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Multivariate analysis of cellular immunity state in rhesus-sensitized women

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Abstract

Implementation of anti -D prophylaxis significantly reduced the degree of allogenic immunization (AI), while this method of prevention is not universal. According to the data of different authors, about 0.3% of Rh (D) - negative women still become Rh (D) AI. We failed to find data as to the state of cellular immunity (CI) in rhesus-sensitized (Rh-S) pregnancy, as well as the possibility of its indicators use for predicting and diagnosing the pregnancy course in the group of women under study. **The objective**: to evaluate the state of CI and identify its most diagnostic important components in the cohort of rhesus-sensitized women. **Materials and methods.** The state of CI (population and subpopulation of lymphocytes) was studied in 82 pregnant women, 37 had Rh-S with different titers of antibodies, control group comprised 45 healthy women without rhesus sensitization. **Results:** The most significant indicators of Rh-S women's CI were distributed among the three main factorial components. Multivariate analysis method was used. Activated subpopulations of T-lymphocytes and T-killers comprised Factor 1. Factor 2 is represented by indices of the lymphocyte population - B-lymphocytes (CD19 +) with a direct correlation of 0.88, as well as T-lymphocytes (CD3 +) with an inverse - 0.92. Factor 3 included the main subpopulations of lymphocytes (T-helpers and T- suppressors), as well as the immunoregulatory index (the coefficient of their ratio).

Conclusion. The most significant indicators for CI study in Rh-S women are: CD19 + (B-lymphocytes), CD3 + (T-lymphocytes), CD3 + HLA-DR + (activated T - lymphocytes), CD3 + CD4 + HLA-DR + (activated T -helpers), CD3 + CD8 + HLA-DR + (activated T-suppressors), NK-cells CD3-CD16 / 56 + (T-killers) and immunoregulatory index CD3 + CD4 + / CD3 + CD8 +.

Key words: rhesus-sensitization, cellular immunity, multivariate analysis.

Introduction. Mother's immune system undergoes complex restructuring during pregnancy which is to ensure fetal development, taking into account its properties as an allograft, and at the same time to preserve adaptive immune mechanisms of protection against pathogenic microflora [1, 2, 3]. Violation of the immunological mechanisms accompanying the development of pregnancy leads to the development of a significant number of complications [3 – 5]. A critical situation arises when the immune system of the Rh (D) -negative pregnant woman meets Rh-alloimmunization, that is, when the maternal immune system is sensitized to the surface antigens of the fetal erythrocyte D (Rh). The risk of alloimmunization depends on several factors, including the degree of fetomaternal transfusion and the maternal immune response [5]. The introduction of anti-D-prophylaxis significantly reduced the degree of alloimmunization but this method of prevention is not universal. According to the data of various authors, about 0.3% of Rh (D) -negative women still become Rh (D) sensitized [5]. Rh (D) -positive fetuses and newborn of Rh (D) -negative mothers are at risk of hemolytic diseases of the fetus and newborn development, which may be associated with a serious perinatal morbidity or mortality [3 – 5].

The need to improve methods for preventing complications of immunoconflict pregnancy determines the relevance of studies of immune responses of pregnant women and the formation of pre- and postnatal diagnostic and prognostic criteria of the pathological process in alloimmunized pregnant women.

We failed to find data as to the state of cellular immunity (CI) in rhesus-sensitized (Rh-S) pregnancy, as well as the possibility of its indicators use for predicting and diagnosing the pregnancy course in the group of women under study and advisability of carrying out pregnancy immunocorrective therapy.

To assess the nature of the pathological process that occurs in the immune system during pregnancy, tests that characterize the functional activity of blood cellular elements are essential. Basically these are methods that study populations and subpopulations of lymphocytes.

The objective of the work presented: to evaluate the state of CI and identify its most diagnostic important components in the cohort of Rh-sensitized women.

Materials and methods. The state of CI (population and subpopulation of lymphocytes) was studied in 82 pregnant women having been under observation and later delivered in maternity clinic N 7 (Odessa) specialized in management of immunoincompatible pregnancy. 37 women had Rh - S with different titers of antibodies. Control group comprised 45 healthy women without Rh - S.

