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Self-Organising Processes in the Immune System: Integration, Disintegration and Cluster Forming

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Abstract: Favorable patient outcomes depend on the individual's ability to adapt to the damaging factors of disease and to accept a therapeutic effect. The immune systems of patients with urgent surgical pathology are affected by multiple damaging factors, which lead to the formation of separate clusters with centres at points corresponding to the optimal characteristics of the immune system. The distance from the centre of the cluster is closely related to the patient disease severity. We examined 442 patients with urgent abdominal pathology. Using the numbers of CD3+, CD4+, CD8+ and CD16+ lymphocytes, we found six clusters of immune status. Lymphopenia was found across the series of clusters, from the first to the sixth. Adaptive loadings were measured by determining in each cluster the share of significant Spearman correlation coefficients (SCS) and correlation graphs (G). Moreover, we calculated the distance between the principal components obtained by factor analysis (S_{PC}) to determine individual adaptive loading. SCS and G levels increased from the first to the fourth clusters. The SCS and G reached critical values in the fourth cluster, after which it was reduced in the fifth and sixth clusters. S_{PC} values highly correlated with SCS and G, but in opposite ways. This was coupled with increasing mortality in the fifth and sixth clusters. We believe that the observed features are the result of a "failure" in adaptation mechanisms and the emergence of distress in the fifth and sixth clusters. S_{PC} values can be used for individual evaluation of the adaptive loading.

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

INTRODUCTION

An important issue in clinical practice is the correct evaluation of the disease severity of the patient, the stability of the patient's condition and prognosis of disease outcome. Favorable patient outcomes depend on the individual's ability to adapt to damaging factors of disease and to accept a therapeutic effect. Integral severity scales allow the estimation of the severity of the patient's immediate condition and disease prognosis. However, the state and opportunities for adaptation is difficult to assess objectively because they cannot be directly measured. Therefore, despite the importance of adaptation, its assessment remains subjective in clinical practice. Furthermore, the concept of adaptation is quite abstract. Current rating adaptability due to the need for monitoring to prevent functional failure and subsequent decompensation of adaptation mechanisms [1-3].

The immune system in patients with urgent surgical pathology is affected by multiple damaging factors. Disorders of the immune status determine the patient wellbeing during the post- operative period, disease outcome and the recovery time [4, 5]. Improving treatments for these patients is not possible without improving their immune status. The development of methods to improve immune disorders requires a more detailed study of the pathogenic features of the immune system.

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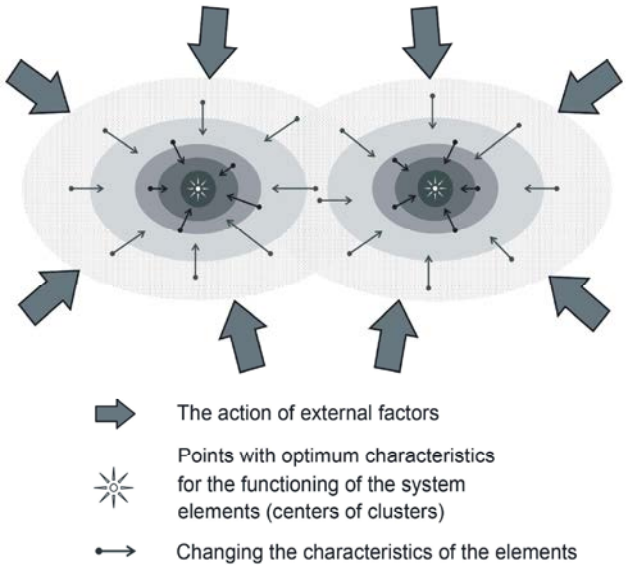

Fig. 1: A simplified scheme for the formation of clusters

As studies of the immune system involve several features, a simplified analysis of its functional organisation is not possible. A comprehensive study of all the diverse immune system relationships is theoretically impossible. As we cannot define the limiting elements of immune system interactions, these systems, exchanging information and energy with the environment, have been named "open systems" by modern science. Therefore, regardless of the completeness of the investigations, we must still consider the immune system as an open system. Modern methods of system analysis facilitate new approaches of understanding the organisation of the immune response, based on synergetics and its branch, chaos theory [6, 7].

Any condition of the organism is the sum of the external and internal factors' actions. The interactions within a system occur with the participation of external factors, which means we must consider all influences outside of our research. To compensate for these external influences, elements within the system seek to acquire optimal performance [8]. As a result of these external influences, areas with optimal characteristics become apparent after condensing the data [6, 7]. Figure 1 represents a cluster consisting of elements of the system with similar characteristics. The distance of indicators from the centre of a cluster (DC) is related to the characteristics of the elements [6, 7].

