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Differences In Immunological Parameters Of Synovial Fluid In Patients With Rheumatoid Arthritis, Depending On The Serological Variant And Stage Of The Disease.

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Absract

The purpose of the study: to study the immunological parameters of SF in RA patients depending on the serological variant and stage of the disease.

Materials and methods. We examined 21 samples of SF in RA. 11 patients had a late stage of the disease, and 10 patients had an early stage. Leukocytes, relative and absolute values of lymphocytes, CD3, CD4, CD8, CD16, CD20, CD23, CD25 and CD95 were determined in the SF. The determination of lymphocyte subpopulations was carried out by the method of monoclonal antibodies. To determine the normative values of subpopulations of lymphocytes of a healthy person, we have developed a mathematical calculation of the values of subpopulations of lymphocytes of a healthy person (theoretical norm).

Results and discussion. There are significant differences in the relative index of CD3%, CD20% and CD25% between seropositive and seronegative variants of RA ($p<0.05$). Significant differences in the relative indices of CD4% and CD8% between the early and late stages of RA ($p<0.05$) are revealed. There is an increase of tens and hundreds of times in the relative and absolute indices of lymphocyte subtypes compared with the normal values, which is associated with the migration of lymphocytes to the focus of inflammation.

Conclusion: Significant differences in a number of relative indicators between the stages and serological variants of the disease are revealed in the SF in RA patients, and at the same time a multiple (tens and hundreds of times) increase in all lymphocyte subpopulations is revealed.

Keywords: rheumatoid arthritis, serological variants, synovial fluid, lymphocyte subpopulations, stages of rheumatoid arthritis.

An important problem of modern medicine is the study of synovial fluid (SF), because it is the main indicator reflecting direct changes in the affected joint(s) in almost all rheumatic diseases [1,2]. Despite the fact that researchers have been studying SF in various rheumatic diseases, and, in particular, rheumatoid arthritis (RA), for almost 90 years, many questions characterizing the features of the composition of SF in RA are still far from their final solution. This is due to a number of factors. Firstly, the physical properties of SF in RA can vary greatly and in many cases, due to the very high viscosity, the extraction of such a liquid will be very difficult. Secondly, often when taking anti-inflammatory drugs and ongoing treatment, SF can dissolve on its own, which makes it impossible to extract it for laboratory testing. Thirdly, the procedure of removing the SF

itself can present significant technical difficulties even when removing it from an easily accessible knee joint. And fourthly, a properly equipped laboratory is required. All of the above causes problems in the study of SF [3,4].

Another important problem in the study of SF is the lack of normative values. This is due to the fact that in a healthy person it is almost impossible to get SF. That is why researchers either compare the obtained indicators with those of a healthy person's blood [4], which is not entirely correct. On the other hand, a number of researchers [5,6,7,8] used for reference values the LV obtained from the joints of corpses of persons who died from accidental causes and did not suffer from joint pathology during their lifetime. However, this method is also not without drawbacks, because unlike the peripheral blood of healthy individuals, the examination of CS samples from corpses is not entirely correct due to the passage of quite a considerable time from the moment of death to autopsy, which also affects the results obtained. It is also not always indicated in the accompanying medical documentation and revealed during external examination and autopsy pathology of the musculoskeletal system at an early stage of the disease (RA, SLE, gout, system sclerosis, etc.), because mortality from rheumatic diseases is relatively small in the structure of total mortality. In addition, for obvious reasons, the study of SF is forced to be carried out on a limited number of deceased persons (usually no more than 10-15), which also affects the reliability of the results obtained, taking into account age-related changes in almost all clinical, immunological and biochemical parameters of healthy individuals.

Currently, the immunological parameters of the peripheral blood of RA have been studied quite well, depending on the stage of the disease and the serological variation, however, these indicators have been studied extremely insufficiently for the above reasons. That is why further study of the immunological parameters of SF in RA is very relevant.

