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Structural and Clinical Features of Immune System Clusters in Patients with Urgent Surgical Pathology

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Abstract: The purpose of the present study was to select individual options (clusters) of immune system organization in patients with urgent surgical abdominal pathology and to assess the features of their organization against the clinical characteristics. The study included 442 patients, using the number of CD3 +, CD4 +, CD8 +, CD16 + lymphocytes six clusters of immune status. To assess the relationship of indicators used correlation and analysis of variance. The leading factor in the formation of immune system cluster organization, clinically associated with development of multiple organ failure syndrome, SIRS, sepsis, abdominal abscess, stress ulcers and perforation of hollow organs, septic pneumonia. Clusters of immune status differ in the number of immune cells and the patients' severity. The structure of the clusters is characterized by more severe condition of patients with increasing distance from the cluster center. Results of the study offer the prospects in the development of new integrated methods for assessing the severity of the patient, prognosis and outcome of disease, choosing the most effective tactics of combined treatment.

Key words: Urgent Surgery · Immune System · Factor Analysis · Cluster Analysis

INTRODUCTION

Treatment of patients with urgent surgical pathology is still actual problem of practical medicine because of the frequent complications and high mortality. The immune system associated with the severity of inflammation, the severity of stress and largely determines the character of the postoperative course, disease outcome and timing of recovery [1].

Modern methods of systems analysis allowed us to consider in new ways, the organization of the immune system [2, 3]. To compensate for external influences, the elements of any system aspire to get optimum performance [4, 5].

The result is a change in mathematical performance data sets. In areas with optimal characteristics occur condensations of data – clusters, consisting of elements of the system with similar properties. The number of clusters depends on the qualitative differences in the interaction of elements with external influences. Distance from the center of cluster (DC) associated with functional characteristics of elements [6]. Such a feature was a common to all formed clusters.

The purpose of the article was to select individual options (clusters) of immune system organization in patients with urgent surgical abdominal pathology and to assess the features of their organization against the clinical characteristics.

MATERIALS AND METHODS

442 patients with pathology of abdominal organs were examined and they are needed urgent operations. Patients with perforated stomach ulcers and duodenal ulcers was 162 (36.6%) patients with penetrating injuries to abdominal injuries - 73 (16.5%), necrotizing pancreatitis - 45 (10.2%), acute adhesive intestinal obstruction - 70 (15.8%), destructive forms of appendicitis - 31 (7.0%). In 104 patients, including the above-mentioned, were a combination of other acute inflammatory processes (23.5%).

Peritonitis and abdominal sepsis was observed in 292 patients (66.1%). However, changing the formula of white blood cells, corresponding to the systemic inflammatory response syndrome (SIRS), are marked at the moment of the study only in 54 patients (12.2%).

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Hospital pneumonia developed in 11 patients (2.5%), multiple organ dysfunction syndrome (MODS) – in 59 patients (13.3%). Recovered patients were 381 (86.2%), in 61 cases (13.8%) disease ended in death. Patients examined within 1-2, 5-7 and 10-12 days after the operation. The study included 949 survey results. Integral assessment of the patient's severity was carried out in the dynamics of Apache II, SAPS II, SOFA, MODS scales.

All patients were operated on within 24 hours of hospitalization. Surgical treatment consisted of laparotomy, revision of the abdominal cavity, removing the effects of trauma or injury, the elimination the source of infection. When can't eliminate the cross-sectional purulent process in the abdominal cavity, were planned relaparotomy with an interval about 48 hours. All patients received the infusion, detoxification and antibiotic therapy in amounts adequate to severity condition.

We used monoclonal antibodies - analogs produced by «Becton Dickinson» adapted for the using with fluorescence microscope - to determine the level of expression in lymphocytes molecules: CD3 (ICO-90), CD4 (ICO-86), CD8 (ICO-31), CD16 (ICO-116), CD20 (ICO-180), CD25 (ICO-105), CD38 (ICO-20), CD95 (ICO-160), the expression of CD16 molecules on neutrophils also studied (CD16+NEUT). The absolute number of these cells (abs) was calculated.

Evaluated the phagocytic index with latex particles (PHI) and calculates the average number of phagocytized particles (PHC). The concentration of total antibodies IgA, IgM, IgG in the serum were measured by enzyme immunoassay. The concentration of circulating immune complexes (CIC) were measured in the light absorbance at a wavelength of 315 nm after incubation of plasma with a solution of polyethylene glycol with molecular weight of 6000.