Mathematical methods allocating clusters and estimating the distance from the cluster centre for each cluster member exist (i.e., using patient data as clusters). However, there are currently no publications where the distances from the cluster centre are both used to determine the membership of a cluster and as a clinical indicator.

We attempted to apply this theory to assess the immune status in patients with urgent surgical pathology. A sufficient number of observations and the heterogeneity of the studied group of patients allowed us to successfully apply these mathematical methods and identify six clusters of immune status organisation. Each cluster was characterised by unique features. For example, system parameters, which are the most informative in characterising the condition of the patients in each cluster, are different and depend on the patient's disease severity [9].

We also describe the relationship between patient disease severity and the distance of their indicators from the centres of the clusters to which they belonged [10]. In patients in the first cluster, Apache II and MODS severity scales positively correlated with the value of the DC [10]. In patients in the second, third and fourth clusters, there was a marked positive relationship between the DC and the disease severity of the patients according to the Apache II, SOFA, SAPS II and MODS scales [10]. In patients in the fifth cluster, there was a marked positive correlation between the DC and the severity of their condition on the SOFA scale [10]. This led to the conclusion that the areas closest to the centres of clusters correlate with decreased severity of the patients' conditions [10].

It is evident that effects of adaptation mechanisms lead to the formation of immune system clusters. Therefore, investigations into the mechanisms of adaptation in each of the clusters are important. The clusters might differ in the features of the adaptive loadings on the immune systems of patients.

To assess the adaptive loadings in complex systems, methods based on determining the correlation between indicators in the groups of studied objects may be used. Common medical data sets show the following features: improved functioning of the system accompanied by increased correlations between indicators, deteriorations of the system accompanied by a decrease in correlations and disintegration of the system as a whole. The clinical outcome of this disintegration is the death of a patient [11, 12]. Unfortunately, these methods are only applicable to investigating group characteristics and cannot be used to evaluate the individual characteristics of adaptation. However, some reports [11, 12] have pointed to the existence of other features of data organisation that depend on adaptive loadings. Along with an increase in correlation, there is a decrease in the rarefaction of the data set [11, 12]. Thus, the use of characteristics of the distance between parameters of the immune status of a patient as an individual characteristic of adaptive loading seems promising.

Therefore, the aim of this study was to find the criteria of evaluating the adaptation loading for patients with urgent surgical pathologies.

MATERIALS AND METHODS

We examined 442 patients with pathologies of abdominal organs in need of urgent operations. There were 162 patients (36.6%) with perforated stomach ulcers and duodenal ulcers, 73 (16.5%) with injuries penetrating the abdominal organs, 45 (10.2%) with necrotising pancreatitis, 70 (15.8%) with acute adhesive intestinal obstruction and 31 (7.0%) with destructive forms of appendicitis. In 104 patients, including the above-mentioned, there was a combination of multiple acute inflammatory processes (23.5%).

Peritonitis and abdominal sepsis were observed in 292 patients (66.1%). However, changes in the ratios of white blood cells, corresponding to the systemic inflammatory response syndrome (SIRS), were noted at the time of the study in only 54 patients (12.2%). Hospital pneumonia developed in 11 patients (2.5%) and multiple organ dysfunction syndrome (MODS) in 59 (13.3%). In 61 cases (13.8%), disease ended in death and there were 381 (86.2%) patients who recovered. Patients were examined within 1-2, 5-7 and 10-12 days after their operation. The study included 949 survey results. Integral assessment of the patients' disease severity was carried out using Mannheim peritonitis index (MPI), Apache II, SAPS II, SOFA and MODS scales. In addition, to estimate the severity, we used the following scales [13]: surgical immune rate (SIR) and SIR predicted death rate (PDR SIR).

All patients were operated on within 24 hours of hospitalisation. Surgical treatment consisted of laparotomy, revision of the abdominal cavity, removal of the effects of trauma or injury and elimination of the source of infection. When surgeons were unable to eliminate the cross-sectional purulent process in the abdominal cavity, re-laparotomy was planned with an interval of approximately 48 hours. All patients received infusion, detoxification and antibiotic therapy in quantities appropriate for the severity of the condition.