The purpose of the study: to study the immunological parameters of SF in patients with RA, depending on the serological variant and stage of the disease.

Materials and methods. We examined 21 samples of SF in RA patients (2 of them men) at the age of 57.24±3.09 years, the average duration of the disease was 5.77±1.57 years. The diagnosis of RA was established according to the ACR/EULAF criteria of 2010. The study of patients was conducted after the patients signed a written informed consent and approval by the Ethics Committee of the Academy of Sciences of the Republic of Uzbekistan. 7 patients were seronegative, and the rest were seropositive. The activity of the disease was established in accordance with the recommendations of V.A. Nasonova and M.G. Astapenko (1989), as well as according to the combined index of activity - DAS28 (Disease Activity Score) recommended by EURAL (A.M. Gestel et al., 1999). The following indicators were used to assess the activity and effectiveness of RA therapy (E.L. Nasonov et al., 2001): assessment of joint pain by the patient on a visual analog scale (VAS) from 0 to 100 mm, the number of painful joints (CHBS, 0-68), the number of inflamed joints (CHVs, 0-66), the duration of morning stiffness in minutes, the severity of the disease according to the patient's assessment (TSP) according to YOUR (0-100 mm) To determine the effectiveness of therapy, the ADAS28 index recommended by EURAL was used (A.M. Gestel et al., 1999). The class of functional joint insufficiency (FC) was assessed according to the Stanford Health Assessment Questionnaire (HAQ) (J.F. Fries, 1982; B.H. Amirdzhanova, 2008). 11 patients had a late stage of the disease (over 2 years from the onset of the disease, the average duration was 10.0±2.37 years), and 10 patients had an early stage (from 1 month to 2 years, the average duration was -1.01±0.32 goals). The diagnosis of RA at an early stage was carried out using ACCP and AMCV tests. All patients had II degree of disease activity (ESR-39.72±2.78 mm/h, C-reactive protein- 18.23±0.9 mg/l). On the day of admission, the patients underwent puncture of the knee joints under aseptic conditions with evacuation of the pancreas, into a test tube with the addition of a solution of sodium citrate in a ratio of 1:10, which was thoroughly mixed. Lymphocytes were isolated from the pancreas by the method of Boyum A. (1991) in a density gradient of gledol

(1,077) (Institute of Plant Chemistry of the Academy of Sciences of the Republic of Uzbekistan). CS lymphocytes were stained with monoclonal antibodies against CD3, CD4, CD8, CD16, CD56, CD19 (Beckman Coulter, USA). After staining, the samples were washed once with an excess of saline solution at 1500 rpm for 7 minutes. The analysis was performed on a CytoFLEX flow cytofluorimeter (Beckman Coulter, USA).. Leukocytes, relative and absolute values of lymphocytes, CD3, CD4, CD8, CD16, CD20, CD23, CD25 and CD95 were determined in the LV. The determination of lymphocyte subpopulations was carried out by the method of monoclonal antibodies. The study of the Russian Federation was carried out by the latex agglutination method using kits from Cypress (Belgium) in accordance with the attached instructions. Determination of the level of C-reactive protein by the latex method using Cypress kits (Belgium) in accordance with the attached information (standard value from 0.5 to 5.5 mg/l). The method of enzyme immunoassay was used to detect ADC. The analysis was performed using the DIASTAT test system (Axis Shield, UK) in accordance with the manufacturer's instructions (standard value less than 5 IU/ml)/. The level of AMCV was determined using a solid-phase enzyme immunoassay using commercial reagents from ORGENTEC (Germany) (the standard value is less than 20 IU/ml). The test tube was delivered to the laboratory no later than 3 hours after sampling.

The calculation method is based on the normal content of leukocytes in the SF (up to 200 in 1 µl), and the content of lymphocytes is about 30% of the total cellular composition, i.e. the absolute number of lymphocytes in the SF is normally 60 cells in 1 µl [3,9].

