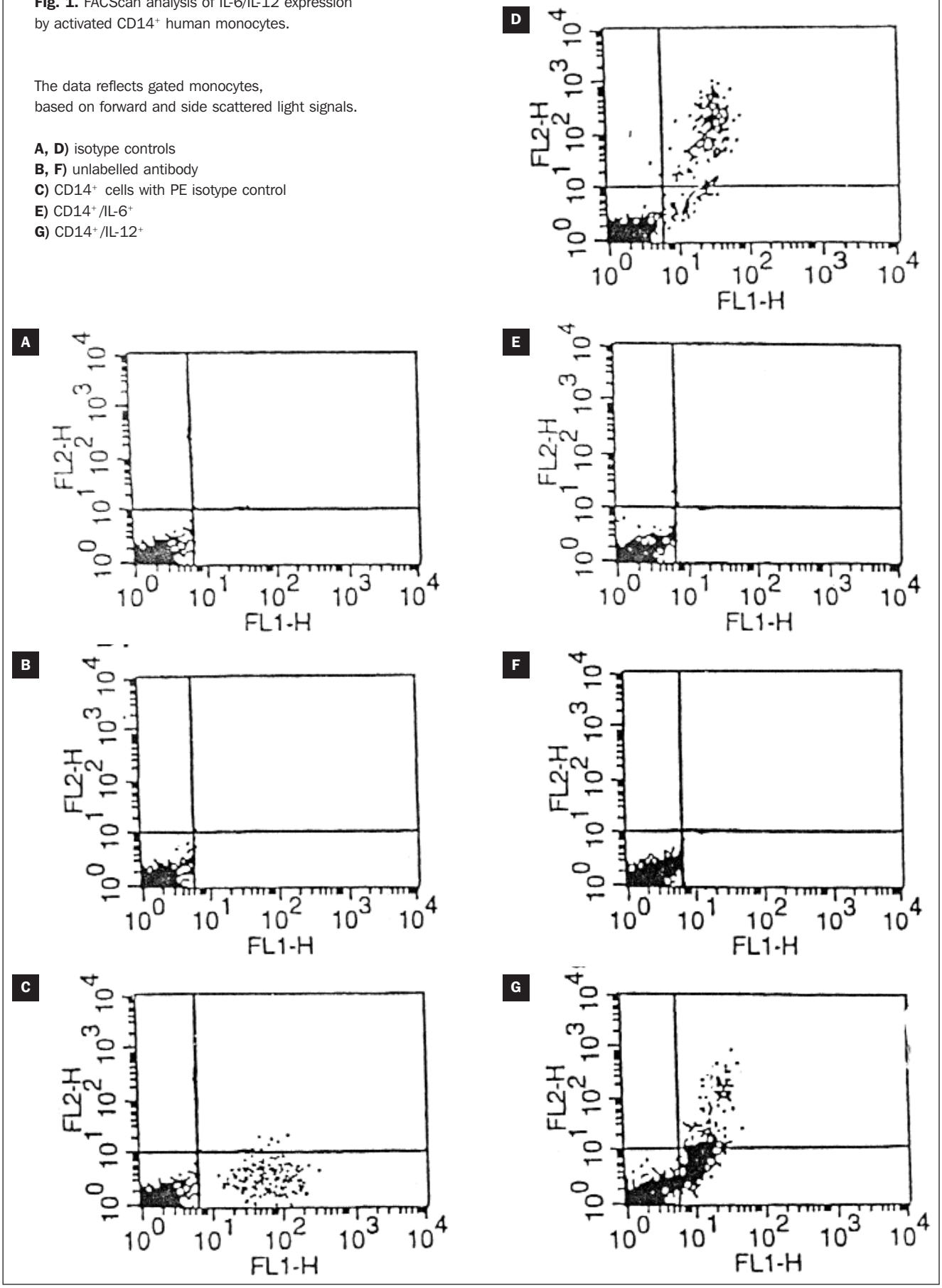

Fig. 2. Relationship between APACHE score and IL-6 (r=0.5602, $P<0.05$).