We used monoclonal antibodies (analogues produced by Becton Dickinson adapted for use with fluorescence microscopy) to determine the levels of expression of lymphocytic molecules: CD3 (ICO-90), CD4 (ICO-86), CD8 (ICO-31), CD16 (ICO-116), CD20 (ICO-180), CD25 (ICO-105), CD38 (ICO-20) and CD95 (ICO-160). The expression of CD16 in neutrophils was also studied (CD16+n). Additionally, the absolute numbers of these cells (abs) were calculated.

We evaluated the phagocytic index with latex particles (PHI), mean number of phagocytosed latex particles (PHN) and calculated the numbers of phagocytosed neutrophils (PNC). The total concentrations of IgA, IgM and IgG antibodies in the sera were measured by enzyme immunoassay. The concentrations of circulating immune complexes (CIC) were measured using light absorbance at a wavelength of 315 nm after incubation of plasma with a solution of polyethylene glycol with a molecular weight of 6000 KDa. Taking into account the number of white blood cells (WBC) and the absolute count of lymphocytes (ALC), we additionally calculated the ratios of the cell populations: leukocyte indexes of intoxication, according to the methods of Ya.Ya. Kalf-Caliph (LII_{KC}), V.K. Ostrovsky (LII_{OS}) and S.F. Khimich that was modified by A.L. Kostyuchenko *et al*. (LII_{KH}) [14]. We also investigated the stress index according to the method of L.H. Harkavy *et al*. (SI) [15], using the following formulae:

$$LII_{KC} = \frac{(4*MYEL + 3*YGN + 2*SNN + SGN)*(PLC + 1)}{(EO + 1)*(LYM + MON)}$$

$$LII_{OS} = \frac{MYEL + NEUT + PC}{EO + LYM + MON};\ LII_{KH} = 0.1*WBC*\frac{NEUT}{100 - NEUT};\ SI = \frac{LYM}{SGN}.$$

The following designations were used: WBC - white blood cells (10^9/l); LYM - lymphocytes (%); NEUT - total neutrophils (%); SGN - segmented neutrophils (%); MON - monocytes (%); EO - eosinophils (%); MYEL - myelocytes (%); YGN - young neutrophils (%); SNN - stab nuclear neutrophils (%); and PLC - plasma cells (%).

The amount of stress reaction symptoms (SR), according to L.H. Harkavy *et al.* [15], was calculated as detailed below. Each sign was estimated by 1 point: (1) WBC count less than $4*10^9$/l or more than $8*10^9$/l; (2) percentage of monocytes less than 4% or more than 7%; (3) percentage of eosinophils less than 1% or more than 6%; (4) ratio of SNN/SGN less than 0.06 or more than 0.07; (5) detection of more than 1% of basophils in the blood; and (6) detection of more than 1% plasma cells in the blood [15]. We also investigated several indicators of autonomic regulation: the Kerdö index (KI) and the volume of heart blood flow per minute (HV) [16].

The serum levels of IL-1-RA, IL-4, TNF-α and interferon-γ (IF-γ) were all evaluated using "Vector-Best" test systems (Novosibirsk, Russia). Biochemical parameters were measured on the analyser “Hitachi-912", using adapted techniques. Measurement of the expression levels of catecholamine-receptor complexes (CA-R) and serotonin-receptor complexes (ST-R) on leukocyte membranes was accomplished using a modified luminescence-histochemical Falk-Hillarp method [17].

To select the most informative indicators for clustering, using methods of factor analysis, the standard procedure for selecting the principal component (PC) is by searching in multi-dimensional space the axes of factors describing the dispersion of the values of the investigated data. Factors were selected using the values of significance testing, as proposed by H.F. Kaiser [18], with eigenvalues λ>1.0. To improve the interpretability of the factors, we used the rotation VARIMAX, allowing us to receive more contrasting factor loadings [19]. We included the following indicators in the data array for factor analysis: WBC; the absolute number (abs) of CD3+, CD4+, CD8+, CD16+, CD16+n, CD20+, CD25+, CD38+ and CD95+ cells; the count of phagocytic neutrophils (PNC) and the concentrations of IgG, IgA and IgM; and the concentration of CIC. The optimal number of clusters was determined on the basis of calculating the values of Euclidean distances between the mean group values [20].

Correlation analysis of the relationships between the 15 indicators of immune status in the 442 patients with urgent surgical pathology allowed us to extract and rank “latent” factors (PCs 1 to 15) based on the degree of their impact on processes in the immune system [9, 10]. The immune systems of the patients studied depended significantly on the influence of four factors, which can be quantified by values of PC 1 to 4 λ>1.0. Thus, the first factor determined 44.85% of all possible states of the immune system of the studied patients, the second 17.32%, the third 8.34% and the fourth 7.47%. In total, these four factors determined the variation in the immune systems of patients at a level of 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 [19].

