EARLY CYTOKINE PROFILE CHANGES IN INTERSTITIAL AND NECROTIC FORMS OF ACUTE PANCREATITIS

Irena Kostic [1], Marko Spasic [2], Bojan Stojanovic [2], Milena Jurisevic [1], Dragce Radovanovic [2], Dragan Canović [2], Srdjan Stefanovic [1], Slobodan Jankovic [1]
[1] Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
[2] Department of General Surgery, Surgical Clinic, Clinical Center of Kragujevac, Kragujevac, Serbia

RANE PROMENE NIVOA CITOKINA KOD INTERSTICIJALNIH I NEKROTIČNIH OBLIKA AKUTNOG PANKREATITISA

Irena Kostić [1], Marko Spasić [2], Bojan Stojanović [2], Milena Jurišević [1], Dragče Radovanović [2], Dragan Čanović [2], Srđan Stefanović [1], Slobodan Janković [1]
[1] Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Kragujevac, Srbija
[2] Odeljenje opšte hirurgije, Hirurška klinika, Klinički centar Kragujevac, Kragujevac, Srbija

Received / Primljen: 04. 07. 2014. Accepted / Prihvaćen: 12. 01. 2015.

ABSTRACT

Acute pancreatitis (AP) is a common, potentially lethal, acute inflammatory process with a highly variable clinical course. The aim of this study was to analyse early changes in the serum concentrations of pro- and anti-inflammatory cytokines in the peripheral blood of patients with the interstitial form of acute pancreatitis (IAP) and necrotic acute pancreatitis (NAP), especially in those patients who had lethal outcomes.

The prospective study enrolled 52 patients who were divided into IAP (65.38% of patients) and NAP (34.62% of patients) groups. The serum levels of interleukins (IL) 6, 8 and 10, together with tumour necrosis factor (TNF)-alpha were measured on the 1st and 3rd day of hospitalisation. Significantly higher values of IL-6, IL-8 and IL-10 were found on day 1 and 3 in NAP than in IAP. IL-6 was significantly higher on both days of measurement, whereas IL-10 on the first day and IL-8 on the third day were significantly higher in the group of patients who did not survive in comparison with patients who had the interstitial form of AP.

In conclusion, the data from this study showed that immune suppression and excessive immune stimulation in the first three days after admission could indicate the development of NAP and a potentially lethal outcome.

Key words: *cytokines; pancreatitis; acute; necrotising; survivors*

SAŽETAK

Akutni pancreatitis (AP) je učestao, potencijalno letalni inflamatorni proces sa vrlo varijabilnim kliničkim tokom. Cilj ovog istraživanja je bio da analizira rane promene u serumskim koncentracijama pro- i anti- inflamatornih citokina u perifernoj krvi bolesnika sa intersticijskom formom akutnog pankreatitisa (IAP) i nekrotičnim oblikom akutnog pankreatitisa (NAP), posebno kod onih bolesnika koji su umrli u toku praćenja.

Prospektivna studija je uključila 52 bolesnika podeljenih u grupe IAP (65.38%) i NAP (34.62%). Serumski nivoi interleukina (IL) 6, 8 i 10, kao i tumorskog nekrotišućeg faktora (TNF)-alfa određivani su u prvom i trećem danu hospitalizacije. Vrednosti IL-6, IL-8 i IL-10 bile su značajno više prvog i trećeg dana u NAP nego u IAP grupi. IL-6 je bio značajno povišen u oba dana merenja, dok su IL-10 prvog i IL-8 trećeg dana dostigli značajno više vrednosti u grupi bolesnika sa letalnim ishodom u poređenju sa grupom pacijenata koji su imali intersticijsku formu AP.

U zaključku, podaci iz naše studije pokazuju da imunska supresija i preterana imunska stimulacija u toku prva tri dana posle prijema u bolnicu mogu da ukažu na razvoj nekrotične forme akutnog pankreatitisa i potencijalno letalni ishod.

Ključne reči: *citokini; pankreatitis, akutni, nekrotični; preživeli*

INTRODUCTION

Acute pancreatitis (AP) is a common, potentially lethal, acute inflammatory process with a highly variable clinical course. AP progresses to a severe form in approximately 10-20% of patients, resulting in systemic inflammatory response syndrome (SIRS), multiple organ failure and a prolonged hospitalisation with significant morbidity and mortality (1).

