World Journal of Medical Sciences 8 (3): 250-262, 2013
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2013.8.3.1116

Clusters of Immune System Function in the Clinic: Theory or Unseen Reality?

[1]Poul V. Sarap, [2]Alexander A. Ostanin and [3]Yuri S. Vinnik

[1]Blood Transfusion Department, N.S. Karpovich Krasnoyarsk
City Clinical Emergency Hospital, Krasnoyarsk, Russia
[2]Laboratory of Cellular Immunotherapy,
Institute of Clinical Immunology RAMS SB, Novosibirsk, Russia
[3]Department of General Surgery, Professor V.F. Voino-Yasenetsky
Krasnoyarsk State Medical University, Krasnoyarsk, Russia

Abstract: The immune systems of patients with urgent surgical pathology are affected by multiple damaging factors. The action of these factors leads to the formation of separate clusters, with centres at points corresponding to optimal characteristics of the immune system. The distance from the centre of the cluster (DC) is closely related to the patient. Were examined 442 patients with urgent abdominal pathology. Using the numbers of CD3+, CD4+, CD8+ and CD16+ lymphocytes, we allocated six clusters of immune status. Depending on the value of DC in each cluster, four quartiles were identified. The results show similar features of relationships between indicators in peripheral and central areas of the clusters as evidence of general principles of organization in different clusters. The peripheral areas of the clusters are characterized by high values of indicators of toxicity and severity of the condition and the greatest values of intensity for immune system and autonomic regulation. Cluster membership and DC are important criteria for evaluating the severity of the patient's condition and to optimize treatment methods.

Key words: Immune system · Urgent surgery · Cluster analysis

INTRODUCTION

In clinical practice, evaluation of a patient's immune status is important for diagnostics, patient monitoring and selection of optimal drug therapy. However, the informative value of the immune status does not always meet expectations. Indicators and their trends are unique to each patient. It appears that the severity of the condition does not always correspond to the values of the parameters. A patient in a critical condition may display more advantageous indicator values than a patient in a less critical condition. The use of immunotropic agents with improved clinical presentation may be accompanied by changes in the values of parameters in an unfavourable direction. While our general ideas about the classical positive or negative changes in the immune system remain faithful, the doctor is concerned with individual patient treatment, guided by the standard statistics. However, in certain cases in clinical practice they are not always justified. Opportunities to study such regularities are hampered by the need to find a theoretical basis for infrequent, yet controversial, evidence. Such self-regulating systems with non-obvious patterns are known as non-intuitive [1, 2]. Some authors are obliged to note the presence of non-intuitive regularities of the immune system and study the principle of neural networks [3].

While improving diagnostic possibilities and the introduction of new laboratory indicators into clinical practice, is not methodologically impeccable, any of the indicators which conjugate with the immune system will behave the same way. We suggest that the most appropriate solution to the problem of interpretation of the results of research of immune status is an attempt to find substantiation of complex reactions of the immune system from the standpoint of adaptation and system analysis. The founder of synergetics, Hermann Haken, describes the challenges we face, *"Information overloads*

Corresponding Author: Sarap, Moscovskaya st., 5a-6, Krasnoyarsk, Krasnoyarsk region, 660069, Russia.

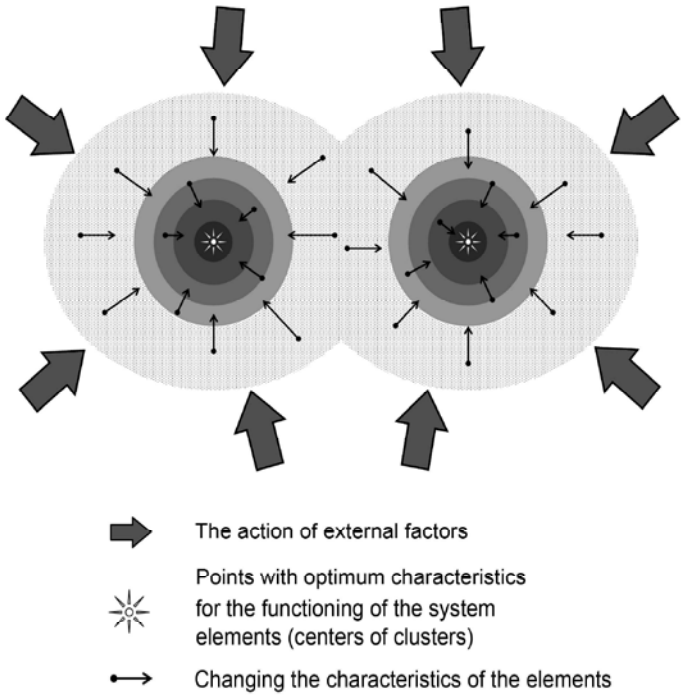

Fig. 1: A simplified scheme for the formation of clusters

lots of details which obscure the merits of the case, it is necessary to compress, making a small number of rules, concepts or ideas" [1].

What could be the benefits of such a treatment of the immune system? We believe that the results of such studies will help us to see the wide principles of the immune system and will relieve researchers and doctors from contradictions, revealing regularities in specific cases and exceptions to the rules. The application of new principles of assessment of the immune system could also improve and optimize diagnostics and treatment effects.

