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Serhiy Ivashchuk
Department of Family Medicine, Bukovinian State Medical University, Chernivtsi, Ukraine

Larysa Sydorchuk
Department of Family Medicine, Bukovinian State Medical University, Chernivtsi, Ukraine

Correspondence
Serhiy Ivashchuk
MD, PhD, Associated Professor of Family Medicine Department, Bukovinian State Medical University, Heroyiv Maydanu Str., 174/3, Chernivtsi, Ukraine

Level of reactive response of peripheral blood neutrophil granulocytes of patients with acute pancreatitis depending on genes polymorphism of CFTR (delF508C), PRSS1 (R122H), IL-4 (C-590T) and TNF-α (G-308A)

Serhiy Ivashchuk and Larysa Sydorchuk

Abstract
The neutrophil granulocytes play the key role in the nonspecific anti-infectious protection and adaptive immunity formation. However, the questions of the reactive response of the peripheral blood neutrophils of the patients with edematous pancreatitis even depending on candidate genes polymorphism stay not studied. The aim of our research was to investigate the level of the reactive response of peripheral blood neutrophils in patients with edematous pancreatitis depending on the polymorphism of genes CFTR (*ΔF508*, rs 113993960), PRSS1 (*R122H* or *G365A,* rs 111033565), IL-4 (*C-590T,* rs 2243250) and TNF-α (*G-308A*, rs 1800629). 123 patients with edematous pancreatitis were involved in the research. It has been established, that the carriers of NN-genotype of the gene CFTR had higher neutrophils reactive response than such of NM-carriers 3.45 times (p<0,01), neutrophil-to-lymphocyte ratio – 1.44 times (p<0,05), neutrophil granulocytes shift index – 2.93 times (p<0,01), ratio index of leukocytes and erythrocyte sedimentation rate – 12.41 times (p<0,001); organism resistance and leukocyte indexes – 1.55 and 1.85 times (p<0,01) correspondingly, however lymphocyte/granulocyte ratio was higher in NM-carriers 1.36 times (p<0,01). The carriers of GA-genotype of gene PRSS1, in comparison with GG-carriers, had lower nonspecific organism resistance 1.98 times (p<0,001), but higher level of neutrophils reactive response – 22.79-28.65 times (p<0,001), bigger neutrophil-to-lymphocyte ratio – 2.02 times (p<0,01), shift indexes of neutrophils and leukocytes – 8.28 and 3.08 times (p<0,001), ratio index of leucocytes and erythrocyte sedimentation rate and leukocyte index – 5.84 and 7.29 times (p<0,001), with lower lymphocyte/granulocyte ratio 3.63 times (p<0,001). Conclusions. The presence of NN-genotype of gene CFTR, GG polymorphic variant of the gene PRSS1 and GG-genotype of gene TNF-α in a patient with edematous pancreatitis is accompanied by a credibly higher level of non-specific resistance of the organism, neutrophils reactive response, and indicates the superiority of immune response by affective cell type and bigger microphage system activity. In the TT-genotype carriers of the gene IL-4, there is lower activity of the factors and mechanisms of anti-infective defence and general organism resistance.

Keywords: Pancreatitis, polymorphism, gene, CFTR *(*ΔF508), PRSS1 (R122H), IL-4 (C-590T), TNF-α (G-308A), neutrophils, reactive response

1. Introduction
Acute pancreatitis (AP) still remains one of the most actual problems of medicine. Among the factors which determine the severity of AP the most important are the etiological factor, the damage of acinar cells, intoxication, organism cell reactivity, adaptive tension level, the reactive response (on the early stages of the disease) of polymorphonuclear neutrophil granulocytes (NG), monocytes/macrophages of peripheral blood, immunological reactivity of patients organism and the infection of pancreatic necrosis nidi [1-10].
NG one of the first immunocompetent cells encounter pathogenic and relatively pathogenic microorganisms, which penetrate the protective barriers of the body. NG are extremely sensitive to the slightest change of homeostasis, can quickly leave the bloodstream (capillaries) and actively attack microbes and other genetically alien bodies. Alongside with this, NG do not have a reliable system of membranes regeneration and so they die when they are overloaded with pathogenic or relatively pathogenic microorganisms. In the presence of severe microbial contamination NG carry out the excess expression of free radicals in the microenvironment. If the antioxidant system of the body is unable to neutralize them, the destruction of the cells producers themselves (NG) takes place [11, 12].
The activated NG secrete, along with grain products, the wide range of preimmune and immune cytokines (cytokines of the first and the second degree).