Previously, AP was considered to be a disease of the pancreas. Currently, however, there is strong evidence for systemic effects of the disease. Localised inflammation in the pancreas is the body's initial physiologic protective response, which is generally strictly controlled at the site of injury. Loss of the local control results in excessive uncontrolled activation of inflammatory cells and mediators, which is called SIRS (2).

DE GRUYTER OPEN
UDK: 616.37-002.1-074:577.85 / Ser J Exp Clin Res 2015; 16 (1): 33-37
DOI: 10.1515/SJECR-2015-0005

Correspondence: Irena Kostic, Faculty of Medical Sciences, University of Kragujevac
Svetozara Markovica 69 Street, 34000, Kragujevac, Serbia
Tel./Fax. +381 64 2529750, E-mail: jecko6yu@yahoo.com

Systemic inflammation in AP is concomitantly associated with rapidly strengthening compensatory anti-inflammatory response syndrome (CARS) (3). There are many mediators included in this interplay between SIRS and CARS. In the clinical setting, early diagnosis and, if possible, assessment of the prognosis of AP is a major interest for the clinician.

The principal cells that modulate immune responses are T helper (Th) lymphocytes, which orchestrate the function of other immune cells by cytokine production. Thus, the aim of this study was to analyse the early changes in the serum concentrations of pro- and anti-inflammatory cytokines in the peripheral blood of patients with necrotic and interstitial AP, especially in those patients who had lethal outcomes.

PATIENTS AND METHODS

Patients

We conducted a prospective study that included 52 subjects who were admitted to the Surgical Intensive Care Unit (SICU) of the Clinical Center of Kragujevac, Serbia from October 2011 to July 2013. All ethical approvals were obtained by the local Ethics Committee of the institution, and the research was conducted in accordance with the regulations governing Good Clinical and Laboratory Practices. Informed consent was obtained from all patients before enrolment in the study.

The diagnosis of AP was based on two of the following three criteria: abdominal pain characteristic of AP, serum amylase and/or lipase ≥ 3 times the upper limit of the normal values, and characteristic findings of AP on a CT/US scan. Patients with underlying chronic pancreatitis, those with acute postoperative pancreatitis, pregnant women with acute pancreatitis, patients transferred from other hospitals or other wards to the SICU of the Clinical Center of Kragujevac after more than 48 hours from disease onset, as well as those under 18 years of age were excluded from this study.

Patients were recruited within 24 hours of the time of hospital admission. Clinical data relating to the severity of the disease, the development of organ dysfunction and/or septic complications were prospectively collected in a standardised fashion according to the revised Atlanta Classification (4). For the purpose of detection of (peri) pancreatic necrosis, a single highly experienced radiologist evaluated and interpreted computerised tomography (CT) scans in a blinded manner with respect to the disease outcome, taking into consideration CT examinations that had been performed at least three days after admission to the SICU.

All patients were divided in two groups: interstitial non-necrotic pancreatitis (IAP) and necrotising AP (NAP). The diagnoses were verified by the findings of non-enhancement in the pancreatic parenchyma after contrast administration exceeding 3 cm in size or 30% of the total gland area and/or by heterogeneous fluid collection within the peripancreatic space containing solid material on the abdominal CT scan.

METHODS

Blood samples were collected following patient enrolment in the study within 30 hours of the onset of pain (1st day of admission) and on the third day of the disease course. Blood clots were cut and centrifuged to separate the serum, and all serum samples were kept at -20°C before use. The serum levels of cytokines were measured using sensitive enzyme-linked immunosorbent assay (ELISA) kits specific for humans (R&D Systems, Minneapolis, MN). The serum levels of tumour necrosis factor (TNF)-alpha, interleukin (IL)-6, IL-8 and IL-10 were determined at the Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac.

Statistical analysis

The results are reported as the mean and standard error (SE) for continuous variables or as frequencies and percentages for categorical data. The differences between compared groups in the means of continuous variables were analysed by an independent T test, a Kruskal-Wallis test with Mann-Whitney U test, or a Wilcoxon test for paired samples (depending on the actual data distribution assessed by the Kolmogorov-Smirnov test for normality), whereas the categorical variables were compared using a Chi-squared test for frequencies. For all analyses, the level of statistical significance was set at an alpha value of 0.05. The statistical analyses were performed using SPSS 13.0 software.