The criteria for clustering the immune status of the indicators used were those most closely associated with the values of the most influential PC, which was PC 1: abs CD3+, abs CD4+, abs CD8+ and abs CD16+ [9, 10].

Six clusters were identified in the data studied using a k-means algorithm. These clusters were variants of the combinations of the indicators of immune status and may characterise the options for the organisation of the immune system [9, 10] (Table 1, Fig. 2). The significance of the differences between the clusters for all the parameters was $p<0.001$ (Table 1).

It should be noted that the intervals of the immune status of the various clusters may overlap. For example, the minimum value of abs CD3 + in the first cluster (972.70 μl^{-1}) was less than the maximum value of abs CD3 + in the second cluster (1313.30 μl^{-1}). To determine whether the immune status of a patient belonged to a particular cluster, the values of the DC for each cluster were calculated; the indicators of patients belonged to the cluster with the lowest DC.

The clusters were allocated using the k-means algorithm. The clusters were considered objects with a shape close to spherical [21] in the 4-dimensional space of the variables abs CD3+, abs CD4+, abs CD8+ and abs CD16+.

Table 1: Characteristics of clusters of immune system function

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+, μl^{-1}	1332.81±64.5	889.7±17.42	630.75±6.86	428.83±4.11	273.18±3.52	127.82±3.70
abs CD4+, μl^{-1}	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+, μl^{-1}	752.8±38.19	537.06±14.78	384.17±5.5	264.93±3.14	164.31±2.41	75.52±2.40
abs CD16+, μl^{-1}	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 significance of the differences between the clusters for all the parameters was $p<0.001$

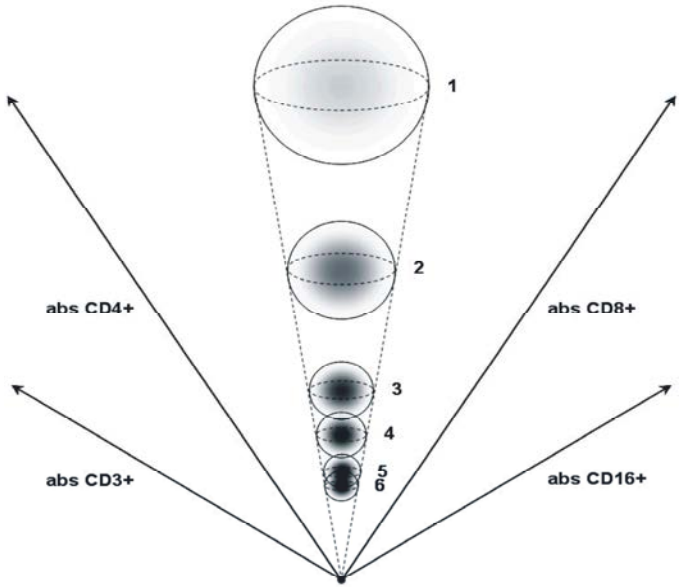

Fig. 2: The ratio of the number of observations and the data density in the clusters of immune system function. Notes: 1- the diameters of the spheres are proportional to the DC values in the clusters; 2 - the intensities of the shading of the spheres are proportional to the data density in the clusters.

The first cluster was the least numerous, had the highest values of the means, the highest dispersion values, the highest values of standard errors and average DC magnitudes. In contrast, the sixth cluster was numerous and had the lowest values of these parameters. Thus, the clusters varied in series from the most voluminous and rarefied first cluster to the densest, most compact and sufficiently numerous sixth cluster. These data are quite natural for the study - the majority of patients with urgent surgical pathologies displayed lymphopenia, while some had a sufficient number of immune cells. Since the clusters were allocated by the level of the indicators – abs CD3+, abs CD4+, abs CD8+ and abs CD16+ – the DC magnitude was also related to the number of lymphocytes. The clusters in the 4-dimensional space of the variables formed a cone in which the data were condensed from the base to the apex (Fig. 2).