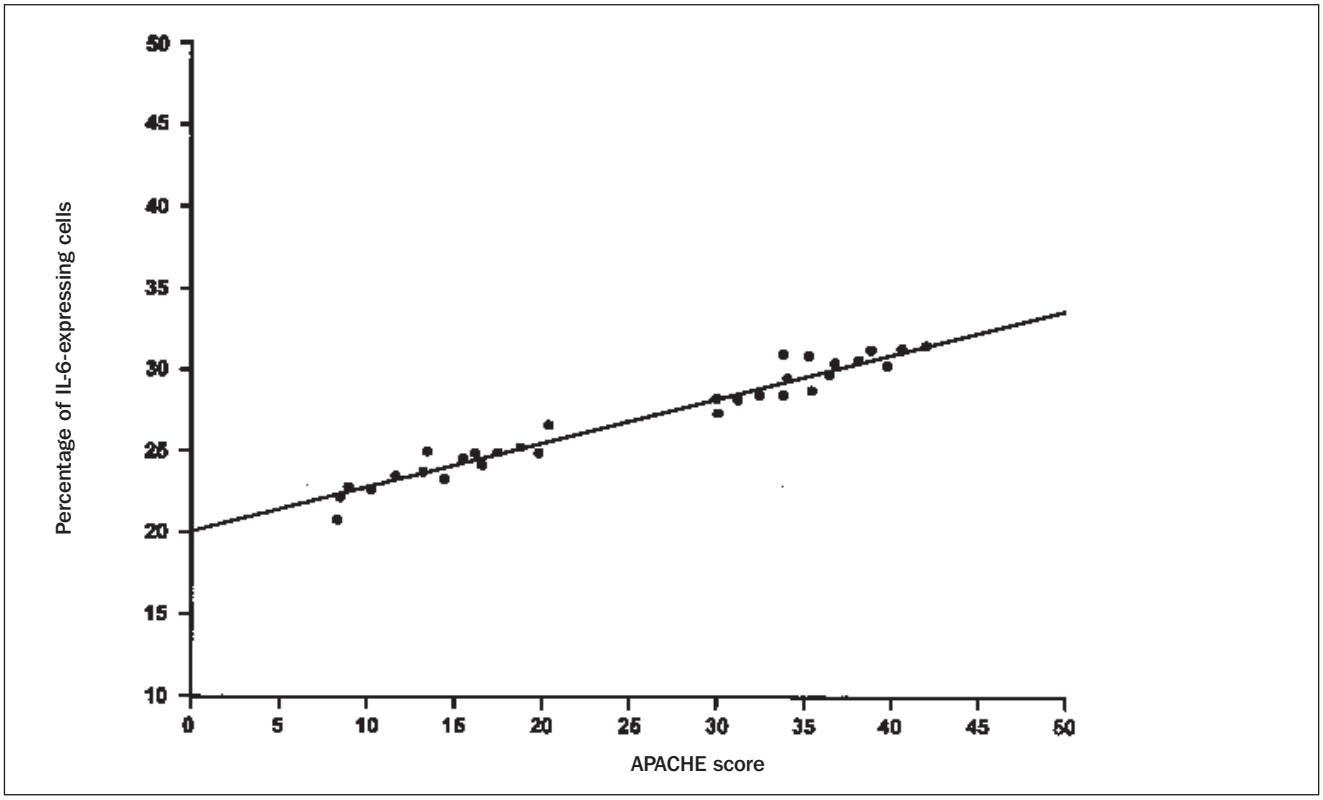

Table 3. Sensitivity, specificity and positive predictive value of IL-6, IL-12 and APACHE III score in patients with acute pancreatitis

	Sensitivity (%)	Specificity (%)	PPV (%)
IL-6 (>25%)	100	100	100
IL-12 (>9%)	70	40	70
APACHE III (>30)	80	100	100

$CD14^+$ cells in the AP group (26.9±2.01%), compared with the control group (5.9±1.2%) ($P<0.01$). The figures for IL-12-expressing $CD14^+$ cells in the AP group were 9.1±1.73%, compared with 3.1±1.02% in the control group ($P<0.05$).