According to the same source, the number of peripheral blood lymphocytes is 4,500 cells per 1 ml. We determine the ratio of lymphocytes in peripheral blood and synovial fluid: 4500:60 = 75. Consequently, the number of lymphocytes in the SF is 75 times less than in the blood. Based on this difference, we calculated the indices of lymphocyte subpopulations in the SF of a healthy person (the theoretical norm). The data obtained make it possible to assess the nature of changes in SF in various diseases accompanied by synovial effusion.

This method of assessment is designed as a rationalization proposal (rationalization proposal No. 8 dated 06/16/11).

Statistical processing of the obtained data was performed using the STATISTICA for Windows software package (version 5.5).The reliability of the obtained values was determined using the Wilkinson U-test in the Mann–Whitney modification. A confidence level of $p<0.05$ was taken as statistically significant changes.

Results. The data obtained by us show, on the one hand, the presence of significant differences in some indicators between serological variants, and, on the other, an increase of tens and hundreds of times of the values obtained in comparison with the theoretical norm. At the same time, in many cases, the degree of increase in comparison with the normative values may differ significantly.

Table 1 shows the immunological differences in the composition of SF in RA patients depending on the serological variant of the disease.

Table 1

Characteristics of immunological parameters of SF in RA patients depending on the serological variant of the disease.

№	indicator	seropositive RA (n=14)	seronegative RA (n=7)	normative values
1	leukocytes,abs	12057,14±2493,17	12642,86±3603,69	200

2	lymphocytes,%	26,93±2,22	29,71±4,14	0,77
3	lymphocytes, abs	3782,57±1108,83	4622,71±2152,32	16
4	CD3%	49,64±2,91	41,43±4,36	0,48
5	CD3, abs	2113,29±772,41	1834,0±818,26	10
6	CD4,%	24,71±1,72	25,28±3,86	0,48
7	CD4, abs	1037,36±377,18	1636±1088,16	10
8	CD8,%	22,71±1,67	22,0±3,75	0,48
9	CD8, abs	948,28±329,19	1460,29±974,00	6
10	CD4/CD8	1,13±0,08	1,18±0,10	1
11	CD16,%	20,71±1,35	20,14±1,35	0,16
12	CD16, abs	859,21±285,41	889,28±382,38	3-4
13	CD20,%	25,21±1,50	22,86±1,62	0,3
14	CD20 abs c	4511,64±3760,14	924,14±325,83	6
15	CD23%	21,0±1,68	23,43±1,69	0,26
16	CD25,%	19,08±1,42	24,0±3,24	0,27
17	CD95,%	21,0±1,6	23,86±1,35	0,37

It can be seen from the data in the table that there are significant differences in the relative values of CD3%, CD20% and CD25% between the seropositive and seronegative variants of RA ($p<0.05$). An increase in the number of leukocytes was found to be 60.3 times in patients with the seropositive variant of RA and 63.21 times in comparison with the standard values. The lymphocyte level was increased 34.98 times in seropositive and 38.6 times in seronegative RA variants. The values of the absolute index of lymphocytes were increased by 236.3 times in SF with seropositive and 288.9 times with seronegative variants of RA in comparison with the standard values.

The values of the relative CD3% index in seropositive variants were increased by 103.4 times, and in seronegative variants by 86.3 times compared with the theoretical norm. The absolute CD3 index increased 211.3 times in seropositive and 183.4 times in seronegative variants, respectively. The values of the relative CD4% index increased 51.5 times for seropositive and 52.6 times for seronegative variants of the disease. The absolute CD4+ index increased 103.7 times in seropositive and 63.6 times in seronegative RA variants, respectively. The level of the relative CD8% index was increased 47.3 times in seropositive and 45.8 times in

seronegative variants of the disease. The absolute index of CD8+ in SF increased 158 times in the seropositive variant and 243.3 times in the seronegative variants of RA.