We chose patients with urgent surgical pathology as the object of our research. This group of patients is similar in severity of manifestations and is characterized by fast dynamics in laboratory parameters and standardized treatment interventions. However, this group of patients is heterogeneous and takes into account the set of clinical signs.

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

To understand the functional organization of the processes of the immune system, we must first attempt to evaluate clinical data impartially and discover new patterns without reference to the well-known views. The result should be a model of immune system organization which fully conforms to the classic concepts, but also explains exceptions.

As studies of the immune system involve several features, a simplified analysis of its functional organization is not possible. A comprehensive study of the full diversity of immune system relationships, with all of its elements together and their interactions with all relevant elements in other body systems is theoretically impossible. Since we cannot define the boundary elements of immune system interactions, such systems, which exchange 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 to understand the organization of the immune response, based on synergetics and its branch, Chaos Theory [6, 7].

The formation of interactions within the system occurs 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 [2]. 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 [8].

We have attempted to apply this theory to the assessment of 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 clusters of immune status organization. Each cluster was characterized by unique features. For example, system parameters, which are the most informative in characterizing the condition of patients in each cluster, are different and depend on the patient's severity [9].

We also described the relationship of patients and the severity of the distance of their indicators from the centres of their own clusters [10]. In patients in the first cluster, Apache II and MODS severity scales positively correlated with the value of DC [10]. In patients in the second, third and fourth clusters, there was a marked positive relationship between DC and the 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 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]. In this context, it becomes possible to further explore the differences in the indicators of patients in the central and peripheral areas of clusters using quartile methods of statistics.

Objective: to investigate the general similarities between the clusters, that are characterized by similar differences in performance in the central and peripheral areas of the clusters.

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 patients (16.5%) with penetrating injuries to abdominal organs, 45 (10.2%) with necrotizing 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 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 patients (13.3%). In 61 cases (13.8%) disease ended in death and there were 381 (86.2%) recovered patients. 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' severity was carried out using the dynamics of Apache II, SAPS II, SOFA and MODS scales.

All patients were operated on within 24 hours of hospitalization. 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 adequate to the severity of the condition.

We used monoclonal antibodies (analogs 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 on 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) and calculated numbers of phagocytized neutrophils (PHN). 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.

Taking into account the number of white blood cells (WBC) and the absolute count of lymphocytes (ALC), we additionally calculated the ratios of populations of cells: leukocyte indexes of intoxication, according to Ya.Ya. Kalf-Caliph (LII_{KC}), by V.K. Ostrovsky (LII_{OS}) and by S.F. Khimich as modified by A.L. Kostyuchenko *et al*. (LII_{KH}) [11]. We also investigated the stress index according L.H. Harkavy (SI) [12], using the following formulae:

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

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

$$SI = \frac{LYM}{SGN}.$$

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

The amount of stress reaction symptoms, by LH Harkavy [12], was calculated as detailed below. Each feature was evaluated in 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) the percentage of eosinophils less than 1% or more than 6%; 4) ratio SNN/SGN less than 0,06 or more than 0,07; 5) detection of more than 1% basophils in the blood; 6) detection of more than 1% plasma cells in the blood [12]. We also investigated several indicators of autonomic regulation: the Kerdö index (KI) and the volume of heart blood flow per minute (HV) [13].

Biochemical parameters were measured on a blood biochemical analyser “Hitachi-912", using adapted techniques. Measurement of the intensity of expression of catecholamine-receptor complexes (CA-R) and serotonin-receptor complexes (ST-R) on leukocytes membranes was accomplished using a modified luminescence-histochemical Falk-Hillarp method [14].

Not every indicator can be used to efficiently allocate clusters. Firstly, among the multitude of indicators we need to find those values that reflect the condition of the whole data set. There are indicators which are sensitive to changes in patient condition, whereas other indicators are more rigid. To separate clusters, it is necessary to estimate the degree of the relationship between indicators and factors that influence the organization of the immune system. It should be noted that the sensitivity of a certain indicator is the main criterion for selection.

Factor analysis allows us to identify external factors that categorize indicators as significant or insignificant, to determine the number of significant factors and quantify the total impact of each factor on the data array. Moreover, factor analysis provides a measure of the values in units of principal components (PC), which are indicators that characterize the influence of factors on the patient data organization at the time of examination. Therefore, the PC reveals alternative information about the patient's clinical characteristics, which is artificially increased due to the mathematical processing of an array of real laboratory parameters. In this study, we obtained the formula for calculating the values of the PC using the values of patient indicators. If a researcher needs to apply the method to a patient from another group, the values of the PC can be calculated. The degree of correlation between the values of the PC and the actual laboratory data allow selection of the most informative indicators. These indicators are systemic and can be used for clustering data arrays.

After selecting the most informative parameters, the procedure for allocation of clusters can be performed. Cluster analysis is essentially a classification procedure. The number of clusters can be set manually, or determined by taking into account the values of the Euclidean distances between the centres of the clusters allocated. We used the latter option, as we did not know prior to analysis how many clusters should be selected. Thus, this mathematical process produces a number of additional indicators, which are not obvious to the researcher : 1) the number of factors that determine the data organization (in our case, the immune system); 2) the significance of each factor for the organization of the data array; 3) values of the PC (complete set of values of each PC for each patient examination); 4) the number of clusters (patterns of functional organization of the immune system); 5) Euclidean distances between the centres of different clusters; 6) indicators belonging to the cluster as the individual characteristic of the patient; 7) DC as the individual characteristics of the patient, which is the most interesting indicator for research and perspective for clinical application.