This gives NG the possibility not only to affect the stimulation of the activity of other immunocompetent cells, and also to regulate specific adaptive immune response. And with microbial contamination, neutrophils realize phagocytosis of microbial cells, forming effective anti-infectious defence. Thus, the NG population plays a key role in nonspecific anti-infectious defence and in the formation of adaptive immunity [13-16].

However, despite the significant function of this population of immunocompetent cells, the questions of the polymorphonuclear NG reactive response (on the early stages of the disease) of peripheral blood of patients with AP in systemic inflammatory response, depending on the genes polymorphism, even with candidate genes remain still not studied and therefore require further research.

1.1 The aim of the research: To study the level of reactive response of polymorphonuclear NG of peripheral blood of patients with acute and exacerbated chronic pancreatitis depending on the genes polymorphism of CFTR (*ΔF508*, rs113993960), PRSS1 (*R122H* or *G365A,* rs111033565), IL-4 (*C-590T,* rs2243250) and TNF-α (*G-308A*, rs1800629).

2. Materials and methods

2.1 Compliance with bioethics

Study was performed in compliance with the Council of Europe Convention on Human Rights and Biomedicine and recommendations of the Committee on Bioethics of the Ministry of Health of Ukraine. Patients' Examination Cards and Patients' Informed Consent Forms were approved by the Biomedical Ethics Commission of Bukovina State Medical University, Ministry of Health of Ukraine (Chernivtsi, Ukraine). All enrolled patients were treated in the Local Emergency hospital (Chernivtsi, Ukraine) during last 4 years (2012-2016 y.y.). Genetic bench study performed at the laboratory of the State institution «Reference centre of molecular diagnostics of the Ministry of Health of Ukraine» (Kyiv) and at the laboratory of Medical Biology and Genetics Department of Bukovina State Medical University. After screening (matching inclusion/exclusion criteria) 123 patients with exacerbation of chronic pancreatitis (ECP) and AP (edematous form) were selected for further examination. The control group included 40 practically healthy individuals who were not relatives with the patients, without reliable differences of sex and age.

2.2 Diagnosis of Acute Pancreatitis and Exacerbation of Chronic Pancreatitis.

The diagnosis of AP and ECP was made on the basis of the existing national and international recommendations criteria [17, 18]. All patients signed an informed consent and underwent a complex of examinations: clinical, laboratory and instrumental according to protocol recommendations.

2.3 Genotyping

The polymorphic variants of genes IL-4 (C-590T), TNF-α (G-308A), PRSS1 (R122H), SPINK1 (N34S) and CFTR (delF508) were studied by polymerase chain reaction (PCR) method using oligonucleotide primers of the company «Metabion» (Germany) according to the modified protocols [19-21]. Amplification products of DNA fragments of genes were digested by hydrolysis using restriction enzyme («Thermo Scientific», USA): enzyme PmlI (Eco72I) for gene PRSS1, AvaII - the gene for IL-4, NcoI - gene for TNFα. The resulting fragments were analysed in agarose gel with the addition of ethidium bromide, molecular weight marker Gene Ruler 50 bp (DNA Ladder, «Thermo Scientific», USA), and further visualization by using transilluminator and Vitran software.

2.4 Reactive Response of Peripheral Blood Neutrophil Granulocytes calculation

The calculation of haematological indexes and ratios were conducted on the basis of the extended general clinical blood test, which was performed on the haematology analyser CELL-DYN 3700 SL (manufacturer – «Abbott Laboratories», USA). The NG reactive response of the organism of patients with AP was evaluated with neutrophil polymorphonuclear leukocytes reactive response index, neutrophil-to-lymphocyte ratio, shift indexes of neutrophils and leukocytes, lymphocyte/granulocyte ratio, leukocyte index, the correlation ratio of leucocytes and erythrocyte sedimentation rate (ESR). Also organism resistance index (ORI) was measured to assess the condition of the body resistance to stressful situation.