Take into account the number of white blood cells (WBC), the absolute count of lymphocytes (ALC). Additionally calculated the ratio of populations of cells - leukocyte indexes of intoxication: by Ya.Ya. Kalf-Caliph (LII_{KK}) by V.K. Ostrovsky (LII_{OS}), by S.F. Khimich modificated by A.L. Kostyuchenko et al. (LII_{KH}) [7] for the formulas:

LII_{KK} = [(4*MYEL+3*YGN+2*SNN+SGN)*(PLC+1)]/[(EO+1)*(LYM+MON)];
LII_{OS} = (MYEL+NEUT+PC)/(EO+LYM+MON);
LII_{KH} = 0.1* WBC* NEUT/(100-NEUT).

The designations: WBC – white blood cells (10^9/l); LYM – lymphocyte (%); NEUT – total neutrophils (%); SGN – segmented neutrophils (%); MON – monocyte (%); EO – eosinophils (%); MYEL – myelocytes (%); YGN – young neutrophils (%); SNN – stabnuclear neutrophils (%); PLC – plasma cells (%).

The indicators of autonomic regulation: the index Kerdö (KI), volume of heart blood flow per minute were investigated (HV) [8].

Calculations carried out using a package of statistical programs «Statistica for Windows 6.0». The main statistical parameters take into account are the arithmetic mean values (M) and standard error (m). The difference between rates in the groups tested using the Mann-Whitney U-test. Analysis of variance was performed with the calculation of F-test and strength of influence of the studied characteristic ($ç^2$) on a data structure. To assess the relationship between indicators of the patient and the DC used the Spearman coefficient of rank correlation (r_s). The critical significance level (p) for verification of statistical hypotheses assumed to be equal 0.05. Values of $p<0.01$ were in the form of the mantissa and exponent. In the case of $p<1.0*10^{-29}$, more than the possibility of measuring «Statistica for Windows 6.0», accepted p = 0.00.

To select the most informative indicators for clustering using methods of factor analysis. The procedure for selection of principal components (PC) is to search in the multidimensional space the axes of factors describing the dispersion of the values of the investigated data. Factors are selected using the values of significance testing, the proposed H.F. Kaiser [9], with eigenvalues ë>1.0. To improve the interpretability of the factors used the method of rotation VARIMAX, allows to receive more contrasting factor loadings [10]. In the data array for factor analysis included indicators: WBC, the absolute number (abs) of CD3+, CD4+, CD8+, CD16+, CD16+NEUT, CD20+, CD25+, CD38+, CD95+, the count of phagocytic neutrophils (PNC), the concentration of IgG, IgA, IgM, CIC. The optimal number of clusters was determined on the basis of calculating the values of Euclidean distances between the mean group values [11].

RESULTS AND DISCUSSION

Analysis of correlation links the 15 indicators of immune status of patients with 442 urgent surgical pathology allowed to extract and rank the "latent" factors (principal components, PC 1-15) the degree of their impact on processes in the immune system (Fig. 1). The immune system of patients studied, significantly depends on the influence of four factors, which can be quantified by values of PC 1-4 ë>1.0.

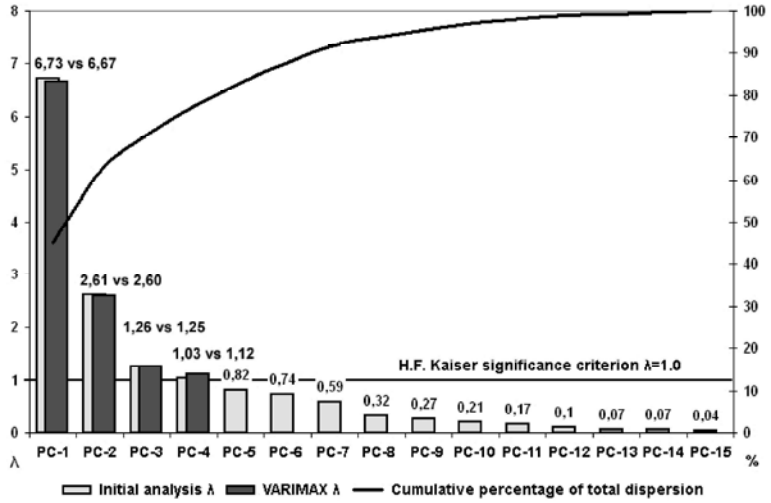

Fig. 1: Factor analysis of immune status indices of patients with urgent surgical pathology (n=442)