The values of the relative CD16% index in SF increased 129.4 times in the seropositive variant of the disease, and 125.8 times in the seronegative variant. The absolute value of CD16+ was increased by 245.4 times in the seropositive and 254 times in the negative variants in comparison with the standard values

The level of the relative CD20% index was increased 84 times in seropositive and 76.2 times in seronegative variants, respectively. The values of the absolute CD20+ index were increased in SF in seropositive 751.8 times and 154 times in seronegative variants of the disease.

The relative CD23% index was increased in SF by 80.76 times in seropositive and 90.1 times in seronegative RA variants. CD25% values increased by 70.6 times in comparison with the normative indicators for seropositive and 88.89 times for seronegative variants of the disease. The CD95% indices, respectively, increased by 56.7 times in seropositive and 64.5 times in seronegative variants of the disease.

Next, the immunological differences in SF were studied depending on the stage of RA. Table 2 shows the differences in the immunological parameters of SF depending on the stage of the disease.

Table 2

Differences in immunological parameters of SF in RA patients depending on the stage of the disease.

№	indicator	early stage RA (n=10)	late stage RA (n=11)	normative values
1	leukocytes,abs	12033,33±2737,55	12681,82±3203,29	200
2	lymphocytes,%	26,55±2,92	29,54±2,93	0,77
3	lymphocytes, abs	3615,67±1268,36	4616,27±1641,38	16
4	CD3,%	45,11±0,95	48,09±4,80	0,48
5	CD3+, abs	1669,11±614,09	2400,45±982,44	10
6	CD4,%	21,66±0,91	27,64±2,90	0,48
7	CD4+, abs	770,11±264,10	1687,73±783,37	10
8	CD8,%	18,44±1,08	25,82±2,61	0,48
9	CD8+, abs	699,22±260,80	1524,18±687,81	6
10	CD4/CD8	1,19±0,08	1,11±0,10	1
11	CD16,%	21,22±1,42	19,45±1,41	0,16
12	CD16+, abs	796,44±297,89	960,64±360,8	3-4

13	CD20,%	23,66±0,78	24,91±2,12	0,3
14	CD20+, abs	903,89±349,85	5543,45±4780,81	6
15	CD23,%	21,44±1,01	21,09±2,03	0,26
16	CD25,%	19,25±1,64	22,1±2,63	0,27
17	CD95,%	21,11±1,34	21,73±1,76	0,37

It can be seen from the table data that significant differences in the relative values of CD4% and CD8% between the early and late stages of RA ($p<0.05$) are revealed. And the level of leukocytes at an early stage was increased by 60 times, and at a late stage by 63 times in comparison with the standard values. The value of the relative index of lymphocytes at the early stage of RA turned out to be increased by 34.5 times, and at the late stage - by 38.4 times. The absolute index of lymphocytes at an early stage turned out to be increased by 226 times, and at a late stage – by 288 times. The values of the relative CD3% index turn out to be 93.7 times higher at an early stage, and 100 times higher at a late stage. The values of the absolute CD3+ index increased 166 times at an early stage, and 240 times at a late stage. The relative CD4% index increased by 45.1 times, and at a late stage by 57.5 times. The absolute CD4+ index increased by 77 times, and at a late stage by 167 times compared to the theoretical norm. The values of the relative CD8% index increased 38.4 times, and at a later stage - 53.8 times. The absolute CD8+ index turned out to be increased by 116.5 times, and at a late stage – by 254 times in comparison with the theoretical norm.

The levels of the relative CD16% index were increased 132 times at an early stage, and 121.5 times at a late stage. The absolute index of CD16+ increased at an early stage by 227.55 times, and at a late stage – by 274.47 times in comparison with the normative values.

The values of the relative CD20% index were increased 78.66 times at the early stage, and 83 times at the late stage, respectively. The absolute CD20+ index turned out to be increased at an early stage by 150.6 times, and at a late stage by -923.9 times.

CD23% values increased 82.5 times in the early and 81.1 times in the late stages of RA. The CD25% level was increased 71.3 times at an early stage and 81.8 times at a late stage, respectively. CD95% indices increased 57 times in the early stages, and 58.7 times in the late stages of the disease. Thus, most indicators of lymphocyte subpopulations were more elevated at a late stage of the disease.