To select the most informative indicators for clustering, using methods of factor analysis, the standard procedure for selection of PC is to search in multi-dimensional space for the axes of factors describing

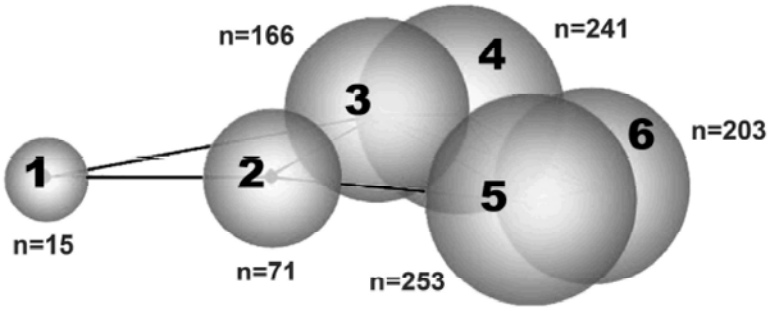

Fig. 2: The ratio of cluster sizes and distances between clusters

Notes:

1.Sizes of spheres are proportional to the number of examinations in clusters.

2.The distances between the spheres are proportional to the distances between the centres of clusters.

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

the dispersion of the values of the investigated data. Factors were selected using the values of significance testing, as proposed by H.F. Kaiser [15], with eigenvalues λ>1.0. To improve the interpretability of the factors, we used the method of rotation VARIMAX, allowing us to receive more contrasting factor loadings [16]. 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, IgM and CIC. The optimal number of clusters was determined on the basis of calculating the values of Euclidean distances between the mean group values [17].

Correlation analysis links to 15 indicators of immune status of the 442 patients with urgent surgical pathology, which allowed us to extract and rank "latent" factors (principal components, PC 1-15) based on the degree of their impact on processes in the immune system [10, 18]. The immune systems of the patients studied significantly depended on the influence of four factors, which can be quantified by values of PC 1-4 λ>1.0. Thus, the first factor determined 44.85% of all possible states of the immune system of the patients studied, the second 17.32%, the third 8.34% and the fourth 7.47%. In total, these four factors determined the variation of the immune system 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 [10, 18].

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 is PC 1: abs CD3+, abs CD4+, abs CD8+, abs CD16+ [10].

Six clusters were identified within the array of data studied using k-means algorithm. These clusters are variants of combinations of indicators of immune status and may characterize the options for organization of the immune system [10] (Table 1, Fig. 2). The significance of the differences between clusters for the values of all parameters 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 (972.7 μl^{-1}) is less than the maximum value of abs CD3 + in the second cluster (1313.3 μl^{-1}). To determine the membership of the immune status of the patient to a particular cluster, it is necessary to calculate the values of DC of each cluster; indicators of patients belong to the cluster which has the lowest DC.

A quite important issue in mathematics is the question of "naturality" of clustering. 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 [19]. In order to check the "naturality" of clustering, the following mathematical similarity criteria were used: Simple Matching coefficient, Rogers and Tanimoto coefficient, Jaccard coefficient, Sokal and Sneath criterion, Dice coefficient and Simple

Table 2: The patient's 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=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	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

Matching criterion [20]. According to the results, the probability of "artificiality" clustering in our study ranged from 0.16 (Sokal and Sneath criterion) to 0.46 (Simple Matching coefficient and Simple Matching criterion). Thus, clusters can be considered as natural structures rather than groups, artificially separated using mathematical algorithms [19]. These results also confirm the validity of the theoretical assumptions required to search for and separate the clusters of immune system function.

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

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

Patient indicators were divided into four quartiles, depending on their distance from the centre of the cluster. We compared DC indicators relating to the first quartile (lower, closest to the centre of the cluster) and fourth quartile (upper, relating to the periphery of the cluster) because they were the most different in magnitude.

We investigated the similarity of the cluster structure in accordance with the theoretical assumptions. As examples, we have shown evidence of a difference between the values of indicators between the central and peripheral areas of the clusters.

It is clear that the relationship between indicators may characterize immune system functions in the centre and the periphery of the clusters. In each quartile of all clusters we investigated the correlations between parameters. We considered similar features of

relationships between indicators in peripheral and central areas of the clusters as evidence of general principles of organization in different clusters.

We chose to represent a pair of clusters by the following features:

- Significant differences in the relationship between indicators in the central and peripheral areas in both of the compared clusters (significant difference in the correlation coefficients between the central area and the peripheral area of each cluster in the pair).
- Authentic indicators of similar organization in the central areas of both clusters (no differences between the correlation coefficients in the central areas of both clusters).
- Authentic indicators of similar organization in the peripheral areas of both clusters (no differences between the correlation coefficients in the peripheral areas of both clusters).
- At least two significant correlation coefficients of the four calculated (two indicators in each of the two compared clusters).

