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Inflammatory Mediators in The Assessment of Severity of Complications Associated With Acute Pancreatitis

Dr.Venkatesan.K Assistant Professor, Department of Surgery, Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry.

Dr.M.Satish Assistant Professor, Department of Surgery, Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry.

Dr.Santosh Raja Erabati Assistant Professor, Department of Surgery, Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry.

ABSTRACT

Acute pancreatitis is an inflammatory process which occurs in a normal organ and which is diagnosed mainly by acute abdominal pain. Usually the injury is mild, but 20% of the patients have a severe injury and, among them, 15-25% will die. Because it is important to predict the severity of the illness as early as possible in order to optimize the therapy and to prevent organ dysfunction and local complications, several scores of severity have been proposed. Criteria of severity such as Ranson , Glasgow , and APACHE scores have been used for a long time. These scores assess the multiple organ dysfunction induced by the disease and consequently, the greater the number of organs injured, the greater the score. New serum markers have recently emerged and their potential for providing additional information on the severity of the disease is currently being evaluated. However, to become useful such markers must be assessed in a large consecutive series of patients, including a significant proportion of severe cases, and the timing of the assessment must be related to the onset of the disease. Moreover, the usefulness of the new marker must be compared with established ones; the results must be reproducible; and the detection of the new marker must be easy to detect in clinical chemistry laboratories. Interestingly, when seeking medical attention usually 12 to 24 h after the onset of pain most patients do not exhibit multiple organ dysfunction, which is likely to emerge by the second or third day and, at admission, numerous mediators can be detected in serum. If the concentration of these biologic factors is correlated to the severity of the disease, and if they are detected before the occurrence of multiple organ dysfunction, it is then conceivable that the therapeutic antagonism of these mediators might prevent or attenuate the severity of the multiple organ dysfunction, and consequently the outcome of the disease. These new factors might be important for the rapid scoring of the disease severity in the acute phase and some of them might be used as potential therapeutic targets. The aim of this study was to evaluate the levels of inflammatory mediators in the assessment of complications associated with acute pancreatitis in the department of surgery at a teaching medical college in over a one year period.

KEYWORDS : Inflammatory markers, abdominal pain, systemic inflammatory response.

BACKGROUND AND AIM OF THE STUDY

Acute pancreatitis (AP) is a common and potentially lethal acute inflammatory disease with an estimated overall mortality rate of 2% to 5%, and a significant burden of morbidity and health care costs . Although usually self-limiting, up to 20% of patients develop a severe form of disease, which can lead to a systemic inflammatory response and multiple organ dysfunction and failure the two prevailing causes of AP are excessive alcohol consumption, most common in men, and gallstones, most common in women, which seem to act through different pathogenic mechanisms to induce pancreatic acinar cell damage. Several multifactorial scoring systems and routine clinical and biochemical parameters measured on admission and during the first 48 hours of hospitalization are used to estimate severity and promptly provide a rational and effective management. Systemic manifestations of a disease initially limited to the pancreas are thought to be mediated by a variety of pro and anti-inflammatory mediators released from the pancreas and various other sources during the course of the disease. Several cytokines play a crucial role in the pathogenesis of AP by driving the additional inflammatory response which leads to tissue damage and organ dysfunction. Local recruitment and activation of inflammatory cells in AP may lead to the production of proinflammatory cytokines, such as interleukins (IL) 6, 8, and tumour necrosis factor alpha (TNF-alpha), as well as anti-inflammatory IL-10. These mediators have been mostly studied as markers of severity of acute pancreatitis. Another commonly applied and one of the first used markers for this purpose was C-reactive protein (CRP). Different studies showed that a CRP value over 200 mg/L obtained at 48 hours after onset of symptoms is highly predictive of pancreatic necrosis

Still, insufficiently is known of the relationship between the clinical course of AP in humans and the dynamic of the major cytokines, in the presence or absence of pancreatic necrosis and distant organ complications. The purpose of our study was to determine the potential clinical value of interleukins (IL-6, IL-8, IL-10), TNF-alpha , pancreatic elastase, and C-reactive protein as biochemical markers for predicting development of systemic complications in patients with AP.

PATIENTS AND METHODS:

A 150 patients with acute pancreatitis were prospectively entered into the study during a two-year period. The diagnosis of AP was made on the basis of a consistent clinical picture combined with a 3-fold increase of serum amylase or a 3-fold increase of serum lipase, and consistent morphological findings obtained by an ultrasound scan and/or computed tomography scan within the first 72 hours of admission. The severity of AP was assessed according to the Atlanta classification. All patients irrespective of disease severity were included in the study. Mild acute pancreatitis (MAP) was defined as confirmed AP without signs of major complications, while severe acute pancreatitis (SAP) was associated with the development of one or more local or systemic complications. Local complications included pancreatic tissue necrosis,acute fluid collections, pancreatic pseudocyst, and abscess. Systemic complications assumed the presence of persistent systemic inflammatory response syndrome (SIRS) and/or developing organ failure. SIRS was defined by 2 or more of the following criteria for >48 hours: heart rate >90 beats/min; rectal temperature 38◦ C; white blood count 12,000 per mm3 ; and respirations >20/min or pCO2 < 32 mmHg. Organ failure was defined as shock (systolic blood pressure < 60 mmHg), renal failure (creatinine >2 mg/dL, despite rehydration), and gastrointestinal bleeding (>500 mL/24 hours).