Table 1

Indexes of cellular immunity in the different groups of women under study, %

index	CD3+	CD3+ CD16/56+	CD3+HLA-DR+	CD3+ CD4+	CD3+CD4+ HLA-DR+	CD3+ CD8+	CD3+CD8+ HLA-DR+	CD3+CD4+/ CD3+CD8+	CD3+CD4+ CD8+	CD3+ CD4-CD8-	CD19+	CD3-CD16/56
Rh-	56,81± 1,69*	4,08± 0,41*	2,83± 0,32*	59,59± 1,39*	4,59± 0,46	16,48± 1,34*	4,91± 0,64*	2,57± 0,11*	1,35± 0,09	3,09± 0,3	18,29± 0,8*	9,74± 1,1
Control	77,93± 1,62	5,85± 0,55	4,28± 0,42	49,55± 1,31	3,47± 0,38	25,88± 1,5	7,18± 0,85	2,21± 0,13	1,28± 0,09	3,06± 0,2	10,07± 0,5	9,85± 1,0

* *Statistical significance of differences with the indexes of control group p<0.05*

Table 2

Indexes of cellular immunity in the different groups of women under study

(absolute data)

Index	CD3+	CD3+CD4+	CD3+CD8+	CD19+	CD3-CD16/56+
Rh-	1,12±0,07*	1,55±0,08*	0,38±0,04*	0,68±0,05*	0,27±0,06
Control	1,76±0,12	1,11±0,09	0,56±0,05	0,31±0,03	0,3±0,06

* *Statistical significance of differences with the indexes of control group p<0,05*

Results and their discussion. The results of cellular immunity study in the groups under examination are presented in Tables 1,2.

In order to classify and reduce the dimensions of lymphocyte population numbers in absolute and relative figures, a multivariate analysis of cellular immunity of Rh -S women was carried out.

To conduct multivariate correlation analysis, the distribution of the studied population and lymphocyte subpopulations was determined (Fig. 1).

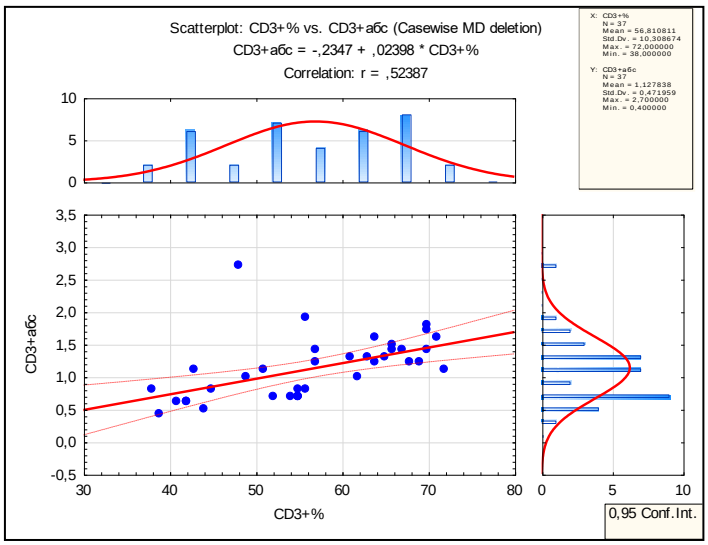

Fig. 1. Scattergram of test for homogeneity of CD+3 cells distribution

When tested all cellular immunity indexes in the women under examination showed relative homogeneity with single "expulsions" (EJECTIONS???).

At the first stage of multivariate correlation analysis the number of external load factors is determined by the principal components method (Table 3). The distribution of aggregate dispersion factors by the principal component method in graphical expression (the "scree" method) is shown in Fig. 2.