2.5 Statistical analysis

The statistical analysis was performed using applications MYSTAT 12 (Systat Software Inc., USA) and Scout 2008 Version 1.00.01 (USA Environmental Protection Agency, USA). The reliability of data for independent samples was calculated according to t-test Student (with the distribution of ranges close to normal), or U-criterion Wilcoxon-Mann-Whitney (with uneven distribution). The analysis of qualitative features was performed according to the χ^2 criterion. The difference was considered reliable at $p<0.05$.

3. Results and Discussion

Prospective Study included 123 patients: 23 (18.7%) women and 100 (81.3%) men. The patients' average age is 45.1 ± 5.19 years for males, 53.2 ± 7.07 years for females (23 to 77).

The results of data calculations of the level of NG reactive response of peripheral blood of patients with AP considering the polymorphic variants of the gene CFTR, are shown in Table 1.

Table 1: The level of reactive response of peripheral blood neutrophils in patients with acute pancreatitis based on ΔF508 CFTR gene polymorphism

№	Parameter	Control group, n=40	Genotype of gene CFTR	
			NN, n=98	NM, n=3
1	Organism resistance index	91.82±7.46	58.92±2.89 $p_c<0.01$	38.07±2.33 $p_c<0.001$ $p_{NN}<0.01$
2	Reactive response of neutrophils (according to S. Kravchenko)	10.53±2.03	176.01±47.82 $p_c<0.01$	206.70±35.48 $p_c<0.01$
3	Reactive response of neutrophils according to Khabirov	10.60±2.10	204.01±49.59 $p_c<0.01$	59.05±4.31 $p_c<0.001$ $p_{NN}<0.01$
4	Neutrophil-to-lymphocyte ratio	2.19±0.27	8.66±0.72 $p_c<0.001$	6.03±0.47 $p_c<0.001$ $p_{NN}<0.01$
5	Neutrophils shift index	0.06±0.009	0.51±0.09 $p_c<0.01$	0.18±0.02 $p_c<0.01$ $p_{NN}<0.01$
6	Leukocytes shift index	1.73±0.09	5.52±0.37$P_c<0.001$	4.59±0.43 $p_c=0.001$
7	Lymphocyte/granulocyte ratio	4.44±0.31	2.14±0.17 $p_c<0.001$	1.57±0.13 $p_c<0.01$ $p_{NN}<0.01$
8	Leukocyte index	0.46±0.04	0.48±0.08	0.26±0.02 $p_c<0.01$ $p_{NN}<0.01$
9	The ratio index of leucocytes and ESR	1.44±0.11	4.22±0.62 $p_c<0.01$	0.34±0.04 $p_c<0.001$ $p_{NN}<0.01$

Notes. p_c - reliability of indexes difference in comparison with control group; p_{NN} - reliability of indexes difference in comparison with carriers of NN-genotype.

In the carriers of the NN-genotype of gene CFTR NG reactive response, determined according to Habirov is higher than control and then such of NN carriers – 19.25 and 3.45 times ($p<0.01$), neutrophil-to-lymphocyte ratio – 3.95 and 1.44 times ($p<0.05$), the neutrophils shift index – 8.5 and 2.83 times ($p<0.01$), the leukocytes shift index – 3.19 times ($p<0.001$) and ratio index of leucocytes and ESR – 2.93 and 12.41 times ($p<0.001$); leukocyte index and the organism resistance index exceeded such ones in carriers of NM-genotype – 1.85 and 1.55 times ($p<0.01$), lymphocyte/granulocyte ratio was lower than in control group – 2.07 times ($p<0.001$), exceeding such of the NM carriers – 1.36 times ($p<0.01$).

Thus, NN-genotype carriers of gene CFTR have significantly higher level of non-specific resistance of organism, NG reactive response of peripheral blood as a marker of nonspecific anti-infectious defence, higher neutrophil-to-lymphocyte ratio, NG and leukocytes shift indexes and the ratio index of leucocytes and ESR, with lower lymphocyte/granulocyte ratio, which indicates the predominance of affective cellular link of immune response, microphage system and active sharp-inflammatory process, that contributes to the induction and formation of additional mechanisms of immune response through humoral type. The carriers of NM-genotype of gene CFTR had significantly lower activity of the factors and nonspecific anti-infectious defence mechanisms and the level of non-specific resistance, than the NN-genotype carriers, that confirms the tendency to chronic inflammation or its primary chronic course.