RESULTS

Demographic and clinical features of the subjects

Fifty-two patients (34 male and 18 female) diagnosed with AP were included in the study (table 1). Necrotic acute pancreatitis (NAP) was observed in 18 (34.62%), patients and the interstitial form of AP was observed in 34 (65.38%) subjects enrolled in our study. There was no difference in the mean age (58.667 ± 18.493 years for NAP and 58.618 ± 14.6 years for IAP), whereas we observed male predominance in both forms of the disease.

During the disease course, 7 (13.46%) patients died, and in 9 (17.308%) subjects, we observed infected pancreatic necrosis as a consequence of a severe form of the disease.

Table 1. Sex distribution in different forms of acute pancreatitis

	Interstitial form (%)	Necrotising form (%)
Male	22	12
Female	12	6
Total	34 (65.38)	18 (34.62)

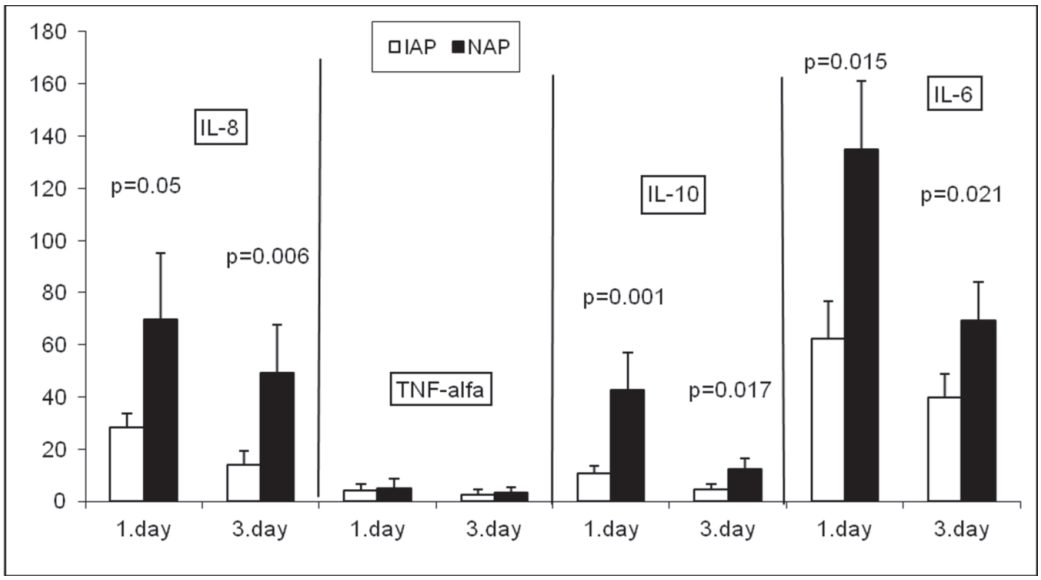

Figure 1. IL-8, TNF-alpha, IL-10 and IL-6 cytokine levels in interstitial and necrotising acute pancreatitis
IAP- interstitial acute pancreatitis
NAP- necrotising acute pancreatitis
p values presented in the figure refer to IAP vs. NAP
Presented cytokine levels are the mean ± SE

Although the patients who died were older than those with the IAP form (64.571 ± 17.175 years for lethal outcome, and 58.618 ± 14.6 years for the interstitial form), the difference in age was not statistically significant.

Cytokine profile

All four measured cytokine levels were significantly higher at the 1st day of AP compared to the values of the 3rd day of the disease course (Wilcoxon test for paired samples). The highest value was for IL-6, which dominated the other cytokine levels, whereas the lowest value was the TNF-alpha concentration (figure 1).

All of the measured pro-inflammatory cytokines, IL-6, IL-8 and TNF-alpha, showed peak serum concentrations on day 1 after the onset of symptoms in both forms of AP, with significantly higher values of IL-6 and IL-8 in necrotising than in interstitial AP. These mediators gradually decreased on the third day of the disease course, but IL-6 and IL-8 remained significantly higher in the NAP group of patients.