Table 3

Determination of the number of factors for multivariate correlation analysis by principal component method

Total Variance Explained						
component	Initial personal data			Rotation load sum of squares		
	Grand total	% dispersion	total %	Total	% dispersion	total %
1	4,163	37,842	37,842	2,741	24,920	24,920
2	1,726	15,693	53,535	2,584	23,493	48,413
3	1,685	15,317	68,852	2,248	20,439	68,852
4	1,239	11,265	80,117			
5	1,028	9,349	89,466			
6	0,576	5,236	94,702			
7	0,266	2,418	97,120			
8	0,158	1,439	98,560			
9	0,086	0,778	99,338			
10	0,050	0,451	99,789			
11	0,023	0,211	100,000			

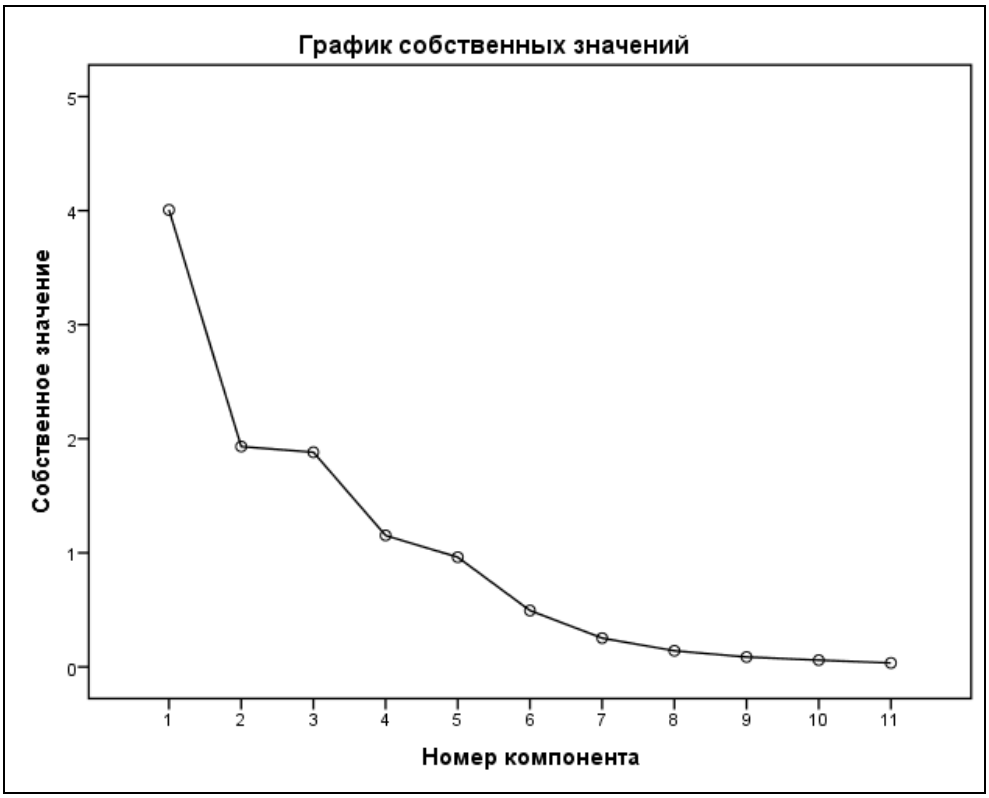

Fig. 2. Graph of total variance proper values (“scree” method)

Based on the results of principal component method, we concluded that it is most expedient to use three factors for the external load in which 68.85% of the load power is selected. The beginning of multivariative correlation analysis in the form of a three-factor load matrix with the use of Varimax raw rotation is presented in Table 4 and Fig. 3.

Table 4

Matrix of factor loads after the rotation by the Varimax raw method of cellular immunity relative indices in women with Rh-conflict

Index	Factor 1	Factor 2	Factor 3
CD3+%	0,307363	0,852303	0,203921
CD3+CD16/56+%	0,061274	0,567778	0,027439
CD3+HLA-DR+%	0,837919	0,218223	0,33936
CD3+CD4+%	0,082246	-0,01761	-0,94404
CD3+CD4+HLA-DR+%	0,763826	-0,40288	0,259719
CD3+CD8+%	0,340533	0,428734	0,732425
CD3+CD8+HLA-DR+%	0,836179	0,220482	0,221323
CD3+CD4+/CD3+CD8+	-0,40271	-0,1162	-0,82817
CD3+CD4+CD8+%	-0,2005	0,005647	-0,22791
CD3+CD4-CD8-%	0,680369	0,365552	0,011241
CD19+%	-0,04477	-0,82268	-0,13725
CD3-CD16/56+%	-0,8236	-0,2167	0,148163