As examples, we present the characteristics of the organization of data in the central and peripheral areas in the third and fifth clusters.

Calculations were carried out using a statistical programs 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 rates within the groups was tested using the Mann-Whitney U-test. To assess the relationships between patient indicators and DC, we used the Spearman coefficient of rank correlation (rS). The comparison of proportions was performed using chi-square calculations.

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 is not possible to measure in the statistical software used, we used p=0.00.

RESULTS

Patients whose exponents fitted in the central area of the third cluster had lower rates of intoxication: WBC (Table 3), LII_{KC} (1.54±0.19 vs. 2.44±0.29; $p=8.33*10^{-3}$), LII_{KH} (2.53±0.69 vs. 3.54±0.52; $p=1.03*10^{-4}$), LII_{OS} and LDG (Table 3).

In the first quartile of the third cluster the levels of sympathetic indicators were lower (KI were: 11.18±1.12% vs. 15.59±1.39%; $p=3.58*10^{-3}$ and HV were: 3562.22±91.00 ml/min vs. 3920.32±106.31 ml/min; $p=1.03*10^{-3}$), as were

Table 3: Differences between quartiles in the third cluster of immune system function

Indicators	Quartile-1, n=58	Quartile-4, n=57	p-value
WBC, 10^9/l	8.72±0.56	11.51±0.79	$8.86*10^{-4}$
ALC, μl^{-1}	2076.10±27.22	2082.46±77.39	0.60
LII_{OS}	2.03±0.12	2.69±0.17	$4.18*10^{-4}$
CA-R,%	425.92±59.07	267.28±68.49	0.06
CD3+,%	30.52±0.40	33.14±1.52	0.38
CD4+,%	17.78±0.26	18.96±0.93	0.99
CD8+,%	18.33±0.28	20.70±1.17	0.42
CD16+,%	20.11±0.33	19.91±0.83	0.40
CD20+,%	18.57±0.30	17.60±0.45	0.03
CD25+,%	16.65±0.21	16.27±0.20	0.22
CD38+,%	15.85±0.20	16.85±0.40	0.02
CD95+,%	17.57±0.33	18.68±0.25	$3.93*10^{-3}$
abs CD16+n, μl^{-1}	1912.78±184.03	2434.17±166.14	$3.97*10^{-3}$
abs CD20+, μl^{-1}	383.83±6.32	357.02±13.04	0.07
abs CD25+, μl^{-1}	345.70±6.19	339.69±14.97	0.15
abs CD95+, μl^{-1}	364.44±8.51	391.29±17.93	0.66
PHI,%	49.81±1.32	47.00±1.38	0.04
PHN, μl^{-1}	2647.47±164.62	3435.43±227.56	$4.06*10^{-3}$
LDG, U/l	486.33±12.39	553.00±9.76	$7.44*10^{-5}$
Creatinine, umol/l	144.36±11.43	153.19±12.47	0.72
Apache II	6.47±0.36	8.95±0.52	$1.90*10^{-4}$
SOFA	1.41±0.17	2.50±0.20	$1.94*10^{-4}$
SAPS II	23.07±0.81	28.16±1.18	$2.09*10^{-3}$
MODS	1.36±0.16	2.46±0.19	$3.10*10^{-5}$

Table 4: Differences between quartiles in the fifth cluster of immune system function

Indicators	Quartile-1, n=80	Quartile-4, n=80	p-value
ALC, μl^{-1}	1083.19±22.27	1274.23±36.43	$3.69*10^{-5}$
LII_{OS}	4.15±0.17	4.22±0.29	0.25
CA-R,%	355.68±46.11	212.24±16.18	0.06
CD3+,%	25.68±0.49	24.35±0.94	0.06
CD4+,%	15.31±0.31	14.18±0.66	0.03
CD8+,%	15.26±0.33	14.38±0.53	0.08
CD16+,%	18.59±0.37	17.48±0.82	0.12
CD20+,%	20.21±0.33	18.84±0.46	0.04
CD25+,%	16.24±0.36	14.65±0.31	$2.91*10^{-3}$
CD38+,%	18.72±0.24	17.71±0.39	$4.89*10^{-2}$
CD95+,%	19.79±0.52	17.56±0.42	$8.75*10^{-4}$
abs CD3+, μl^{-1}	271.65±3.02	293.24±8.49	0.02
abs CD20+, μl^{-1}	217.89±5.27	235.48±9.13	0.11
abs CD25+, μl^{-1}	174.34±4.46	184.43±6.78	0.17
abs CD38+, μl^{-1}	202.32±4.94	225.24±8.27	0.02
abs CD95+, μl^{-1}	213.65±7.19	221.85±9.14	0.43
LDG, U/l	502.80±14.25	500.08±11.43	0.38
Creatinine, umol/l	116.61±8.65	157.16±13.47	0.05
SOFA	2.49±0.19	3.19±0.22	0.03
MODS	2.47±0.19	2.94±0.18	0.08

the levels of stress indicators (SI were: 0.46±0.02 vs. 0.34±0.02; $p=2.30*10^{-5}$ and SR were: 2.66±0.14 vs. 3.14±0.15; p=0.03).

