

Research letter

Development of lymphocytes apoptosis at the first phase of acute pancreatitis

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Abstract: *Introduction* — Acute pancreatitis takes the main place in the structure of surgical abdominal diseases. Endothelial dysfunction is one of the development mechanisms of the severe acute pancreatitis, which is accompanied by the formation of leucocytal and platelet microparticles, and massive cells apoptosis.

Aim — To study lymphocytes apoptosis depending on severity of acute pancreatitis and area of pancreas and retroperitoneal cellular tissue affection.

Material and Methods — We examined 40 patients with acute pancreatitis of IA phase of the disease, who were divided into two groups: with severe and non-severe course of the disease. Condition of lymphocytes plasma membrane in peripheral blood was estimated at admission of the patients by phase-contrast microscopy. Condition of pancreatic glandular tissue and retroperitoneal space were estimated by computed tomography angiography.

Results — In most cases, non-severe acute pancreatitis was not associated with development of necrotic affection of pancreatic glandular tissue. In the event of severe acute pancreatitis structural changes of the pancreatic glandular tissue and retroperitoneal cellular tissue were in 95% of the cases. In cases of severe acute pancreatitis, the total blebbing of lymphocytes in terms of 100 cells was 33.5 (27.1, 39.7), terminal – 21.9 (14.6, 28.3) (data presented as median with lower and upper quartiles). There were high positive correlations between the number of lymphocytes at terminal blebbing and affection in pancreatic gland tissue ($r=0.76$, $p<0.001$), also terminal blebbing and retroperitoneal space affection ($r=0.82$, $p<0.001$).

Conclusion — Lymphocytes apoptosis in peripheral blood reflects the severity of acute pancreatitis.

Keywords: acute pancreatitis, blebbing, apoptosis, lymphocytes

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Introduction

Severe acute pancreatitis and its complications stay currently one of the important problems in modern surgery. Severe forms of the disease are diagnosed in approximately 15-25% of people with pancreatitis [1-3]. Release of a great number of biologically active compounds, which starts cascade of pathological reactions, plays leading role in pathogenesis of severe acute pancreatitis. The first phase of the disease is characterized by development of dangerous complications such as early multiple organ failure and shock [4-5].

Severe acute pancreatitis is associated with massive cells apoptosis, which is caused by oxidative stress [6]. Microparticles of leucocytal and platelet origin are the activators of endotheliocytes apoptosis [7]. Massive blebbing of lymphocytes plasma membrane is caused by oxidative stress due to necrosis of pancreatic glandular tissue. It shows indirectly the severity of acute pancreatitis [8].

Severe acute pancreatitis is accompanied by immunodeficiency and impairment cellular immunity due to

peripheral lymphocytes apoptosis. All this leads to development of subsequent infectious complications of acute pancreatitis.

Aim of the study: to study lymphocytes apoptosis depending on severity of acute pancreatitis and area of pancreas and retroperitoneal cellular tissue affection.

Material and Methods

Forty patients with acute pancreatitis of IA phase of the disease underwent medical treatment at the Railway Hospital at Krasnoyarsk station of Russian Railways from 2015 to 2016.

Patients were divided into two groups: the 1st group contained 20 patients with no severe acute pancreatitis; the 2nd group had 20 patients with severe acute pancreatitis. We determined severity of acute pancreatitis in conformity with IAP/APA (International Association of Pancreatology / American Pancreatic Association) evidence-based guidelines for the management of acute pancreatitis [9-10]. The study was made in accordance with the Good Clinical Practice and principle of the Declaration of Helsinki. All participants signed informed consent before the study.

Table 1. Pancreas affection in patients with acute pancreatitis

Parameters	No severe acute pancreatitis, n (M±m, %)	Severe acute pancreatitis, n (M±m, %)	p-level (t-test)
Interstitial edema of pancreatic gland tissue	15 (75.0±9.68%)	1 (5.0±4.87%)	p<0.001
Small-focal pancreatonecrosis (V<30%)	4 (20.0±8.94%)	7 (35.0±10.67%)	p=0.215
Large-focal pancreatonecrosis (V=30-50%)	1 (5.0±4.87%)	8 (40.0±10.95%)	p=0.011
Subtotal pancreatonecrosis (V=51-75%)	0 (0%)	3 (15.0±7.98%)	-
Total pancreatonecrosis (V≥75%)	0 (0%)	1 (5.0±4.87%)	-

V, volume; n, number; P, percentage; m, standard error of percentage.