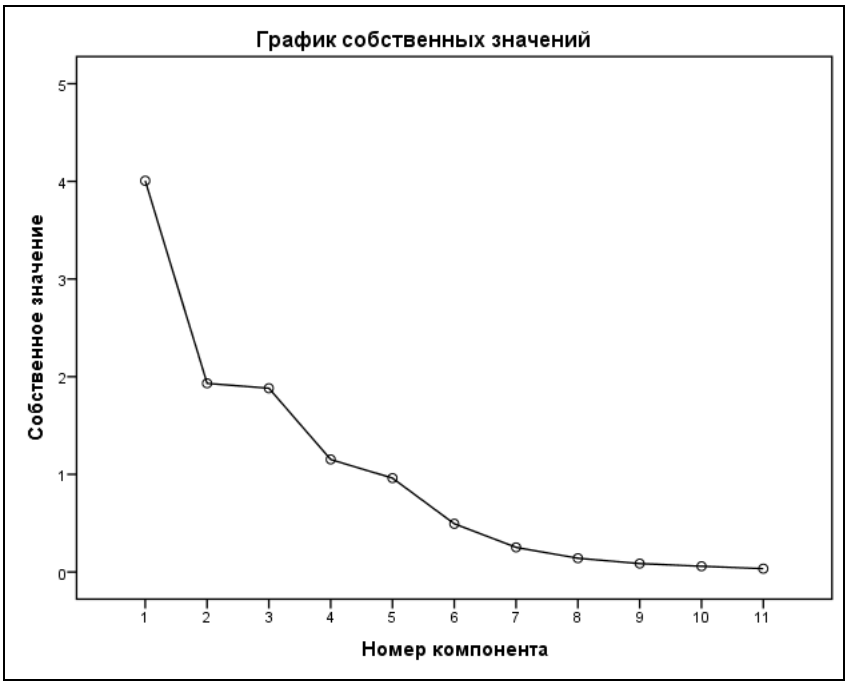

Fig. 3. Graphic reflection of the beginning of a three-factor analysis of cellular immunity relative indices in Rh - conflict women (Varimax raw rotation method)

The result of the multivariative correlation analysis is presented in Table 5 and Figure 4.

Table 5

Matrix of factor loads after turning by Varimax raw of cellular immunity relative indexes in Rhesus conflict women (summarized version)

Index	Factor 1	Factor 2	Factor 3
CD3+%	0,225234	-0,92876	0,154775
CD3+HLA-DR+%	0,841573	-0,23507	0,310538
CD3+CD4+%	0,018942	-0,00722	-0,95976
CD3+CD4+HLA-DR+%	0,837795	0,256569	0,214113
CD3+CD8+%	0,315341	-0,54916	0,694435
CD3+CD8+HLA-DR+%	0,839679	-0,21829	0,20668
CD3+CD4+/CD3+CD8+	-0,39788	0,218478	-0,78839
CD19+%	0,02316	0,888906	-0,08781
CD3-CD16/56+%	-0,77143	0,426827	0,256838

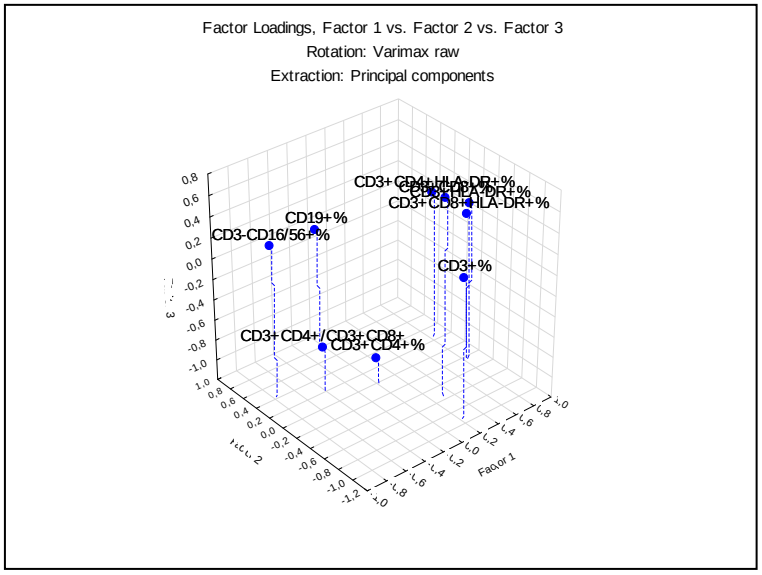

Fig. 4. Graphical reflection of the results of a three-factor analysis of cellular immunity relative indices in Rh-conflict women (Varimax raw rotation method)