METHODS. Blood samples from patients were obtained on admission and after 48 hours. Samples for IL-6, IL-8, IL- 10, IL-15, IL-17, and TNF alpha were collected in portions and stored at −20◦ C, not longer than two months. Measurements were performed using a commercially available ELISA kit (R&D Systems Inc., Minneapolis, USA) on a standard ELISA reader according to the manufacturer's instructions. Pancreatic elastase was analysed using a commercially available ELISA kit (ScheBo-Biotech, Giessen, Germany). Levels of CRP and other routine laboratory assessments were completed on biochemistry analyser Olympus AU 640 (Mishima Olympus, Japan). Measurements

of interleukins and TNF alpha were performed on samples obtained on the first day of admission. CRP and pancreatic elastase levels were assessed on admission and after 48 hours.

STATASTICS. All variables are expressed as medians with 95% confidence intervals (95% CI). Mann Whitney *U* test was used for comparison of independent samples. For differences between values of same parameters obtained on admission and after 48 hours Wilcoxon pair test for dependent samples was used. Receiver operating characteristic (ROC) curves and respective areas under curve (AUC) were established for biochemical prognostic factors. Cut-off values were chosen as values that achieved the highest sensitivity and specificity, as well as positive (PPV) and negative predictive values (NPVs). The proportion of patients without systemic complications was used as a measure of prevalence in performing ROC analysis. A value of $P < 0.05$ was considered statistically significant.

RESULTS:

A total of 150 patients with AP (71 male; median age 63; range 20–91) were included in the study. AP was considered severe in 28 patients (19%) and mild in 122 patients (81%). The etiology of AP was biliary in 68 patients (45%), alcoholic in 51 patients (34%), and other possible causes in 31 patients (21%). The average value of IL-6 measured in the group of patients was higher than the upper limit of reference range recommended by the manufacturer (29 vs12.5 pg/mL, resp.), whereas average values of other measured cytokines were within normal ranges. CRP measured on the first and third day of admission was above the upper limit of normal, as well as the average value of pancreatic elastase measured on the first day. Average values of pancreatic elastase measured on the third day were within the boundaries of recommended values . In the assessment of disease severity, average values of CRP and pancreatic elastase differed significantly between the first and third day of hospitalization, with a significant increase in CRP values and a significant decrease in serum concentrations of pancreatic elastase. We found a significant difference between the values of IL-6, IL-8, IL-10, and TNF-alpha evaluated on the first day of admission, and a significant difference between CRP and elastase values analyzed from samples taken on the third day. No significant difference was noted in the values of CRP and elastase on the first day between these two groups of patients. The effectiveness of the investigated biochemical parameters in the early recognition of patients with and without systemic complications was assessed using ROC analysis. Considering the area under the ROC curve, values of cytokines measured on the first day were statistically significant indicators for development of systemic complications. CRP and pancreatic elastase measured on the third day also reached statistical significance. The largest area under the curve was for IL-6 (AUC = 0.71) and elastase on the third day (AUC = 0.70) . Elastase had a fairly high sensitivity of 92%, but a rather low specificity of 43%. The highest specificity (84%) was calculated for the marginal value of CRP measured on the third day, with a sensitivity of 54%. CRP and elastase measured on the first day had the lowest predictive value (AUC = 0.51 and 0.56, resp.) not reaching statistical significance.

Average values of biochemical parameters in patients with acute pancreatitis.

Parameters	Median(95% CI)	Reference Values
IL-6(1st DAY)	29 (18-46)	03.13-12.5 pg/mL
IL-8(1st DAY)	30 (23-39)	<31.2 pg/mL
IL-10(1st DAY)	7.4 (3.9-11.2)	<7.8 pg/mL
TNF-alpha(1st DAY).	1698 (1419-1998)	749-1966 pg/mL
CRP(1ST DAY)	15.2 (12-27)	< 5 mg/L
CRP(3RD DAY)	103.6 (80-139)	< 5 mg/L
ELASTASE (1ST DAY)	5.9 (4.3-7.5)	< 3.5 ng/ml
ELASTASE(3RD DAY)	1.8 (1.6-2.2)	< 3.5 ng/ml

Values of biochemical parameters in patients without systemic complications and systemic complications

Parameters	Patients without systemic complications(N=122)	Patients with systemic complications (N=28).	***P* value**
IL-6(1st DAY)	20 (16-34)	104 (51-139)	0.001
IL-8(1st DAY)	27 (19-34)	53 (33-98)	0.012
IL-10(1st DAY)	5.1 (2.6-8.9)	26 (8-58)	0.010
TNF-alpha	1520 (1220-1886)	2220 (1873-2722)	0.004
CRP(1ST DAY)	14.4 (11.3-28.3)	15 (5.1-78.8)	0.954
CRP(3RD DAY)	85 (66.7-122)	216 (105-257)	0.002
ELASTASE (1ST DAY)	5.7 (4.2-7.5)	7.5 (3.6-10.9)	0.249
ELASTASE (3RD DAY)	1.7 (1.4-1.9)	2.5 (2.1-5.1)	0.001