Regarding the anti-inflammatory cytokine, IL-10, we found significantly higher values of this cytokine in NAP compared to interstitial AP on both days of the disease course (figure 1). Whereas the IL-6, IL-8 and IL-10 levels were 2-3 times higher in the necrotic form of AP in comparison to interstitial AP, TNF-alpha was only moderately increased. Anti-inflammatory IL-10 was significantly lower on the third day in the NAP group of patients compared to the concentrations of the pro-inflammatory cytokines IL-6 and IL-8 ($p<0.01$ and $p=0.041$, respectively).

Subsequently, we compared the early cytokine levels of the 7 patients who died during the disease course with the group of patients who had only the interstitial form of AP. As shown in figure 2, all pro-inflammatory cytokines, IL-6, IL-8 and TNF-alpha, showed peak serum concentrations on day 1 after the onset of symptoms and were gradually decreased on the third day of the disease course, with significantly higher values of IL-6 and IL-8 in the lethal form of AP compared to IAP.

Anti-inflammatory cytokine IL-10 was significantly higher on the first day of the disease course in lethal AP compared to IAP (figure 2). However, its concentration decreased and became significantly lower in comparison with the pro-inflammatory IL-6 and IL-8 levels on the 3rd day of the disease course in patients who died ($p=0.001$ and $p=0.04$, respectively).

DISCUSSION

Acute pancreatitis is no longer considered only a disease of the pancreas because there is strong evidence for systemic effects of the disease. Localised inflammation in the pancreas is the body's initial physiologic protective response. Loss of the local control results in excessive uncontrolled activation of the pro-inflammatory response (hyperinflammation), which is called systemic inflammatory response syndrome or SIRS (2). SIRS can result in host defence failure, expressed by multiple organ failure (MOF) and a lethal outcome. During the SIRS phase, there was up-regulation of the pro-inflammatory factors, such as TNF-alpha, IL-6 and IL-1beta. Systemic inflammation in AP is concomitantly associated with compensatory anti-inflammatory response syndrome or CARS (3). An anti-inflammatory response may be sufficient to control SIRS, and the patient may survive without complications. However, CARS may be excessive, leading to immune suppression and the activation

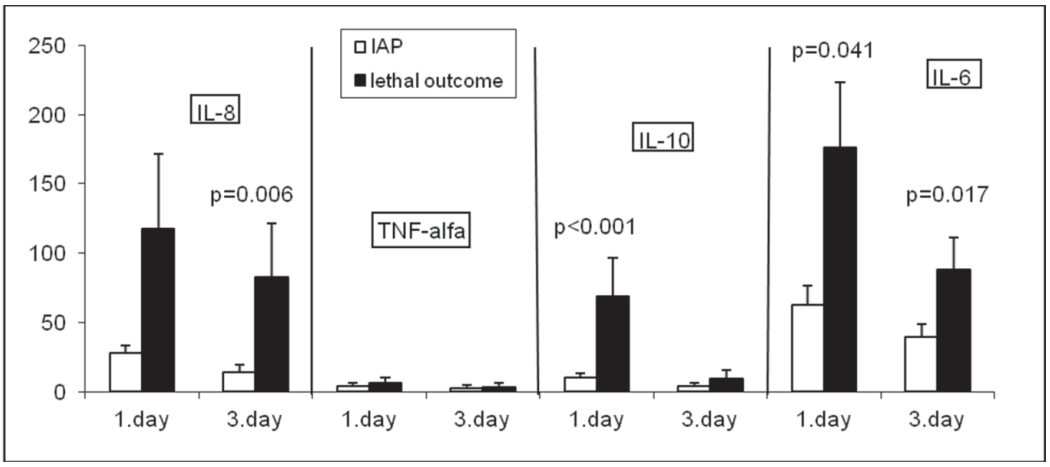

Figure 2. IL-8, TNF-alpha, IL-10 and IL-6 cytokine levels in the IAP group of patients and AP patients with a lethal outcome
IAP-interstitial acute pancreatitis
p values presented in the figure refer to survivors vs. lethal outcome

of circulating cells of the immune system, including monocytes and CD4+ T helper lymphocytes that shift to Th2 response (5-8).