Results and their discussion. According to the results of multivariate analysis with rotation of Varimax raw, it can be noted that the most significant indicators of cellular immunity in Rh - sensitized women were distributed among the three main factor components. The first sector (factor 1) comprised activated subpopulations of T-lymphocytes and T - killers. CD3 + HLA-DR + (activated T - lymphocytes) with correlation coefficient of 0.84, CD3 + CD4 + HLA-DR + (activated T - helpers) with correlation coefficient of 0.83, and CD3 + CD8 + HLA-DR + (activated T -suppressors) with a similar correlation of 0.83 are presented with direct dependence.

NK cells CD3-CD16 / 56 + (T-killers) with coupling force coefficient of - 0.77 are presented with inverse dependence in the group.

Factor 2 is represented by indices of lymphocytes population – B - lymphocytes (CD19 +) with direct correlation of 0.88, as well as T-lymphocytes (CD3 +) with an inverse one of - 0.92.

Factor 3 includes the main subpopulations of lymphocytes (T-helpers and T-suppressors), as well as the immunoregulatory index (the coefficient of their ratio). CD3 + CD8 + (T-suppressor) with coefficient of 0.69 are presented with direct dependence. CD3 + CD4 + (T-helpers) -0,95 and immunoregulatory index CD3 + CD4 + / CD3 + CD8 + with correlation coefficient of -0.78 have negative dependence. All indexes of coupling force are significant at $p < 0.05$.

Cluster analysis, the purpose of which was also the identification of classification regularities of cellular immunity during rhesus-sensitization of pregnant women, revealed

similar patterns of correlation dependences in the subpopulations of lymphocytes under study. The results of the graphical reflection of the cluster analysis of cellular immunity at Rh-sensitization are presented in Fig. 5.

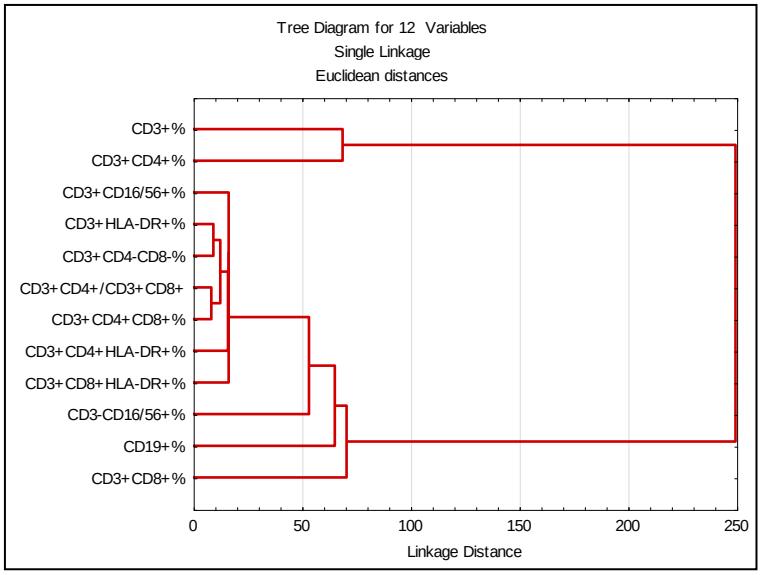

Fig. 5. The result of cluster analysis of the relative indices of cellular immunity in the women with Rh-isosensitibilization

Conclusion:

Thus, CD19 + (B - lymphocytes), CD3 + (T - lymphocytes), CD3 + HLA-DR + (activated T-lymphocytes), CD3 + CD4 + HLA-DR + (activated T-helpers), CD3 + CD8 + HLA-DR + (activated T-suppressors), NK-cells CD3-CD16 / 56 + (T-killers) and immunoregulatory index CD3 + CD4 + / CD3 + CD8 + are the most interesting indicators for the further study of cellular immunity in Rh-sensitized women.

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