DISCUSSION

In this study we examined the value of IL-6, IL-8, IL-10, TNF, CRP, and pancreatic elastase as predictors of systemic complications in AP. The need for an early risk recognition and determination of best possible treatment modalities led to a series of investigations trying to establish an objective, rational, and clinically manageable severity assessment tool in patients with AP. The initial acinar cell damage in the early stage of acute pancreatitis of any etiology is caused by a hypersecretion of pancreatic proteolytic enzymes. As a result there is an overproduction of inflammatory mediators and free oxygen radicals. Tissue macrophages are the main source of proinflammatory and anti-inflammatory cytokines that attract neutrophils and more macrophages, and induce the production of proteases, elastases, and phospholipases. These enzymes, as well as free oxygen radicals cause tissue damage, mainly vascular endothelial necrosis which leads to circulatory stasis. The increase of pro inflammatory and decrease of anti-inflammatory cytokines are crucial factors in the progression of inflammation of severe acute pancreatitis. The largest studies have focused on the role of TNF-alpha, IL- 1, IL-6, and IL-10. Most of these studies have shown that the levels of proinflammatory cytokines (TNF-alpha, IL-1, IL-6) are higher in severe forms of AP, while levels of IL- 10, which is anti-inflammatory agent are higher in patients with mild disease. The systemic manifestations of severe acute pancreatitis are not only caused by local inflammatory processes, but also by an excessive production and systemic spreading of inflammatory mediators. We performed data analysis by dividing our patients into two groups. In one group we had patients who developed systemic complications (*N*= 28), and in the other those who had none (*N*=122). Our results show that the average value of IL-6 in patients with AP was above the upper limit of reference range, while average levels in controls were within normal ranges. ROC (Receiver operating characteristic) analysis was performed to evaluate the prognostic value of IL-6 to distinguish patients with systemic complications from those without, showing that patients with IL-6 concentrations greater than 37.9 pg/mL can be considered high risk in terms of developing systemic complications. We found a sensitivity of 82%, and a specificity of 65%. The role of proinflammatory IL-8 in prediction of severity of acute pancreatitis seems less valuable than IL-6. Although it reached statistical significance ($P < 0.0008$) in the differentiation of mild and severe disease forms at a threshold value of 42.5 pg/mL, it achieved a modest sensitivity and specificity of 68% and 67%, respective. IL-10 has an anti-inflammatory role inhibiting the synthesis and release of other proinflammatory cytokines and free oxygen radicals from macrophages and T-helper lymphocytes. On the other hand, it shows a positive effect on the proliferation and differentiation of B lymphocytes promoting the production of immunoglobulins. Our results show that a limit for IL-10 that can be said to separate milder from more severe forms of AP is 7.2 pg/mL, with a sensitivity of 75%, and a specificity of 56% . However, two other studies found significantly lower values of IL-10 in patients with severe AP . Authors speculated that an impaired immune response to inflammation could be a possible cause. It seems that a balance between pro- and anti-inflammatory cytokines is the key process in the course of AP and development of systemic complications. A reduced functional reserve of IL- 10 and

a higher IL-6/IL-10 ratio could lead to SIRS and a worse prognosis. However, this is still a matter requiring further investigations. Results of TNF alpha analysis, as another proinflammatory cytokine, were consistent with significant elevation of serum concentrations in patients with AP. The research included the analysis of two parameters that can be considered as valuable indicators of the course of disease pancreatic elastase(E1) as a specific enzyme secreted by pancreatic acinar cells, and CRP as an acute phase protein, both increasingly produced and released in a state of acute inflammation. Elevated concentrations of E1 were measured in both groups of patients on admission, but without significant difference between the groups. However, we noticed a significant decline in concentration of E1 between the first and third day ($P < 0.001$), with a significant difference in values between the two groups patients ($P= 0.001$). Our results show that patients with a value of E1 below a cutoff value of 1.5 ng/mL measured on third day of admission could be considered potentially at low risk of development of systemic complications. The concentrations of CRP differed significantly between day one and three ($P < 0.001$). Patients who developed systemic complications showed significantly higher levels of CRP ($P= 0.002$).We confirmed that CRP and elastase analyzed on the third day of admission, in addition to the evaluation of IL-6, IL- 8, IL-10, and TNF on the first day, represent a valuable diagnostic tool in the assessment of severity and course of disease in patients with acute pancreatitis. Nevertheless, CRP is still the only recommended and standardized method for a fast and relatively inexpensive determination of severity of AP. Routine use of proinflammatory cytokines as predicting factors of severity of acute pancreatitis is still not feasible in most hospitals, due to high costs and inaccessibility of analytic methods. Therefore, development of new and more accessible laboratory equipment, as well as methods of analysis could help the clinicians in the early recognition of development of systemic complications and improve the management of severe acute pancreatitis.

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