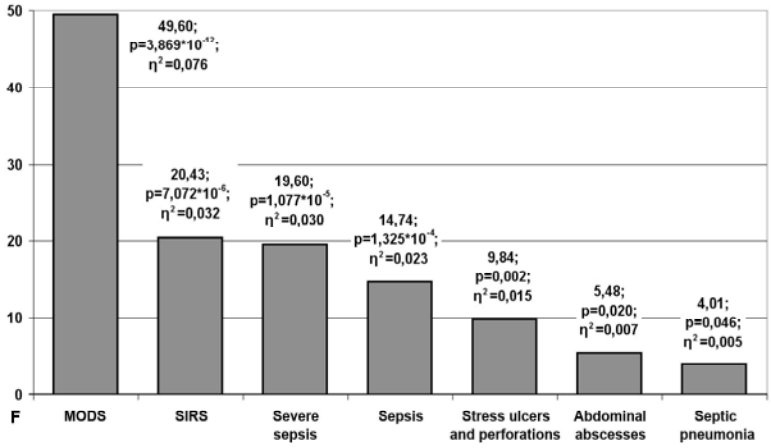

Fig. 2: The relationship of PC and clinical conditions in patients with urgent surgical pathology (F, ç2, n=442)

Thus, the first factor determines 44.85% of all possible states of the immune system of patients studied, the second - 17.32%, third - 8.34%, the fourth - 7.47%. In total, these four factors determine the variation of the immune system of patients at 77.52%. In accordance with the canons of statistics fluctuations in the values of other factors are not significant in terms of changes in the immune system.

The values of the most influential PC-1 is most significant correlated with the cellular branch of the immune system (Fig. 1). Factor loadings were highest for indicators: abs CD3+ (r_S=0.93; p=0.00), abs CD4+ (r_S=0.92; p=0.00), abs CD8+ (r_S=0.92; p=0.00), abs CD16+ (r_S=0.91; p=0.00). These indicators are sensitive to pathogenic influences in postoperative period: emotional stress and the development of complications, the development of purulent surgical infection or multiple organ failure [12-14]. Indicators: abs CD3+, abs CD4+, abs CD8+, abs CD16+ were used as criteria for clustering the immune status data. Were identified 6 clusters - variants of organization the immune system (Table 1). These variations in the usual sense of the term can not be called groups, since the cluster membership of the immune status is determined by the characteristics of the organization's immune system and the value of DC.

The relationship between the values of principal components and the presence or absence of clinical features of patients studied makes it possible to evaluate the pathogenetic factors in the organization characteristics of the immune system. According to the results of analysis of variance, PC-1 is the most markedly associated with development of the MODS, SIRS, sepsis (Table 1). Also found significant associations of the PC-1 with the development of abdominal abscesses, stress ulcers and perforations of hollow abdominal organs, septic pneumonia (Fig. 2).

Table 1:Characteristics of the clusters of immune system (M±m, ul^{-1})

Indicators	Cluster 1, n=15	Cluster 2, n=71	Cluster 3, n=166	Cluster 4, n=241	Cluster 5, n=253	Cluster 6, n=203
abs CD3+	1332.81±64.5	889.7±17.42	630.75±6.86	428.83±4.11	273.18±3.52	127.82±3.7
abs CD4+	811.2±60.95	510.72±11.41	363.86±4.47	263.46±3.54	161.11±2.35	71.62±2.14
abs CD8+	752.8±38.19	537.06±14.78	384.17±5.5	264.93±3.14	164.31±2.41	75.52±2.4
abs CD16+	977.73±84.66	570.28±24.59	412.57±9.42	296.06±6.46	198.5±4.21	116.33±3.29

Note: The reliability of differences between clusters for all indicators $p<0.001$.

Table 2:The Indicators of the severity scales of patients in clusters

Indicators	Cluster 1, n=15	Cluster 2, n=71	Cluster 3, n=166	Cluster 4, n=241	Cluster 5, n=253	Cluster 6, n=203
Apache II, score	8.73±1.97	7.72±0.46	8.42±0.39	9.71±0.39; p2=0.03; p3=0.04	12.31±0.34; p1=0.001; p2=7.78*10^{-12}; p3=2.42*10-^{14}; p4=3.46*10^{-8}	15.74±0.39; p1=1.35*10^{-5}; p2=1.72*10^{-19}; p3=6.83*10^{-26}; p4=8.25*10^{-20}; p5=2.77*10^{-8}
SOFA, score	2.53±0.45	1.94±0.15	2.20±0.13	2.50±0.12; p2=0.03	3.05±0.11; p2=3.03*10^{-7}; p3=1.45*10^{-7}; p4=2.46*10^{-4}	3.59±0.12; p1=0.01; p2=3.50*10^{-11}; p3=5.45*10^{-13}; p4=5.02*10^{-9}; p5=0.005
SAPS II, score	27.67±3.61	25.08±0.80	27.34±0.76	30.14±0.73; p2=1.37*10^{-3}; p3=5.11*10^{-3}	33.77±0.65; p1=1.49*10^{-3}; p2=2.34*10^{-11}; p3=1.34*10^{-13}; p4=1.59*10^{-6}	38.98±0.76; p1=1.27*10^{-5}; p2=4.22*10^{-19}; p3=6.33*10^{-24}; p4=2.31*10^{-16}; p5=2.67*10^{-6}
MODS, score	2.47±0.46	1.79±0.14	2.11±0.12	2.42±0.11; p2=6.34*10^{-3}	2.94±0.10; p2=1.27*10^{-8}; p3=5.81*10^{-8}; p4=2.25*10^{-4}	3.57±0.11; p1=0.007; p2=1.08*10^{-13}; p3=7.37*10^{-15}; p4=8.98*10^{-11}; p5=2.29*10^{-4}
Mortality	1 (6.67%)	8 (11.27%)	14 (8.43%)	32 (13.28%)	44 (17.39%)	70 (34.48%)