Table 2. Retroperitoneal cellular tissue affection in patients with acute pancreatitis

Parameters	No severe acute pancreatitis, n (M±m, %)	Severe acute pancreatitis, n (M±m, %)	p-level (t-test)
Without cellular tissue affection	16 (80.0±8.94%)	2 (10.0±6.71%)	p<0.001
Affection of less than 3 regions	3 (15.0±7.98%)	6 (30.0±10.25%)	p=0.307
Affection of 4 - 6 regions	1 (5.0±4.87%)	8 (40.0±10.95%)	p<0.001
Affection of 7 and more regions	0 (0%)	4 (20.0±8.94%)	-

Table 3. Lymphocytes apoptosis in peripheral blood in cases of severe acute pancreatitis

Parameters	No severe acute pancreatitis (n=20)	Severe acute pancreatitis (n=20)	p-level
Lymphocytes at initial blebbing in terms of 100 cells	6.8 (5.4, 9.0)	13.4 (12.6, 15.3)	p<0.001
Lymphocytes at terminal blebbing in terms of 100 cells	3.1 (1.7, 3.9)	21.9 (14.6, 28.3)	p<0.001
Total blebbing of lymphocytes in terms of 100 cells	11.2 (8.0, 13.4)	33.5 (27.1, 39.7)	p<0.001

Table 4. Correlation coefficients between lymphocytes apoptosis in peripheral blood and type of affection in pancreatic gland and retroperitoneal space

	Necrosis in pancreatic gland tissue	Retroperitoneal cellular tissue affection
Lymphocytes at initial blebbing in terms of 100 cells	rS=0.46, p<0.001	rS=0.57, p<0.001
Lymphocytes at terminal blebbing in terms of 100 cells	rS=0.76, p<0.001	rS=0.82, p<0.001
Total blebbing of lymphocytes in terms of 100 cells	rS=0.68, p<0.001	rS=0.73, p<0.001

rS, Spearman correlation coefficient; p, double tail significance.

Signs of pancreatic glandular tissue and retroperitoneal space affections were evaluated not earlier than the 14th day of the disease with computed tomography (CT) angiography on multispiral 4 multislice tomograph «Lightspeed» (General Electric, USA). The pancreas affection was described by the relative value of pancreatonecrosis focus volume. By 3D-modelling we found out the volume of pancreatic gland (ml), the volume of pancreatonecrosis (ml), and we calculated relative pancreatonecrosis focus volume (%). Retroperitoneal cellular tissue affection was estimated based on the number of anatomical regions of retroperitoneal cellular tissue with infiltration.

To estimate the activity of lymphocytes apoptosis in peripheral blood we took blood samples from the patients at the admission. The study was made at Research Institute of Molecular Medicine and Pathobiochemistry of Voino-Yasenetsky Krasnoyarsk State Medical University (Krasnoyarsk, Russia). Lymphocyte culture was selected by the standard method: centrifugation of heparinized blood by density gradient with Lympholyte-H CL5010 (Cedarlane Laboratories Limited, Canada). The plasma membrane condition was estimated by phase-contrast microscopy with microscope Olympus BX41 (Olympus Corp., Japan). We estimated such indicators as lymphocytes at initial blebbing (small vesicles less than 1/3 of cell radius) in terms of 100 cells, lymphocytes at terminal blebbing (large vesicles more than 1/3 of cell radius) in terms of 100 cells and total blebbing of lymphocytes (sum of the lymphocytes at initial blebbing and the lymphocytes at terminal blebbing) at more than 10 fields of view.