The results of the study of reactive response level of peripheral blood neutrophils in patients with AP taking into account gene polymorphism PRSS1 (G365A) are shown in Table 2.

Table 2: The level of reactive response of peripheral blood neutrophils of patients with acute pancreatitis based on polymorphic variants of gene PRSS1 (G365A)

№	Parameter	Control group, n=40	Genotype of gene PRSS1	
			GG, n=117	GA, n=6
1	Organism resistance index	91.82±7.46	59.24±2.56 $p_c<0.01$	29.97±2.28 $p_c<0.001$ $p_{GG}<0.001$
2	Reactive response of neutrophils (according to S. Kravchenko)	10.53±2.03	85.87±8.77 $p_c<0.001$	2460.30±279.07 $p_c<0.001$ $p_{GG}<0.001$
3	Reactive response of neutrophils according to Khabirov	10.6±2.1	107.96±15.92 $p_c<0.01$	2460.33±280.13 $p_c<0.001$ $p_{GG}<0.001$
4	Neutrophil-to-lymphocyte ratio	2.19±0.27	8.23±0.63 $p_c<0.001$	16.60±1.65 $p_c<0.001$ $p_{GG}<0.01$
5	Neutrophils shift index	0.06±0.009	0.39±0.06 $p_c<0.01$	3.23±0.35 $p_c<0.001$ $p_{GG}<0.001$
6	Leukocytes shift index	1.73±0.09	5.08±0.28 $p_c<0.001$	15.67±1.42 $p_c<0.001$ $p_{GG}<0.001$
7	Lymphocyte/granulocyte ratio	4.44±0.31	2.18±0.15 $p_c<0.001$	0.60±0.07 $p_c<0.001$ $p_{GG}<0.001$
8	Leukocyte index	0.46±0.04	0.38±0.06	2.77±0.25 $p_c<0.001$ $p_{GG}<0.001$
9	The ratio index of leucocytes and ESR	1.44±0.11	3.43±0.48 $p_c<0.01$	20.04±2.31 $p_c=0.001$ $p_{GG}<0.001$

Notes. p_c - reliability of indexes difference in comparison with control group; p_{GG} - reliability of indexes difference in comparison with carriers of GG-genotype.

In GA-genotype carriers of PRSS1 gene, patients with AP, compared with the GG-genotype carriers, there is a lower non-specific organism resistance 1.98 times ($p<0.001$), with the higher level of NG reactive response of peripheral blood of patients with AP (according to S. Kravchenko) – 28.65 times ($p<0.001$), according to Habirov – 22.79 times ($p<0.001$), higher neutrophil-to-lymphocyte ratio – 2.02 times ($p<0.01$), shift indexes of neutrophils and leukocytes – 8.28 and 3.08 times ($p<0.001$), respectively, leukocyte index and the ratio index of leucocytes and ESR – 7.29 and 5.84 times ($p<0.001$),with a lower lymphocyte/granulocyte ratio – 3.63 times ($p<0.001$).

Thus, the presence GA polymorphic variant of gene PRSS1 in patients with AP is associated with significantly more pronounced laboratory manifestations of acute inflammatory autoimmune process in pancreas, which is accompanied by increased NG reactive response of peripheral blood, higher neutrophil-to-lymphocyte ratio, higher NG and leukocytes shift indices, bigger leukocyte index, that confirms the increased cell reactivity and immune response preference "on damage" according to affective cell type (lymphocyte/granulocyte ratio reduction), in addition indicating both exogenous and endointoxication, including infectious nature, and the high risk of pancreas cells autolysis.

The level of NG reactive response of peripheral blood in patients with AP depending on the gene IL-4 (C-590T) polymorphic variants is presented in Table 3.