Also, the central area of the third cluster had higher cholesterol levels (4.25±0.04 mmol/l vs. 3.97±0.08 mmol/l; $p=1.66*10^{-4}$) and higher levels of expression of several lymphocyte markers: CD20, CD38 and CD95. This is consistent with lower values on the Apache II, SOFA, SAPS II and MODS severity scales (Table 3). The intensity of immune system phagocytes was higher in the periphery of the third cluster, as shown by the values of PHI and PHN (Table 3). The abs CD16+n was also higher, indicating a more intense load on innate immunity.

At the periphery of the fifth cluster, the values of several key parameters were lower than in the central area: CD4+, CD20+, CD25+, CD38+ and CD95+. However, the quantitative indicators (abs CD3+, abs CD38+ and ALC) were higher in the periphery of the fifth cluster (Table 4). In the periphery of the fifth cluster there were also higher values of intoxication: creatinine (Table 4), MM (490.50±55.42 U vs. 600.13±41.72 U; $p=4.44*10^{-3}$) and indicators of the SOFA scale (Table 4). There were also reduced levels of total protein (59.93±0.98 g/l vs. 55.52±0.94 g/l; $p=1.08*10^{-3}$) and a higher intensity of cytolysis, as evidenced by indicators of alanine-aminotransferase (41.54±5.18 U/l vs. 56.07±5.50 U/l; p=0.04). Indicators of activity of sympathetic regulation were higher in the centre of the fifth cluster, as evidenced by the values of KI (22.28±0.92% vs. 16.44±2.25%; p=0.01) and HV (4707.78±68.90 ml/min vs. 4559.88±128.89 ml/min; $p=5.30*10^{-3}$).

Despite the fact that each cluster was different, we were able to identify the similarities of data organization within the various clusters. The theoretical assumption of the cluster organization of the immune system is supported by the combination of differences between clusters and simultaneously the similarities between the clusters. Many of the features of the relationship between the indicators were observed in several clusters. Some of the indicators (shown in Tables 3 and 4) were similar in the first and fourth quartiles. However, not only the absolute values, but the features of the relationship between the indicators determine the similarities and differences between the central and peripheral areas of the clusters.

In the centres of the third and fifth clusters the ALC indicators were negatively correlated with the values of CD16 + (Table 5); this rule was also valid for the fourth cluster. At the same time, the ALC indicators were positively correlated with the values of CD95 + at the peripheral areas of the third and fifth clusters (Table 5); this rule was also valid for the sixth cluster.

In the centre of the third cluster, levels of intoxication (estimated using the index LII_{OS}) were negatively associated with the values of CD3 +. The relationship between these parameters differed significantly at the peripheral areas of the clusters. The same relationships were found for the pair of indicators LII_{OS} and CD4 + (Table 5). Such features were typical for clusters 2-5.

Table 5: Similarities and differences in the relationships between the first and fourth quartiles

				p-value		
Indicators	Quartiles	Cluster-3	Cluster-5	1st vs. 1st, 4th vs. 4th quartiles	cluster-3 1st vs. 4th quartiles	cluster-5 1st vs. 4th quartiles
ALC & CD16+	1	rS=-0.70; p=1.23*10^{-9}	rS=-0.63; p=2.57*10^{-9}	0.47	9.58*10^{-10}	1.36*10^{-7}
	4	rS=0.38; p=4.49*10^{-3}	rS=0.18; p=0.14	0.22		
ALC & CD95+	1	rS=-0.15; p=0.26	rS=-0.07; p=0.54	0.67	0.01	0.03
	4	rS=0.33; p=0.02	rS=0.29; p=0.01	0.83		
CD3+ & LII_{OS}	1	rS=-0.36; p=5.78*10^{-3}	rS=-0.15; p=0.21	0.20	5.53*10^{-3}	9.62*10^{-4}
	4	rS=0.15; p=0.26	rS=0.38; p=5.93*10^{-4}	0.17		
CD3+ & CD16+	1	rS=0.53; p=1.96*10^{-5}	rS=0.55; p=3.65*10^{-7}	0.86	6.75*10^{-9}	6.95*10^{-11}
	4	rS=-0.53; p=2.88*10^{-5}	rS=-0.49; p=8.82*10^{-6}	0.76		
CD4+ & LII_{OS}	1	rS=-0.33; p=0.01	rS=-0.20; p=0.09	0.44	1.70*10^{-3}	2.18*10^{-3}
	4	rS=0.26; p=0.06	rS=0.29; p=9.05*10^{-3}	0.84		
CD4+ & CD16+	1	rS=0.61; p=3.68*10^{-7}	rS=0.60; p=2.54*10^{-8}	0.88	7.32*10^{-10}	1.34*10^{-9}
	4	rS=-0.51; p=7.57*10^{-5}	rS=-0.37; p=1.41*10^{-3}	0.32		
CD4+ & creatinine	1	rS=0.30; p=0.10	rS=0.42; p=4.10*10^{-3}	0.57	2.25*10^{-4}	3.13*10^{-4}
	4	rS=-0.53; p=1.70*10^{-4}	rS=-0.30; p=0.03	0.15		
CD8+ & CD16+	1	rS=0.47; p=2.11*10^{-4}	rS=0.41; p=3.51*10^{-4}	0.66	2.40*10^{-7}	2.84*10^{-6}
	4	rS=-0.48; p=1.79*10^{-4}	rS=-0.36; p=2.15*10^{-3}	0.38		
CD16+ & abs CD20+	1	rS=-0.31; p=0.02	rS=-0.43; p=1.79*10^{-4}	0.46	6.34*10^{-4}	5.61*10^{-6}
	4	rS=0.33; p=0.02	rS=0.31; p=6.57*10^{-3}	0.94		
CD16+ & abs CD25+	1	rS=-0.51; p=4.42*10^{-5}	rS=-0.36; p=1.63*10^{-3}	0.30	1.19*10^{-7}	5.15*10^{-5}
	4	rS=0.47; p=3.49*10^{-4}	rS=0.30; p=0.01	0.28		