There are many pro- and anti-inflammatory mediators included in this interaction between SIRS and CARS. In this paper, we showed that early changes in the serum cytokine profile could distinguish NAP from IAP and could also indicate a lethal outcome. In the clinical setting, early diagnosis and, if possible, assessment of the prognosis of AP, is a major interest for the clinician. Indeed, early aggressive treatment in patients who will develop the necrotising form of the disease could potentially change the outcome.

In our study, all examined cytokines were higher in the necrotising compared to the interstitial form of AP. Their levels gradually decreased on the third day of the disease course, with significantly higher values of IL-6, IL-8 and IL-10 in the NAP group (figure 1).

Our results are similar to the findings in patients with acute alcoholic and severe biliary pancreatitis, in which the serum IL-6 level reached its peak on admission and was significantly higher in the severe form during the whole observational period (9,10). In the study conducted by Dambrauskas Z, et al. (11), a severe form of AP was associated with a typical SIRS and a 2-5-fold increase in the expression of pro-inflammatory cytokines (IL-6, IL-8, macrophage inhibitory protein-MIF), together with the induction of a compensatory and regulatory mechanism (IL-10). The study also revealed that the serum IL-6 concentration is a good predictor of the necrotising form of the disease and systemic complications (SIRS, MOF). This marker can also be utilised for the stratification of patients with necrotising AP and those with a possible fatal outcome.

Regarding TNF-alpha, we did not find a significant difference between the necrotising and interstitial forms in our AP patients. TNF-alpha plays a pivotal role in NAP, acting early in the disease course, and is quickly cleared. As a result of its rapid clearance, the TNF-alpha serum levels are less useful than downstream cytokines (e.g. IL-6) as biomarkers of early events (12).

We examined the early changes of another pro-inflammatory cytokine, IL-8, produced by macrophages and epithelial cells, which exerts chemotactic activity on neutrophils. In turn, activated neutrophils are a significant source of IL-8 (13). In our study, patients with NAP had significantly higher IL-8 levels on both days, whereas those with a lethal outcome had significantly higher levels on the third day of the disease course, indicating the persistence of hyperinflammation (figures 1 and 2). This result was in agreement with the previous studies that showed significantly higher mean values of IL-8 and neutrophil elastase in patients with complicated pancreatitis (10,14). In another clinical study, the role of serum IL-8 in predicting lethal AP was confirmed (15).

In the study conducted by Li, et al. (16), there was an increase of Th2 cells (IL-4+) in the beginning of NAP, especially in the first week. They also showed decreased monocyte surface expression of HLA-DR antigen in the first week of the disease course, and this decreased expression correlated positively with decreasing levels of TNF-alpha, IL-6 and IL-10 during that period of NAP. IL-10, the most potent anti-inflammatory cytokine, could be responsible for the decreased monocyte HLA-DR expression in these subjects with increased risk of secondary infections and MODS (17). A high level of anti-inflammatory cytokine IL-10 may follow the increase in the pro-inflammatory factors TNF-alpha and IL-6, which might be a part of the CARS (18). In our study, statistically higher levels of IL-10 and IL-6 on the admission day and at the third day of the disease course were found in the NAP group as well as in a group of patients who had lethal outcomes (figures 1 and 2), which confirms the previous results.

The network of various cytokines and other molecules participating in the regulation of the inflammatory process-

es is very complex, and the precise timing of the release and activation of these mediators is not known. SIRS and CARS at a certain stage of the disease might even develop simultaneously (11). In our study, pro- and anti-inflammatory cytokines were significantly higher on the 1st day of acute pancreatitis in the NAP group and in the group of patients with lethal outcomes compared to the values in the IAP group (figures 1 and 2). Anti-inflammatory IL-10 decreased more than IL-6 and IL-8 and was significantly lower than pro-inflammatory cytokines on the 3rd day of the disease course in the necrotising AP form and in the group of patients who died. These findings could indicate that if SIRS and pro-inflammatory cytokines dominate the immune response, the patients will develop the necrotising form of acute pancreatitis with a potentially lethal outcome.

This is the first study in which these cytokines (IL-6, IL-8, IL-10 and TNF-alpha) were tested on the same group of patients. We found that IL-6, IL-8 and IL-10 are good indicators of the presence of necrosis and also of a potentially lethal outcome.