A relatively important issue in mathematics is the question of whether clustering is "natural". Assuming that "naturally" separated clusters are not fused together, then under favourable conditions of observation, the researcher can easily classify objects without using mathematical data processing [21]. To check whether the clustering was "natural", the following mathematical similarity criteria were used: the Simple Matching coefficient, the Rogers and Tanimoto coefficient, the Jaccard coefficient, the Sokal and Sneath criterion, the Dice coefficient and the Simple Matching criterion [22]. According to the results, the probability of "artificial" clustering in our study ranged from 0.16 (Sokal and Sneath criterion) to 0.46 (Simple Matching coefficient and Simple Matching criterion). Thus, our clusters can be considered as natural structures rather than groups, artificially separated using mathematical algorithms [21]. These results also confirmed the validity of the theoretical assumptions required to search for and separate the clusters of immune system function.

The allocation of the clusters of immune system function presupposed different patient disease severities and different outcomes in each cluster. Most indicators of the patient's severity in the fifth and sixth clusters were higher than those in clusters 1 to 4 (Table 2).

Table 2: The patients' disease severity in the clusters of immune system function

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	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=1.02*10^{-3}; 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	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	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	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=7.19*10^{-3}; 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%); p3=0.01	70 (34.48%); p1=0.03; p2=1.91*10^{-4}; p3=2.91*10^{-9}; p4=1.21*10^{-7}; p5=2.80*10^{-5}

Notes:

1. The table shows significant differences, $p<0.05$.
2. p1-p5 – the significant differences between the performances of the clusters.

The values of the scales for Apache II and SAPS II consistently increased for patients in clusters 4 to 6 (Table 2). Mortality rates were comparable in clusters 1 to 5, with the exception of a high mortality rate in the fifth cluster compared to the third cluster. Mortality rate in the sixth cluster was significantly higher than that of patients in clusters 1 to 5 (Table 2).

To estimate the intensity and characteristics of adaptive loadings, we used three methods: calculating the shares of significant correlations, correlation graphs [23] and the rarefaction of the indicators of the investigated data. Calculations were carried out using a statistical programme package (Statistica for Windows 6.0). The main statistical parameters taken into account were arithmetic mean values (M) and standard errors (m). The difference between the rates within the groups was tested using the Mann-Whitney U-test. To assess the relationships between patient indicators, we used the Spearman coefficient of rank correlation (rS).

The 68 indicators used in the study were divided into 4 groups.

Immunity Indicators: CD3+, CD4+, CD8+, CD16+, CD16+n, CD20+, CD25+, CD38+, CD95+, ALC, abs CD3+, abs CD4+, abs CD8+, abs CD16+, abs CD16+n, abs CD20+, abs CD25+, abs CD38+, abs CD95+, PHI, PHN, PNC, IgA, IgM, IgG, CIC, IL-1-RA, IL-4, IL-8 and TNF-a; and ratios: abs CD4+ / abs CD8+, abs CD25+ / abs CD38+, abs CD25+ / abs CD95+, abs CD38+ / abs CD95+, abs CD3+ / WBC, CD25+ / IL-4, IL-1-RA / TNF and IL-4 / IL-8. This gives a total of 38 indicators.

Autonomic Regulation: SI, SR, KI, HV, CA-R and ST-R. This gives a total of 6 indicators.

Toxicity Indicators: WBC, Hb, erythrocyte sedimentation rate, LII_{KC}, LII_{OS}, LII_{KH}, total bilirubin, urea, creatinine, amylase, alanine-aminotransferase, aspartate-

aminotransferase, LDG, total protein, cholesterol, choline-esterase and intermediary molecules. This generates a total of 17 indicators.

Severity Score Indicators: MPI, Apache II, SAPS II, SOFA, MODS, SIR and PDR SIR. This produces a total of 7 indicators.

Values of rS were calculated for all possible pairs of indicators used in the study, using the principle of "all-to-all". The number of pairs of indices, for which the values of rS were calculated, was 2278. In the first cluster, rS values were calculated only for 2198 pairs of indicators. This was a consequence of insufficiency and a mosaic distribution of some data.

We estimated the number of pairs of indicators with valid values of rS ($p<0.05$). To assess the level of the relationships between the elements, the shares of significant correlations in the central and peripheral areas of the clusters were taken into account. We calculated the proportion of significant rS using the formula:

$$SCS_{p<0.05} = \frac{SCN_{p<0.05}}{TCN}, SCS_{p<0.001} = \frac{SCN_{p<0.001}}{TCN}.$$

The following designations were used: $SCS_{p<0.05}$ and $SCS_{p<0.001}$ - significant correlation shares of rS with prescribed levels of validity; $SCN_{p<0.05}$ and $SCN_{p<0.001}$ - significant correlation numbers with prescribed levels of validity; and TCN - total number of correlations.