Table 5: Continued

Indicators	Quartiles	Cluster-3	Cluster-5	p-value: 1st vs. 1st, 4th vs. 4th quartiles	p-value: cluster-3 1st vs. 4th quartiles	p-value: cluster-5 1st vs. 4th quartiles
CD16+ & abs CD95+	1	rS=-0.46; p=3.18*10^{-4}	rS=-0.46; p=4.89*10^{-5}	0.99	5.27*10^{-5}	2.03*10^{-3}
	4	rS=0.29; p=0.03	rS=0.03; p=0.83	0.13		
CD25+ & CD95+	1	rS=0.42; p=1.07*10^{-3}	rS=0.38; p=9.30*10^{-4}	0.79	0.03	5.64*10^{-3}
	4	rS=0.03; p=0.85	rS=-0.06; p=0.59	0.62		
CD25+ & CA-R	1	rS=0.30; p=0.04	rS=0.40; p=5.82*10^{-4}	0.52	0.03	0.02
	4	rS=-0.17; p=0.32	rS=0.03; p=0.81	0.34		
SOFA & LDG	1	rS=0.36; p=5.75*10^{-3}	rS=0.41; p=2.69*10^{-4}	0.72	0.02	0.03
	4	rS=-0.09; p=0.51	rS=0.08; p=0.48	0.33		
MODS & LDG	1	rS=0.33; p=0.01	rS=0.44; p=8.69*10^{-5}	0.46	0.03	0.03
	4	rS=-0.08; p=0.56	rS=0.12; p=0.31	0.27		

Notes. The table used the following abbreviations:

1. rS - Spearman correlation coefficients.
2. p - the reliability values of rS.

Relationships between CD16 + and the values of the other parameters were found, depending on whether they belonged to central or peripheral areas of clusters. For indicators CD3+, CD4+ and CD8+, marked positive relationships with the values of CD16+ at the central areas of the third and fifth clusters and negative relationships at the periphery of the clusters were found (Table 5). For the pair of indices CD3+ and CD16+, these features were valid in clusters 2, 3 and 5 and for the pair CD3+ and CD16+ these features were valid in clusters 2-5. Contradictory regularities were found for pairs of indices – CD16+ & abs CD20+ (valid in clusters 3-5), CD16+ & abs CD25+ (valid in clusters three to six), CD16+ & abs CD95+ (valid in clusters three to five); at the central areas of clusters correlation coefficients were negative, but at the periphery of the clusters a positive correlation was revealed (Table 5).

At the central areas of the third and fifth clusters, the values of CD25+ positively correlated with CD95+ and CA-R indicators. At the peripheral areas of clusters this dependence was not observed (Table 5). The conditions of similarities and differences in the organization of clusters complied with the values of severity scales. In the central areas of the third and fifth clusters, the severity of the patients, estimated using the SOFA and MODS scales, correlated with the activity of LDG (Table 5).

DISCUSSION

The results of this study highlight the differences between patients belonging to the centres and the peripheries of the clusters of immune status functional organization. The peripheral areas of the clusters are characterized by high values of indicators of intoxication and high values on the severity scales. These indicators show greater intensity of the immune system and autonomic regulation in the peripheral areas of the clusters.

As the numbers of CD3+, CD4+, CD8+ and CD16+ lymphocytes changed, the severity of the patient's condition changed in a stepwise fashion. Clusters of immune status are a manifestation of the organizing

stages of the immune system. Each cluster has a central area, which can be characterized as a set of optimal characteristics of the immune system in response to the adverse action of pathogenic factors. Indicators of patients in relatively favourable conditions belong in the central areas of the clusters. The organization of the data in the central areas of different clusters has common features. On the contrary, the condition of patients whose indices belong in the peripheral areas of clusters cannot be described as optimal. In the peripheral areas of different clusters, similar properties were also found.

Perhaps in patients in the fifth and sixth clusters, the severity of the condition is determined not so much by the performance indicators used in the traditional scales of severity, as by changes in cellular processes and intercellular cooperation, thereby reducing the reliability of correlations between DC and the severity performance scales. A small number of distinct indices showed disruption of the functioning of the immune system, not only at the periphery but also in the central areas of the fifth and sixth clusters. This hypothesis was confirmed by the decline in the numbers of immune cells in the series between the first and sixth clusters.