Table 3: Association of C-590T polymorphism of gene IL-4 with the level of reactive response of peripheral blood neutrophils of patients with acute pancreatitis

№	Parameter	Control group, n=40	Genotype of gene IL-4		
			CC, n=58	CT, n=34	TT, n=9
1	Organism resistance index	91.82±7.46	57.71±2.68 $p_c<0.01$	61.36±6.44 $p_c<0.01$	27.93±2.15 $p_c<0.001$ $p_{CC}<0.001$ $p_{CT}<0.01$
2	Reactive response of neutrophils (according to S. Kravchenko)	10.53±2.03	245.94±83.25 $p_c<0.01$	95.06±22.58 $p_c<0.01$	118.20±13.08 $p_c<0.001$
3	Reactive response of neutrophils according to Khabirov	10.6±2.1	236.40±83.45 $p_c<0.01$	157.41±44.58 $p_c<0.01$	118.22±13.29 $p_c=0.001$
4	Neutrophil-to-lymphocyte ratio	2.19±0.27	8.41±0.98 $p_c<0.01$	7.48±1.00 $p_c<0.01$	22.50±2.01 $p_c<0.001$ $p_{CC}<0.01$ $p_{CT}<0.001$
5	Neutrophils shift index	0.06±0.009	0.69±0.15 $p_c<0.01$	0.28±0.07 $p_c<0.01$ $p_{CC}<0.05$	0.18±0.02 $p_c<0.01$ $p_{CC}<0.01$
6	Leukocytes shift index	1.73±0.09	5.23±0.49 $p_c<0.001$	5.38±0.63 $p_c<0.01$	10.11±1.89 $p_c<0.01$ $p_{CC}<0.05$ $p_{CT}<0.05$
7	Lymphocyte/granulocyte ratio	4.44±0.31	2.04±0.20 $p_c<0.001$	2.37±0.32 $p_c<0.01$	0.44±0.11 $p_c<0.001$ $p_{CC}<0.001$ $p_{CT}<0.01$
8	Leukocyte index	0.46±0.04	0.65±0.13	0.27±0.06 $p_c<0.05$ $p_{CC}=0.01$	0.18±0.02 $p_c<0.01$ $p_{CC}<0.01$
9	The ratio index of leucocytes and ESR	1.44±0.11	4.22±0.83 $p_c<0.01$	3.89±1.12 $p_c<0.05$	3.84±1.06 $p_c<0.05$

Note. p_c - reliability of indexes difference in comparison with control group; p_{CC} - reliability of indexes difference in comparison with carriers of CC-genotype, p_{CT} - reliability of indexes difference in comparison with carriers of CT-genotype.

Unidirectional dependence of NG reactive response of the peripheral blood in patients with AP depending on the polymorphic variants of gene IL-4 (C-590T) was not established. Thus, the resistance index in CC and CT genotypes carriers of gene IL-4 is significantly higher than in TT-genotype carriers - 2.07 and 2.20 times ($p<0.01$), the NG shift index and leukocyte index – 3,83 and 3.61 times ($p<0.01$) respectively, lymphocyte/granulocyte ratio – 4.64 and 5.39 times ($p<0.01$). At the same time, the neutrophil-to-lymphocyte ratio in TT genotype carriers was higher than in C allele carriers of gene IL-4 - 2.68 and 3.01 times ($p<0.001$) and the leukocytes shift index – 1.93 and 1.88 times ($p<0.05$) respectively.

Thus, in patients with AP carriers of the TT genotype of gene IL-4 is observed significantly lower non-specific reactivity and organism resistance with compensatory higher neutrophil-to-lymphocyte ratio and leukocytes shift index, which confirms the high activity of the inflammatory process and preferred cell link of immune response. While in the C allele carriers of the gene IL-4 was determined the higher ORI, NG reactive response, lymphocyte-granulocyte ratio, leukocyte index, which also indicate the presence of acute inflammation but with a dual mechanism of implementation, not only through affective cell link of anti-infectious defence, but and (mostly) by macrophage, humoral effector link. The received results also demonstrate lower activity of factors and mechanisms of anti-infectious defence and total organism resistance of patients with AP - carriers of TT-genotype of gene IL-4.

Results of changes NG reactive response of peripheral blood of patients with AP depending on the polymorphic variants of the gene TNF-α (G-308A) are shown in Table 4.