Table 2 shows the mean IL-6/IL-12 values and APACHE III scores in AP. IL-6 and IL-12 values did not correlate. Percentages of IL-6/IL-12-positive cells were significantly higher in all AP patients. The magnitude of the IL-6 response differed between the mild and severe AP patients, but this was not seen with IL-12. In the severe AP group, mean IL-6 value was 29±5.32%; in the mild group it was 21±3.14%. These differences were highly significant when compared to the control group ($P<0.001$). IL-12 values were higher in alcoholic AP (9±1.07%), compared to patients with biliary pathology (8±1.22%) ($P<0.05$).

Mean APACHE score was 31.0+7.72. Six of the eight patients with mild AP showed a mean score of 14.8±5.11. In severe AP the mean was 34.2±8.01. The difference between these two groups was statistically significantly ($P<0.05$).

In severe AP, there was a corresponding increase in IL-6 value with increase in APACHE III score. Pearson's correlation coefficient (Figure 2) showed a significant positive correlation between the two (r=0.56, $P<0.05$). Values of 25% for IL-6 and 30 for APACHE III score were the best cut-off values to differentiate mild from severe AP.

Table 3 shows the sensitivity, specificity and PPV for IL-6, IL-12 and APACHE III score in predicting the severity of AP. With IL-6 values >25%, both sensitivity and specificity were 100%; whereas APACHE scores of >30 showed a sensitivity of 80% and specificity of 100%.

Discussion

Pancreatitis is a serious disease with high morbidity and mortality rates. Some 80% of attacks are mild and rapid recovery is achieved with conservative treatment alone. The remaining 20% are severe, with a protracted course that needs intensive care and specialised management.[25] Early assessment of severity is essential for proper management.

In acute AP it has been proposed that inflammatory cells are activated, resulting in the release of the inflammatory mediators responsible for the severity of the disease.[27] Pivotal to this process is increased secretion of the pro-inflammatory cytokines IL-6, IL-8, TNF-α and IL-1β from activated monocytes and mononuclear phagocytes. IL-6 is the principal mediator of acute-phase protein response and has proved to be a predictable marker of severity and outcome of disease.[8,12,13,28]

The value of cytokines in pancreatitis was first proposed by Rinderknecht,[1] and several studies have demonstrated the utility of IL-6 in determining severity in AP. Results from a number of studies[3,4,5] have shown that serum IL-6 levels from patients with acute AP correlate with disease severity.

Norman *et al*.[10] found that experimental pancreatitis was associated with release of IL-6 into the serum within an hour of onset of the pancreatic insult. Messmann *et al*.[29] showed that disease severity is reflected not only by higher peaking of IL-6 concentration but also by earlier peaking. In the present study, the percentage of IL-6-positive cells showed a significant rise in all cases of acute AP, and IL-6-positive cell levels >25% were associated with severity in AP.

APACHE III is regarded as a good prognostic scoring system in acute pancreatitis. The ability to use it serially and sequentially is its greatest advantage. In the present study, APACHE III score showed significant differences in mild and severe AP, and it correlated well with severity. We found that an APACHE III score of 30 was a good cut-off point to differentiate between mild and severe cases, and this accords with previous studies of its use in AP. Williams *et al*.[23] found that an APACHE III score >30 indicated a much higher morbidity and mortality rate. In the present study, APACHE evaluation proved very suitable for serial monitoring of patients and gave an objective indication of progress in the individual patient. In patients with severe disease, correlation between IL-6 value and APACHE score was seen.

IL-12 values were raised significantly in patients with AP but no correlation was found with disease severity. Pezzilli *et al*.[15] measured IL-12 in AP and found increased serum concentrations. In the present study, IL-12 values correlated well with aetiological factors (alcoholic and biliary pathology) associated with AP.

In conclusion, this study has demonstrated that IL-6-positive PBMC levels reflect the severity of AP. Thus, it would seem logical to use IL-6 assessment with APACHE III scoring in the assessment of AP, as their combination would appear be a better marker of disease severity. In addition, IL-12 appears to correlate with aetiology in acute pancreatitis; however, further study is necessary to clarify these results. □

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