In the centres of the clusters, the ALC and natural immunity indicator (CD16 +) were negatively associated. The number of lymphocytes at the periphery of clusters increased due to pre-apoptotic CD95+ cells. In the centres of the clusters, we also observed a typical pattern of negative relationships between toxicity and the expression of CD3 + molecules. As we did not evaluate the double CD-markers, we cannot exclude the possibility that the positive relationship between LII_{OS} and CD3 + at the periphery of the clusters could be due to the joint expression of receptors on CD3+CD95+ cells.

At the periphery of the clusters, we observed a negative correlation between indices of adaptive and natural immunity. This is likely due to a lack of resources, or the action of processes associated with stress. At the periphery of clusters, we also observed processes associated with simultaneous load on the immune system associated with the CD16+ cells, the synthesis of immunoglobulins (CD20+ cells), the pro-inflammatory influences of IL-2 (CD25 + cells) and the number of pre-apoptotic (abs CD95+ indicator) cells.

With an increase in toxicity at the peripheral areas of clusters, co-operation of humoral immune system and CD16+ cells showed a higher load on the immune system. Expression of IL-2 receptors (CD25 + cells) is directly related to the number of pre-apoptotic cells and leukocyte sensitivity to catecholamines. We can assume that in the central areas of the third and fifth clusters, the expression of these molecules on the cells' membranes occurs simultaneously. This can be explained by the fact that the functional load on the immune system is lower than in the periphery. Therefore, in the central regions of clusters immune cells have the capacity to synthesize opposing signalling molecules without the consumption of cells in the immune response. At the periphery of clusters this relationship disappears.

In the central regions of the third and fifth clusters, the severity of the patients is associated with the activity of LDG. Because the LDG activity in the cells of the immune system is high, it is likely that the severity of the patients increases at the central areas of clusters not only by glycolysis, but also due to cytolysis and intoxication. At the periphery of clusters, disorders may be associated not only with the mechanisms of acute intoxication, but also with the increase of multiorgan dysfunction. This is confirmed by values of Apache II, SOFA, SAPS II, MODS in the third cluster (Table 3) and values of SOFA and MODS in the fifth cluster (Table 4).

The most significant relationships were observed in the centres of clusters. At the periphery of clusters, these interactions were lost or changed their direction. Obviously, the mechanisms of innate immunity and antibody-dependent cellular cytotoxicity (CD16+ indicators) are involved to a lesser degree with the pathogenesis of urgent surgical pathologies. This is perhaps the reason that the relationship of CD16+ with other indexes is used as an indicator of differences in the interactions in the centres and the peripheries of the clusters.

In the centres of the clusters, we observed the simultaneous expression of many different receptors (including those associated with the various branches of the immune response) on cell surfaces, which is clearly a sign of more opportunities to adapt. In the peripheral areas of the clusters, a lack of resources and multiple organ failure have a great influence on the immune system.

A necessary condition for recovery is to reduce toxicity and repair damaged systems. Recovery of the immune system is accompanied by an increase in the number of lymphocyte populations and as a result the change of the cluster membership of a patient's indicators. In addition, the need to "move" the patient's

indicators to the area with better characteristics within the cluster is necessary for recovery. The existence of a number of clusters, in which the mortality rate increases from cluster one to cluster six, combined with the dependence of the patient's condition on the magnitude of the DC, could be the reason for apparent discrepancies between the immune status and clinical condition of the patient.

Medical research demonstrates that a fundamental property of living matter is the desire for self-regulation. To identify patterns in self-regulation, a large array of data and heterogeneity of the objects of study is necessary. We are conscious that a change in the number of objects and their properties, including those associated with features of disease and treatments and the number and names of system indicators, as well as the number of clusters, may result in a drift of the coordinates of the centres of clusters and cluster boundaries. However, the principles of data organization will remain unchanged. It is important to note that the methodology we have used to consider medical data does not contradict the traditional approach to the formation of groups based on nosological criteria.

CONCLUSIONS

Clinical immunology is a complex field of research and studies in this area primarily address the clinical contradictions and aim to solve the practical problems of this type of research. Modern studies of the immune system require the application of methods of mathematical processing. Clusters of functional organization of the immune system can be identified by considering the complex self-regulating systems within the immune system. Affiliation with a cluster and DC are new informative characteristics of the patient. Perspective areas of the application of knowledge about these features of the patients, depending on their distance from the centres of clusters, can provide an estimate of severity of the patient's condition and assist with the selection and optimization of drug therapy.

It is clear that at present our proposed approach is more important than the study of observed private characteristics. In the future, medical technology and changes in patients' lifestyles will also affect factors influencing patients' immune systems. This will entail changes in the number and composition of self-regulating system indicators, which will lead to a drift of the centres of the clusters. However, the principles of the functional organization of the immune system, as well as other functional self-regulating systems, will remain the same.

We believe that new computer technology, to recognize patterns and improve computer performance, will in the future be used to classify indicators of patients and thus facilitate informed decisions based on a set of characteristics that are difficult to understand and are deeply concealed in the group characteristics of patients' indicators.

ACKNOWLEDGEMENTS

Special thanks to Academic Proofreading Services (www.proof-reading-services.org) for proof reading this article.