Table 4: The level of reactive response of peripheral blood neutrophils of patients with acute pancreatitis based on G-308A polymorphism of TNF-α gene

№	Parameter	Control group, n=40	Genotype of gene TNF-α	
			GG, n=9	GA, n=2
1	Organism resistance index	91.82±7.46	59.60±3.87 $p_c<0.01$	37.95±2.81 $p_c<0.001$ $p_{GG}<0.05$
2	Reactive response of neutrophils (according to S. Kravchenko)	10.53±2.03	69.32±11.00 $p_c<0.01$	206.70±27.29 $p_c<0.001$ $p_{GG}<0.01$
3	Reactive response of neutrophils according to Khabirov	10.6±2.10	69.32±11.01 $p_c<0.01$	59.05±8.67 $p_c<0.01$
4	Neutrophil-to-lymphocyte ratio	2.19±0.27	11.55±3.11 $p_c<0.01$	6.11±0.83 $p_c<0.01$
5	Neutrophils shift index	0.06±0.009	0.27±0.06 $p_c<0.01$	0.18±0.03 $p_c<0.01$
6	Leukocytes shift index	1.73±0.09	6.16±0.71 $p_c<0.01$	4.59±0.32 $p_c<0.001$
7	Lymphocyte/granulocyte ratio	4.44±0.31	1.25±0.17 $p_c<0.001$	1.57±0.21 $p_c<0.001$
8	Leukocyte index	0.46±0.04	0.27±0.06 $p_c<0.05$	0.26±0.05 $p_c<0.01$
9	The ratio index of leucocytes and ESR	1.44±0.11	10.38±2.87 $p_c<0.01$	0.34±0.11 $p_c<0.001$ $p_{GG}<0.05$

Note. p_c - reliability of indexes difference in comparison with control group; p_{GG} - reliability of indexes difference in comparison with carriers of GG-genotype.

Clear unidirectional correlation of all reactive response parameters of peripheral blood neutrophils of patients with AP depending on polymorphic variants of gene TNF-α (G-308A) was not determined. However, GG-genotype carriers had a higher than the GA-variant carriers ORI – 1.57 times ($p<0.05$) and the ratio index of leucocytes and ESR – 30.41 times ($p<0.001$) with lower NG reactive response (according to S. Kravchenko) – 2.98 times ($p<0.001$).

The obtained data show that patients with AP – carriers of GG-genotype of gene TNF-α have higher level of non-specific reactivity and organism resistance with lower NG reactive response of peripheral blood (according to S. Kravchenko) than GA-genotype carriers, that indicates the active microphage cell reaction of immunity in response to acute inflammation of pancreas with the advantage of endogenous origin autointoxication.

Everything described above indicates a significant activation of factors and mechanisms of nonspecific anti-infectious defence with edematous pancreatitis that is a defensive reaction to the inflammatory process.

4. Conclusion

The presence of NN-genotype of gene CFTR (rs113993960), GG polymorphic variant of gene PRSS1 (rs111033565) and GG-genotype of gene TNF-α (rs1800629) in a patient with AP is accompanied by a significantly higher level of non-specific organism resistance, reactive response of peripheral blood NG, as a marker of nonspecific anti-infectious defence, higher neutrophil-to-lymphocyte ratio, NG and leukocytes shift indexes and the ratio index of leucocytes and ESR, with lower lymphocyte/granulocyte ratio, that indicates the preference of immune response by affect cell type, the bigger activity of microphage system, confirming in addition exo- and endo-intoxication including infectious origin and the high risk of pancreas cells autolysis.

The implementation of anti-infectious defence mechanisms for acute pancreas inflammation in C allele carriers of gene IL-4 (rs2243250) realize in two ways: through the increased activity of affect cell link of immunity and (mostly) by macrophage, humoral effector link, which was confirmed by the higher ORI, NG reactive response, lymphocyte/granulocyte ratio and leukocyte index. The results also demonstrate the lower activity of anti-infectious defence factors and mechanisms and total organism resistance in patients with AP – in the TT-genotype carriers of gene IL-4.

In perspective we plan to analyse the association of analysed genes' haplotypes with metabolism parameters disorders and cytokines level.

4.1 Limitations of the Study: The study was limited by the number of enrolled patients.

4.2 Conflict of Interest: None declared.

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