There is an increase of tens and hundreds of times in the relative and absolute values of lymphocyte subtypes compared with the standard values, which is associated with the migration of lymphocytes to the focus of inflammation.

The data obtained indicate that the main process occurs precisely in the affected joints and these changes are many times greater than in peripheral blood.

Discussion. According to Kovalenko V.N. et al.(2007) [11] there is a significant increase in CD3+% levels, which is associated with the migration of T lymphocytes to the inflammatory focus, i.e. from peripheral blood to the pancreas. Another important participant in the chain of pathological reactions are natural killers – CD16+, which directly migrate to the lesion [2,13]. According to the results of our study, there is a significant increase in their level in the SF. There is also a natural increase in the level of natural killers - CD16 in the pancreas. This is due to the destructive and proliferative processes occurring in the affected joints, direct

cytotoxic effect on synovial cartilage and subchondral bone. The results obtained coincide with the data of most authors [2, 4, 12, 13, 15, 16]. It is obvious that CD16+ plays an important role in joint damage in RA.

In patients with RA, there is a significant decrease in the articular liquor of neutrophilic granulocytes and lymphocytes with CD95 receptors, which indicates the accumulation of apoptosis cells in the focus of inflammation (Klubova G.F., 2003) [11]. According to Loginova T.K. (2007) [13], an increase in differentiated forms of B lymphocytes occurs in the SF already in early RA against the background of T-lymphocytopenia.

However, differences in the degree of increase in both lymphocyte and leukocyte subpopulations, depending on the serological variant, are probably due to the fact that currently seropositive and seronegative variants of the disease are currently recognized, according to ICD 10, as independent diseases. This fact is supported by the fact that currently there are quite a lot of immunological and genetic differences between the variants of the disease on the one hand, and on the other - different degrees of response to treatment with biological genetically engineered drugs, depending on the serological variant of the disease. It is also necessary to take into account the influence of rheumatoid factor on immunological parameters in both peripheral blood and SF.

In RA, the entire T-cell pool is redistributed from the peripheral blood to the focus of inflammation – the joint, with an increase in all indicators tenfold. The greatest increase occurs with natural killers, which are the most important participant in the development of bone destruction, acting both directly and by producing cytokines in the focus of inflammation - the joint [16]. Apoptosis is important, and in the affected joints it increases tenfold compared to the normal value. This indicates the predominance of apoptosis processes in the affected joints [16]. An increase in the level of B cells indicates their important role in the development of local immune response. This indicates the predominance of local immune disorders in the affected joint in RA over systemic ones occurring in peripheral blood [1,17,18,19,20].

A significant increase in the absolute index of almost all populations at a late stage of the disease indicates the formation of autonomous follicle-producing centers in the affected joints, producing all subtypes of lymphocytes in the cavity of the affected joint, which indicates a kind of autonomization of the inflammatory process in the affected joints, which can often occur independently at a late stage of the disease, while poorly amenable to systemic treatment as basic drugs (methotrexate, sulfosalazine, azathioprine), and biological genetically engineered drugs (rituximab, remicade, humira, actemra) [19, 21, 22, 23, 24, 26].

Thus, in patients with RA, there is a multiple increase in all subtypes of lymphocytes in the SF compared to normal values. The data obtained indicate that it is in the joints that the most significant changes occur. This suggests that joints are the main springboard for the development of the disease [1,2,12].

Conclusion. Significant differences in the relative values of CD4% and CD8% between the early and late stages of RA and the relative values of CD3%, CD20% and CD25% between seropositive and seronegative variants of the disease are revealed in the LV in RA patients. There is a multiple (tens and hundreds of times) increase in all subpopulations of lymphocytes in comparison with the standard values for both serological variants and at all stages of the disease, and there are differences in a number of indicators in the degree of increase.

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