Abbreviations:

Abs	:	The absolute number of specified cells
ALC	:	The absolute number of lymphocytes
CA-R	:	The intensity of expression of catecholamine-receptor complexes on leukocytes membranes
CD16+n	:	The expression of CD16 molecules on neutrophils
CIC	:	The concentration of circulating immune complexes
DC	:	The distance of indicators from the centre of the cluster
HV	:	The volume of heart blood flow per minute
KI	:	The Kerdö index
LII_{KC}	:	The leukocyte index of intoxication by Ya.Ya. Kalf-Caliph
LII_{KH}	:	The leukocyte index of intoxication by S.F. Khimich
LII_{OS}	:	The leukocyte index of intoxication by V.K. Ostrovsky
MM	:	The level of middle molecules
PC	:	Principal components
PHI	:	The phagocytic index
PHN	:	The number of phagocytized neutrophils
PNC	:	The absolute number of phagocytic neutrophils
SI	:	The stress index by L.H. Harkavy
SR	:	The result of calculation of features of stress response by L.H. Harkavy
ST-R	:	The intensity of expression of serotonin-receptor complexes on leukocytes membranes.

REFERENCES

1. Bezruchko, B.P., A.A. Koronovskii, C.I. Trubetskov and A.E. Hramov, 2010. Way to Synergetics: Guided of ten Lectures (2nd ed.). Moscow: Librocom, pp: 304.
2. Ryll, A., R. Samaga, F. Schaper, L.G. Alexopoulos and S. Klamt, 2011. Large-scale Network Models of IL-1 and IL-6 Signalling and their Hepatocellular Specification. Molecular Biosystems, 7(12): 3253-3270.
3. Prigogine, I. and I. Stengers, 1986. Order from Chaos: a New Dialogue with Nature. Moscow: Progress, pp: 432.
4. Bantel, H. and K. Schulze-Osthoff, 2009. Cell Death in Sepsis: a Matter of How, When and Where. Critical Care, 13(4): 173.
5. Oberholzer, A., S.M. Souza and S.K. Tschoeke, 2005. Plasma Cytokine Measurements Augment Prognostic Scores as Indicators of Outcome in Patients with Severe Sepsis. Shock, 6: 488-493.
6. Freeman, W.J., R. Kozma and P.J. Werbos, 2001. Biocomplexity: Adaptive Behavior in Complex Stochastic Dynamical Systems. Biosystems, 2: 109-123.
7. Manian, F.A., 1997. Modern Medicine and Chaos Theory. JAMA, 279(11): 1399-1400.
8. Zhang, X., M. Fu, J. Xiao and G. Hu, 2006. Self-Organization of Chaos Synchronization and Pattern Formation in Coupled Chaotic Oscillators. Physical Review R. Statistical Nonlinear and Soft Matter Physics, 7: 1520-1522.
9. Sarap, P.V., Y.S. Vinnik and A.A. Ostanin, 2010. Investigation of the Factors in the Phasic Organizing of Immune Response in Patients with Urgent Surgical Pathology. Cuban Medical Research Journal, 7: 127-132. In Russian.
10. Sarap, P.V., A.A. Ostanin and Y.S. Vinnik, 2012. Structural and Clinical Features of Immune System Clusters in Patients with Urgent Surgical Pathology. World Applied Sciences Journal, 16: 207-212.
11. Kostyuchenko, A.L., A.N. Belskych and A.N. Tulupov, 2000. Intensive Care of Postoperative Wound Infections and Sepsis. St. Petersburg: Foliant, pp: 448.
12. Harkavy, L.H., E.B. Kvakina and M.A. Ukolova, 1990. Adaptive Response and Resistance of the Organism. Rostov-on-Don: Phoenix, pp: 100.
13. Vein, A., T.G. Voznesenskaya and L.A. Golubev, 1991. Diseases of the Autonomic Nervous System. Ed. A.M. Vein. Moscow: Medicine, pp: 624.
14. Sarap, P.V., Y.S. Vinnik, N.S. Minoranskaya, E.A. Chikhachev and E.V. Chizhikova, 2009. The Method for Quantitative Evaluation of the Expression Level of Catecholamine- and Serotonin-receptor Complexes on the Surfaces of White Blood Cells. Russian Federation patent RU 2377565. 2009 Dec 27.
15. Kaiser, H.F., 1960. The Application of Electronic Computers to Factor Analysis. Educational and Psychological Measurement, 20: 141-151.
16. Kaiser, H.F., 1958. The Varimax Criterion for Analytic Rotation in Factor Analysis. Psychometrika, 23: 187-200.
17. Leonchik, E.J. and O.V. Savastru, 2007. Cluster Analysis. Terminology, Methods and Objectives. Odessa: Odessa National I.I. Metchnikoff University, pp: 208.
18. Sarap, P.V., Y.S. Vinnik and A.A. Ostanin, 2010. The Factorial Analysis of Indicators of the Immune Status in Patients with Urgent Surgical Pathology. Siberian Medical Journal (Irkutsk), 8: 30-33. In Russian.
19. Orlov, A.I., 2002. Econometrics. Moscow: Examen, pp: 576.
20. Xu, R. and D.C. Wunsch 2nd, 2010. Clustering Algorithms in Biomedical Research: a Review. Reviews in Biomedical Engineering, 3: 120-154.