More studies are needed to identify the immunological phenomenon that may lead to the development of immunomodulatory treatments in necrotising AP. A better understanding of the physiology of AP is necessary to accurately identify those patients with necrotising AP in need of monitoring and treatment in intensive care units.

AKNOWLEDGEMENTS

This study was partially financed by grant No III41010 ("Preclinical examination of bioactive substances") given by the Serbian Ministry of Education, Science and Technical Development, and also by project No 13/11, entitled "The analysis of cytokine profile in patients with acute pancreatitis treated in intensive care unit", that is currently being implemented in cooperation between the Faculty of Medical Sciences, University of Kragujevac, and the Clinical Center of Kragujevac.

REFERENCES

1. Isenmann R, Beger HG. Natural history of acute pancreatitis and the role of infection. *Baillieres Best Pract Res Clin* 1999; 13: 291–301
2. Bone RC. Toward a theory regarding the pathogenesis of the systemic inflammatory response syndrome: what we do and do not know about cytokine regulation. Crit Care Med 1996; 24: 163-172
3. Mentula P, Kylanpaa ML, Kemppainen E, et al. Plasma antiinfiammatory cytokines and monocyte human leucocyte antigen-DR expression in patients with acute pancreatitis. Scan J Gastroenterol 2004; 39: 178-187
4. Banks PA, Bollen TL, Dervenis C, et al. Acute Pancreatitis Classification Working Group. Classification of acute pancreatitis-2012: revision of the Atlanta classification and definitions by international consensus. Gut 2013; 62: 102-111
5. Ni Choileain N, Redmond HP. The immunological consequences of injury. Surgeon 2006; 4: 23-31
6. Pietruczuk M, Dabrowska MI, Wereszczynska-Siemiatkowska U, Dabrowski A. Alteration of peripheral blood lymphocyte subsets in acute pancreatitis. World J Gastroenterol 2006; 12: 5344-5351
7. Smith JW, Gamelli RL, Jones SB, Shankar R. Immunologic responses to critical injury and sepsis. J Intensive Care Med 2006; 21: 160-172
8. Kylanpaa ML, Repo H, Puolakkainen PA. Inflammation and immunosuppression in severe acute pancreatitis. World J Gastroenterol 2010; 16: 2867-2872
9. Panek J, Kusnierz-Cabala B, Dolecki M, Pietron J. Serum proinflammatory cytokine levels and white blood cell differential count in patients with different degrees of severity of acute alcoholic pancreatitis. Pol Przeglad Chirur 2012; 84: 230-237
10. Mayer J, Rau B, Gansauge F, Beger HG. Inflammatory mediators in human acute pancreatitis: clinical and pathophysiological implications. Gut 2000; 47: 546-552
11. Dambrauskas Z, Giese N, Gulbinas A, et al. Different profiles of cytokine expression during mild and severe acute pancreatitis. World J Gastroenterol 2010; 16(15): 1845-1853
12. Malleo G, Mazzon E, Siriwardena AK, Cuzzocrea S. Role of tumor necrosis factor-alfa in acute pancreatitis: from biological basis to clinical evidence. Shock 2007; 28: 130-140
13. Strieter RM, Kosahava K, Allen RM, et al. Cytokine-induced neutrophil-derived interleukin-8. Am J Pathol 1992; 141: 397-407
14. Gross V, Andreesen R, Leser HG, et al. Interleukin-8 and neutrophil activation in acute pancreatitis. Eur J Clin Invest 1992; 22: 200-203
15. Rau B, Steinbach G, Gansauge F, Mayer J, Grunert A, Beger HG. The potential role of procalcitonin and interleukin 8 in the prediction of infected necrosis in acute pancreatitis. Gut 1997; 41: 832-840
16. Li JP, Yang J, Huang JR, et al. Immunosuppression and the infection in patients with early SAP. Frontiers in Bioscience 2013; 18: 892-900
17. Fumeaux T, Pugin J. Role of interleukin-10 in the intracellular sequestration of human leukocyte antigen-DR in monocytes during septic shock. Am J Resp Crit Care Med 2002; 166: 1475-1482
18. Smith JW, Gamelli RL, Jones SB, Shankar R. Immunologic responses to critical injury and sepsis. J Intensive Care Med 2006; 21: 161-172