The obtained results were processed by statistical software package SPSS 17.0. The frequency and the character of the pancreas affection and the retroperitoneal cellular tissue affection are presented as absolute value with percentage and its standard error – n (P±m%). T-test for relative value was used for comparisons of studying groups. Based on the Shapiro–Wilk test, all examined parameters of the apoptosis did not correspond to

the normal distribution. These data are presented as median with lower and upper quartiles – Me (LQ, UQ). Mann–Whitney U test was used for pairwise comparisons of studying groups of patients. If P<0.05, differences between groups were recognized as statistically significant. Association between parameters was revealed based on the Spearman correlation test (rS).

Results

The severity of acute pancreatitis depends on the area of pancreatic glandular tissue affection. Based on CT-angiography findings, interstitial edema of tissue without signals of necrotic affection dominated in cases of non-severe acute pancreatitis in 75.0±9.68% of cases, necrotic affection less than 30% of pancreas area was in 20.0±8.94% of cases. In case of severe acute pancreatitis necrotic affection of pancreatic glandular tissue was in 95.0±4.87%, the domination of large-focal affection was in 40.0±10.95% of cases (Table 1).

In cases of no severe acute pancreatitis retroperitoneal cellular tissue was not involved in pathological processes in 80±8,94% of cases, in other cases, infiltration of retroperitoneal cellular tissue accompanied the formation of necrosis in pancreatic glandular tissue. In 90.0±6.71% cases of severe acute pancreatitis retroperitoneal cellular was involved in pathological processes. The vast affection of cellular tissue dominated, it included 4-6 anatomical regions (Table 2).

The severe acute pancreatitis was characterized by massive lymphocytes apoptosis in peripheral blood. The total blebbing of lymphocytes in terms of 100 cells was 33.5 (27.1, 39.7), it was 3 times more than the index in cases of no severe acute pancreatitis (p<0.001). Number of cells at terminal blebbing in cases of severe acute pancreatitis was 21.9 (14.6, 28.3), in cases of no severe – 3.1 (1.7, 3.9) (p<0.001) (Table 3).

The most interesting value was correlation between indicators of lymphocyte membrane apoptosis in peripheral blood and pathological changes at pancreatic glandular and retroperitoneal space. There were high positive correlations between the number of lymphocytes at terminal blebbing and necrosis in pancreatic gland tissue ($rS=0.76$, $p<0.001$), also terminal blebbing and retroperitoneal space affection ($rS=0.82$, $p<0.001$). Total blebbing of lymphocytes had moderate positive correlations with area of pancreas affection ($rS=0.68$, $p<0.001$) and involvement of retroperitoneal cellular tissue in pathological processes ($rS=0.73$, $p<0.001$) (Table 4).

Discussion

Development of severe acute pancreatitis is accompanied distinct oxidative stress, which causes the cascade of biochemical reactions, the base of pathogenesis of pancreatonecrosis [11]. In case of severe acute pancreatitis there is an endothelial dysfunction, it causes the increase of vessel walls permeability, impairment of hemostasis. Many authors connect development of severe complication, such as acute kidney injury and shock, with development of endothelial dysfunction [12]. Lymphocytes apoptosis in peripheral blood is non-specific marker, it shows intensity of functional condition of endothelium impairment.

Kataoka et al. (2004) showed how important to study role of apoptosis in severe acute pancreatitis and how hard to make a clinical study in this field [13]. The obtained data about the correlations between severity of acute pancreatitis and lymphocytes apoptosis intensity in peripheral blood coheres with multiple findings in this field, such as Qin et al. [14] findings and experimental findings by Pinhu et al. [15], and Weis et al. [16].

A distinctive feature of this study is high positive correlations between activity of lymphocyte apoptosis in peripheral blood and necrosis in pancreatic gland tissue, and infiltration of retroperitoneal cellular tissue, which was revealed by CT-angiography.

Conclusion

The apoptosis due to the development of oxidative stress accompanies the severe clinical course of acute pancreatitis, destructive changes in pancreatic glandular and retroperitoneal cellular tissue. Lymphocytes apoptosis in peripheral blood reflects the severity of acute pancreatitis; it directly correlates with the area of pancreas and retroperitoneal cellular tissue affection.

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Conflict of interest

The authors of the article have no conflicts of interest to disclose. Authors of the article confirm the absence of sponsorship.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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