The comparison of ratios was performed using chi-square calculations.

To assess the level of adaptive loading, we used the values of the correlation graphs - the sums of the Spearman correlation coefficients provided confidence levels at $p<0.05$. For the calculations, we used the formula [23]:

$$G = \sum | rS_{xi} |$$

The following designations were used: G - the correlation graph and rS_{xi} - the Spearman correlation coefficient ($p<0.05$) for a pair of indicators *x* and *i*.

To assess the intensity of the rarefaction of the data, the magnitudes of the standard deviations between the values of PC-1 to PC-4 (S_{PC}) were used. The relationships between the PC values can also be seen as indicators of the balance of the factors that determine the functional organisation of the patients immune systems. The four principal components formed the six pairs of indicators of distances between them. The standard deviation between the pairs of PCs was calculated by the formula:

$$S_{PC} = \sqrt{\frac{1}{6} \sum_{i=1,x=1}^{4} (PC_i - PC_x)^2}$$

The following designations were used: PC_i, PC_x - the principal components PC-1 to PC-4.

The critical significance level (p) for verification of statistical hypotheses was assumed to be 0.05. Values of $p<0.01$ were in the form of the mantissa and exponent. In the case of $p<1.0*10^{-29}$, which was not possible to measure in the statistical software used, we used $p=0.00$.

RESULTS AND DISCUSSION

In the central areas of the clusters, the shares of significant ($p<0.05$) correlations increased in the series from the first to the fourth cluster (Fig. 3, Table 3). The highest SCS values were recorded in the fourth cluster. In the central areas of clusters 5 and 6, the SCS values progressively decreased (Fig. 3, Table 3).

The results showed similar trends in the $SCS_{p<0.05}$ and $SCS_{p<0.001}$ indicators between the clusters. Levels of $SCS_{p<0.05}$ and $SCS_{p<0.001}$ indicators in the second cluster were significantly higher than those in the first cluster (Fig. 3, Table 3). The third cluster exhibited high values of $SCS_{p<0.05}$ and $SCS_{p<0.001}$ compared to the first and second clusters. The $SCS_{p<0.05}$ and $SCS_{p<0.001}$ values in the fourth cluster were higher than those in the first, second and third clusters (Fig. 3, Table 3). The $SCS_{p<0.05}$ and $SCS_{p<0.001}$ values in the fifth cluster were higher than those in the first and second clusters and were comparable to those in the third cluster, but were significantly lower than those in the fourth cluster (Fig. 3, Table 3). In the sixth cluster, the level of $SCS_{p<0.001}$ was higher than that in the second cluster, but the $SCS_{p<0.05}$ value was similar to that in the second cluster (Fig. 3, Table 3). Furthermore, the $SCS_{p<0.05}$ and $SCS_{p<0.001}$ values in the sixth cluster exceeded the values in the first cluster, but were lower than the SCS values in the third, fourth and fifth clusters (Fig. 3, Table 3).

The values of the correlation graphs varied in the clusters the same way as the indicators $SCS_{p<0.05}$ and $SCS_{p<0.001}$. In the series from the first to the fourth cluster, the values of the G indicators increased (Fig. 3), reaching the highest numbers in the fourth cluster. Furthermore, from the fourth to the sixth cluster, the G values decreased progressively (Fig. 3). The $SCS_{p<0.05}$, $SCS_{p<0.001}$ and G indicators characterised the adaptive loadings in the clusters in a similar manner.

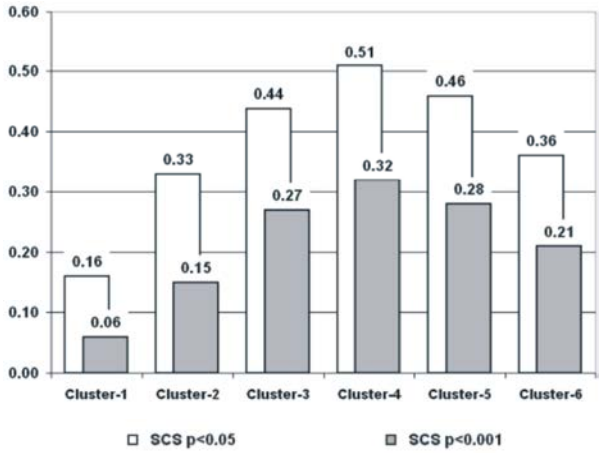

Fig. 3: The $SCS_{p<0.05}$ and $SCS_{p<0.001}$ values in the clusters of immune system function