Note: p1, p2, p3, p4, p5 – reliability of differences in rates of 1-5 clusters, respectively.

Table 3:The Spearman correlation coefficients of cluster distance and severity of patients

Indicators	Cluster 1, n=15	Cluster 2, n=71	Cluster 3, n=166	Cluster 4, n=241	Cluster 5, n=253	Cluster 6, n=203
Apache II	0.50; p=0.02	0.48; p=2.16 *10^{-6}	0.32; p=9.99 *10^{-6}	0.26; p=3.84*10^{-5}	0.04; p=0.55	-0.03; p=0.66
SOFA	0.44; p=0.05	0.51; p=3.60 *10^{-7}	0.26; p=5.51 *10^{-4}	0.25; p=4.90*10^{-5}	0.13; p=0.04	-0.06; p=0.41
SAPS II	0.35; p=0.13	0.63; p=6.64 *10^{-11}	0.32; p=1.14 *10^{-5}	0.24; p=1.59*10^{-4}	-0.05; p=0.42	0.03; p=0.63
MODS	0.45; p=4.81 *10^{-2}	0.52; p=2.79 *10^{-7}	0.30; p=4.63 *10^{-5}	0.26; p=3.40*10^{-5}	0.09; p=0.14	-0.08; p=0.26

It is evident that these clinical conditions are accompanied by severe immunosuppression and lymphopenia can be regarded as a sign of MODS [12-14].

There was no difference between the values of parameters of severity scales in patients 1-4 clusters (Table 2). Most indicators of the patients' severity of fifth and sixth clusters is higher than values of 1-4 clusters. The exception is the Apache II scale, whose values are increased in patients in 3-6 clusters (Table 2). In the selected clusters, the probability fatal outcome varies from 6.67% to 34.48%. Despite the very high reliability of the differences ($F=11.67$; $p=5.85*10^{-11}$), predictive value of the separation patients into clusters is not large enough to predict the outcome: the power of influence of clustering $ç^2=0.06$ and a probabilistic forecast fatal outcome in the sixth cluster reaches only 34.48%.

Indicators of the patients severity in the first cluster, calculated using the Apache II and MODS scales, correlated positively with the value of DC (Table 3). Noticed negative correlation for DC and CD16+NEUT ($r_S=-0.55$; $p=0.03$), PNC ($r_S=-0.48$; $p=0.03$); positive correlation DC and abs CD4+ ($r_S=0.53$; $p=0.02$). Let us see how this trend persists in considering the principles of the organization of other clusters of immune status.

In patients in the second cluster marked positive correlations of the DC with the value of the of Apache II, SOFA, SAPS II and MODS scales (Table 3) and expression of molecules CD3 ($r_S=0.33$; $p=1.85*10^{-3}$),

CD4 (r_S=0.23; p=0.03), CD8 (r_S=0.30; p=5.51*10^{-3}), CD38 (r_S=0.27; p=1.89*10^{-3}), proportion abs CD38+/abs ÑD95+ (r_S=0.32; p=4.92*10^{-3}). Marked negative correlation values DC and the expression of CD25 molecules (r_S=-0.24; p=0.03), proportion abs CD25+/abs CD38+ (r_S=-0.32; p=5.23*10^{-3}), the concentration of IgA (r_S=-0.24; p=4.90*10^{-3}). Thus, in the second cluster of immune system on the periphery of the cluster severity of the patient higher, combined with higher levels of expression of CD-molecules by lymphocytes.