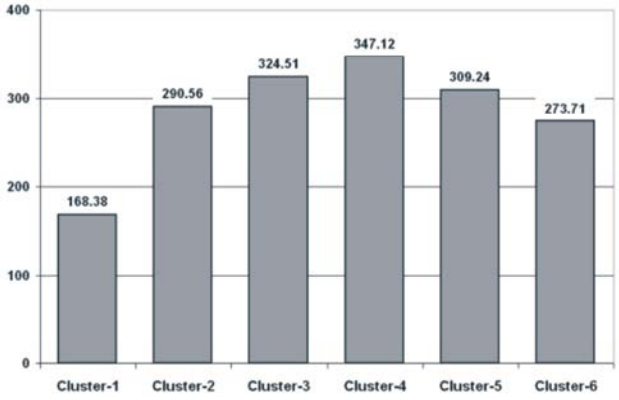

Fig. 4: The values of the correlation graphs in the clusters of immune system function

Table 3: The significance of differences between SCS in the clusters of immune system function

$SCS_{p<0.05}$		Cluster-2	Cluster-3	Cluster-4	Cluster-5	Cluster-6
x^2; p	cluster-1	184.17; 0.00	423.74; 0.00	606.49; 0.00	479.89; 0.00	230.93; 0.00
	cluster-2		55.02; $1.19*10^{-13}$	138.39; 0.00	78.06; $1.00*10^{-18}$	2.83; 0.09
	cluster-3			19.02; $1.29*10^{-5}$	1.96; 0.16	33.35; $7.68*10^{-9}$
	cluster-4				8.96; $2.75*10^{-3}$	102.75; $3.80*10^{-24}$
	cluster-5					51.8; $6.16*10^{-13}$
$SCS_{p<0.001}$		Cluster-2	Cluster-3	Cluster-4	Cluster-5	Cluster-6
x^2; p	cluster-1	101.81; $6.11*10^{-24}$	366.86; 0.00	483.15; 0.00	390.49; 0.00	211.28; 0.00
	cluster-2		98.9; $2.65*10^{-23}$	171.54; 0.00	112.82; $2.36*10^{-26}$	23.19; $1.47*10^{-6}$
	cluster-3			10.33; $1.31*10^{-3}$	0.44; 0.51	27.36; $1.69*10^{-7}$
	cluster-4				6.68; $9.73*10^{-3}$	71.35; $2.99*10^{-17}$
	cluster-5					35.05; $3.22*10^{-9}$

Note: In the case of $p<1.0*10^{-29}$, we used p=0.00.

Table 4: The S_{PC} values in the clusters of immune system function

Clusters	Cluster-1, n=16	Cluster-2, n=73	Cluster-3, n=182	Cluster-4, n=238	Cluster-5, n=211	Cluster-6, n=137
S_{PC}	6.50±0.40	3.23±0.10; p1=$2.19*10^{-9}$	2.05±0.05; p1=$3.46*10^{-11}$; p2=$7.30*10^{-23}$	1.36±0.05; p1=$2.46*10^{-11}$; p2=0.00; p3=$2.17*10^{-26}$	1.77±0.06; p1=$4.90*10^{-11}$; p2=$2.42*10^{-26}$; p3=$3.25*10^{-9}$; p4=$1.28*10^{-8}$	2.86±0.07; p1=$2.42*10^{-10}$; p2=$3.69*10^{-4}$; p3=$1.94*10^{-19}$; p4=0.00; p5=0.00

We also evaluated the possibility of assessing the adaptive characteristics of patients using the individual indicator of the patient`s data rarefaction in clusters - S_{PC}. There was a significant progressive decrease in the S_{PC} indicators from the first to the fourth cluster (Table 4). The fourth cluster demonstrated the lowest value of S_{PC} in relation to the other clusters. From the fourth to the sixth cluster, S_{PC} values progressively increased (Table 4). The values of S_{PC} in the fifth cluster were lower than those in the first, second and third clusters. In the sixth cluster, the values of S_{PC} were lower than those in the first and second clusters, but higher than that in the third cluster (Table 4).

Thus, there is evidence of close associations between the indicators of adaptive loadings in the clusters. The S_{PC} index describes the balance of factors that determine the functional organisation of the patients` immune systems and is negatively associated with the indicators of group characteristics of adaptation in the clusters. Moreover, it is an individual indicator of the adaptive loading.