In patients in the 3rd cluster marked positive correlation DC with the rates severity on Apache II, SOFA, SAPS II, MODS scales (Table 3), with the rates on LII_{OS} (r_S=0.15; p=4.53*10^{-2}) and LII_{KH} (r_S=0.15; p=4.47*10^{2}). While DC positively associated with the expression level of activation molecules CD38 (r_S=0.16; p=0.04) and indicators of abs CD3+ (r_S=0.16; p=0.03), abs CD8+ (r_S=0.19; p=0.01). The results show an increase in the severity of the patients while increasing the functional load on the immune system by increasing the values of DC.

In patients in the 4th cluster observed a positive correlation of DC with the severity of Apache II, SOFA, SAPS II, MODS scales (Table 3). With increasing distance of patients indicators from the cluster center increased values of WBC (r_S=0.15; p=0.02), abs CD38+ (r_S=0.13; p=4.95*10^{-2}), PNC (r_S=0.13; p=4.76*10^{-2}); while expression of CD20 (r_S=-0.15; p=0.02), CD25 molecules (r_S=-0.19; p=2.30*10^{-3}) are reduced. Lack of links most of the cellular branch indicators and DC may be a manifestation of the lack of adequate response to the action of pathogenic factors. This confirms the rather high mortality rates in the 4 cluster - 13.28% (Table 2).

Patients in the 5th cluster noted a positive correlation between DC and the severity of the condition on SOFA scale (Table 3), ALC (r_S=0.18; p=3.40*10^{-3}). Conversely, the level of CD16+NEUT (r_S=-0.15; p=0.02) and the expression of CD95 molecules on lymphocytes (r_S=- 0.17; p=7.48*10^{-3}) are reduced to the periphery of the 5th cluster. Probably significant roles in the functional organization of the 5th cluster of immune status are the processes associated with antibody-dependent cellular cytotoxicity (CD16) and apoptosis (CD95).

Results of the study of structural organization of the 6th cluster showed that with the increase of DC reduced sympathetic influence: KI (r_S=-0.13; p=0.04), HV (r_S=-0.20; p=1.90*10^{-3}), WBC (r_S=-0.19; p=0.04) and indexes LII_{KK} (r_S=-0.54; p=3.31*10^{-25}), LII_{KH} (r_S=-0.69; p=0.00), LII_{OS} (r_S=-0.59; p=2.06*10^{-30}), increase in the proportion of lymphocytes CD3+ (r_S=0.44; p=9.19*10^{-16}), CD4+ (r_S=0.48; p=4.27*10^{-19}), CD8+ (r_S=0.44; p=2.90*10^{-16}), CD25+ (r_S=0.57; p=1.14*10^{-26}), CD95+ (r_S=0.45; p=8.42*10^{-16}); serum concentration of IgG (r_S=0.50; p=1.07*10^{-19}), IgA (r_S=0.53; p=1.42*10^{-22}), CIC (r_S=0.33; p=3.88*10^{-9}). Indicators abs CD4+ (r_S=-0.17; p=1.57*10^{-2}) è abs CD8+ (r_S=-0.17; p=1.32*10^{-2}), the expression of CD20 molecules (r_S=-0.12; p=0.04) è CD38 (r_S=-0.60; p=0.00), PNC (r_S=-0.13; p=0.02) negatively correlated with the DC. Taking into account these features, it should be noted that the immune system is closely related to the pathogenetic influences and severity of patients belonging to the 6th cluster. Mortality in patients of 6th cluster (34.48%) is highest (Table 2). This demonstrates the low effectiveness of this type of response. Perhaps the severity of the patients of the 5th and 6th clusters not only can be described as characteristics of indicators used by the traditional scales of severity, as changes in cellular processes and intercellular cooperation. This assumption is in agreement with a reduction in the number of immunocompetent cells in the range from 1st to 6th clusters (Table 1).

The data indicate a more severe condition of patients at the periphery of clusters and this is associated with the characteristics of the immune system that can be interpreted as a manifestation of a higher functional load. This confirms the hypothesis of a more rational organization of the immune system in patients whose exponents closest to the centers of clusters.

CONCLUSION

The leading factor in the formation of immune system cluster organization clinically associated with development of MODS, SIRS, sepsis, abdominal abscess, stress ulcers and perforation of hollow organs, septic pneumonia. The most significant relationship noted between PC-1 and indicators of cell branch of the immune system. Clusters of immune status differ in the number of immune cells and the patients' severity. The structure of the clusters is characterized by more severe condition of patients with increasing distance from the cluster center. Results of the study offer prospects in the development of new integrated methods for assessing the severity of the patient, prognosis and outcome of disease, choosing the most effective tactics of combined treatment.

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