The main criterion for assessing the validity of our conclusions should be the level of mortality and the disease severity of the patients, whose exponents belong to each cluster. Since the share of significant values of rS in the total number of correlations characterises the relationship between the elements of the functional systems of the body and, as a consequence, the level of functional loading on the adaptation mechanisms, we can draw the following conclusions.

Firstly, the features of the organisation of the immune system in clusters 1 to 3 can be characterised as optimal. In the fourth cluster, the functional loading on adaptation processes peaks, affecting the peripheral and central areas of the cluster. In the series of clusters from 1 to 4, the levels of mortality are comparable. In the fifth cluster, the functional loading is reduced and is lower in the central area of the cluster than in the periphery. The mortality level in the fifth cluster is similar to those in the first, second and fourth clusters; the only exception is the increase in mortality compared to the third cluster. In the sixth cluster, the functional loading continued to decrease and similar SCS values were observed in the central and peripheral areas. The mortality rate in the sixth cluster was the highest and was significantly higher than those in the first five clusters. We suggest that the revealed features are the result of “failure” of adaptation mechanisms and the emergence of distress in the fifth and sixth clusters. Consequently, the critical changes in the structure of the functional organisation of the immune system did not occur in the fifth cluster, which was characterised by a significant increase in mortality, but rather in the fourth cluster, which displayed large values of the following indices: abs CD3+, abs CD4+, abs CD8+ and abs CD16+ (Table 1).

The load level on the adaptation processes in cluster 4 was the highest, allowing for a favourable outcome in the patients studied. This is supported by two factual findings. First, the SCS in the fourth cluster was the highest among all the clusters in both the central and peripheral areas. Second, in the fourth cluster, functional loading “switches” from the central areas (in the second and third clusters) to the peripheral areas (fifth cluster) were prevalent.

In our case, the clusters of immune system function were allocated based on the results of factor analysis of the overall data set. According to the results of the study, clusters were characterised by different levels of adaptive loadings. These findings are consistent with the level of mortality in the clusters. Patient data from the fourth to the sixth cluster are in complete agreement with the conclusions reached by the authors of that research [11, 12]. We believe that our study surveyed a very wide range of adaptive loadings as seen by the increase, peak and then decrease in the adaptive loadings in our series of clusters. The results of this study allowed us to identify the other side of effects: increases in the intensity of adaptive loadings (clusters 1 to 3), the maximum and critical level of intensity of the adaptation processes (cluster 4) and further disintegration of the connections between the elements of the system (clusters 5 and 6).

It is important to note that the trend of extreme characteristics of the patients in the fourth cluster can be observed not only in the values of the $SCS_{p<0.05}$, $SCS_{p<0.001}$ and G indicators (that characterize significant share of relationships), but also in the S_{PC} indices, which characterise the relationship between the main components. Relationships between them were deeper and stronger than expected. Obviously, the effect of external factors on the immune system of patients, which can be traced to the relationship between the principal components, were hidden preconditions for the formation of interactions between elements of the body systems and determined its intensity. In a broad sense, factor analysis allows the exploration of a limited set of indices within an open system to obtain information about the entire state of the open system– outside the "horizon of research" [24]. Therefore, factor analysis of clinical data can be used as an important practical tool for the integrated assessment of patients. The present study confirmed that

the conclusions reached in the analysis of the immune status should be extended to the set of relationships that go beyond the practical capacity of the researcher.

To compensate for the negative effect of external factors, the immune systems of patients have to be integrated closely with other body systems. The existence of several optimum variants of integration produces clusters – the functional organisation stages of the immune system – related to the number of immune cells and severity of lymphopenia. Active processes of integration and disintegration continues as seen within the clusters. The clusters differ in the levels of functional loading on the interactions between the components of body systems. Since integration is to increase the intensity of the interaction between the elements of body systems, it is obvious that it requires energy. Depletion of functional reserves leads to the impossibility of maintaining a high level of integration between systems and the "failure" of adaptation mechanisms. Disintegration clinically manifests itself as increased mortality.

CONCLUSION

The application of modern statistical methods for data processing allows us to reveal new features of immune system organization. Using fundamental principles to evaluate adaptive processes enabled us to find signs of critical reconstructions in functional connections in a series of clusters. An important property of the S_{PC} indicator is the ability to assess individual adaptive loadings, involving a set of parameters of other functional systems. Factor analysis, cluster analysis and calculation of the relationship between principal components are important tools for assessing functional loading on the immune system and evaluating adaptation features for the development of methods to correct immune or adaptation disorders in patients with urgent surgical pathologies.

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