International Journal of
Health Systems and Medical Sciences

ISSN: 2833-7433
Volume 2 | No 2 | February -2023

Comparative Analysis of Indicators of Changes in Cells of the Immune System during Acute Osteomyelitis in Experimental Studies in Laboratory Animals

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Abstract: Purpose: study of changes in the cells of the central and peripheral organs of the immune system in the acute form of osteomyelitis in experimental laboratory animals, comparative analysis of the obtained results and changes.

Material and methods: Thymus, spleen, small intestinal lymph nodes and femur were collected from 72 purebred male mice. Methods of experimental study and statistical analysis of changes in central and peripheral immune system cells in laboratory animals.

Resultats: Results and indicators of changes in cells of the central and peripheral organs of the immune system obtained in the experimental study of the dynamics of acute osteomyelitis in laboratory animals are presented.

Conclusion: In experimental studies, quantitative and qualitative changes in the dynamics of acute osteomyelitis in experimental laboratory animals of cells of the central and peripheral organs of the immune system. The study of the level of influence on the indicators is dedicated to determining the indicators of quantitative changes in the cells of the immune system of the body, namely: thymus, bone marrow and lymph nodes during acute osteomyelitis. Also, indicators of changes in cells of the central and peripheral organs of the immune system and other immunocompetent cells during the course of the pathological process in acute osteomyelitis and study results are presented.

Keywords: immune system, spleen, lymph nodes, experimental studies, leukocytes, thymus, bone marrow, osteomyelitis.

Research results and discussion

All ethical principles of working with experimental material and international rules of biological safety were followed in this scientific work, assuming that experimental researches involving laboratory animals are carried out using clinical, laboratory-instrumental methods. To achieve this goal, experiments were conducted on 72 purebred mice. Taking into account reproductive changes in adult female mice and the effect of this condition on the purity of the experiment, only 3-month-old white mice weighing at least 25 g were recruited [**2**, **6**, **7**].

White male mice involved in the experiment were divided into 3 groups:

Control group - intact white mice without various osteomyelitis, n=36;

The main group - non-white mice with acute osteomyelitis, n=36;

Also, the control and main groups, in turn, were divided into subgroups:

1a - 7 days after acute osteomyelitis (n=12);

2a - 14 days after acute osteomyelitis (n=12);

3a - 21 days after acute osteomyelitis (n=12);

These periods were determined according to the periods of formation and resolution of acute osteomyelitis of various forms.

After the laboratory animals were euthanized during the study period, the thymus, spleen, lymph nodes of the small intestine and femur were removed from them. The thymus, spleen, and lymph nodes were then cleaned of adipose tissue and homogenized in a 199 medium mixture in a glass homogenizer.

All cell suspensions were separately passed through a filter made of three-layer, the femur was cleaned of muscles and tendons, the epiphyses were cut, and the bone marrow was washed out of the femoral canal with 199 medium using a thin needle syringe.

Yerne and local hemolysis methods were used to determine the amount of antibody-producing cells (APC). A certain number of lymphoid tissue cells (spleen, lymph nodes) of white mice immunized with sheep erythrocytes were mixed and lysed with sheep erythrocytes in vitro without affecting cell viability in agar. The mixture was placed in a Petri dish, and after incubation in a thermostat at 37°C in the presence of complement, the number of hemolysis zones ("plaques" formed) on the agar plate was visually counted. The number of lymphoid cells obtained for seeding on agar, a certain number of fixed karyocytes (for example, 1 million or 10 million) or the number of APC for a whole organ (spleen) was calculated. The amount of APC in the spleen was determined on the 5th day after immunization with sheep erythrocytes (QE) by the method of local hemolysis in agarose according to Jerne N. and Nordin A. [**5**, **10**]. APC was counted and recorded for the whole spleen (absolute value) and for $1x10^6$ spleen cells (relative value).

In cell suspensions of all isolated immune system organs, the number of splenic nucleated cells (SNC) was counted using a Goryaev chamber and recalculated relative to the whole organ. Using this method, the total number of cells in the central (thymus, bone marrow) and peripheral (spleen, lymph nodes) organs of the immune system was determined.

All studies were conducted in experimental dynamics, and the results were observed on the 7th, 14th and 21st days of the experiment.

The first (main) group (n=36) - white mice with acute osteomyelitis; they, in turn, were divided into three subgroups: group 1a - laboratory animals whose results were studied on the 7th day after acute osteomyelitis (n=12); Group 1b - laboratory animals whose results were studied on the 14th day after acute osteomyelitis (n=12); Group 1 - laboratory animals (n=12) whose results were studied on the 21st day after acute osteomyelitis [**8**].

The second (control) group (n=36) - intact white mice without acute osteomyelitis; they were euthanized on the 7th (n=12), 14th (n=12) and 21st (n=12) days of the experiment, according to the main group, and were compared.

Both groups were representative of each other and had the same symptoms, except for acute osteomyelitis caused by a pathogenic microorganism (*Staphylococcus aureus*). When conducting experimental studies, it was achieved that they were randomized and fully followed the principles of evidence-based medicine, and that the groups being compared were representative of each other [**1**, **3**, **4**].

In experimental studies, it was found that on the 7th day of the experiment after the development of acute experimental osteomyelitis, the number of antibody-producing cells of the spleen was 1097±134 cells in the main group, and it was found to be 1.97 times lower than in the control group (2156±148 cells) - $R<0.001$ (Table 4-1).

This reduction was also assessed as the effect of stimuli introduced into the organism to induce acute experimental osteomyelitis. Also, in the number of antibody-producing cells per 1 million cells (APC per 1 million cells), a quantitative decrease trend was observed - 11±2 cells against 17±2, respectively (by 1.55 times, R<0.001). As for the results of the nuclear storage cells of the spleen, a convincing reduction was observed in the main group (121±5 cells) compared to the parameters of the control group (146±4 cells) (by 1.21 times, R<0.05). The significant decrease in the amount of these three cells in the main group compared to the control group was explained by the effect of the acute osteomyelitis process in the body of the experimental animals.

Table 1.1. Comparative indicators of quantitative changes of immune system cells in laboratory animals during the course of acute osteomyelitis on the 7th day of the experimental study

Indicators	Control group, n=12	1a main group, n=12
Antibody-producing cells	2156±148	1097±134* ↓
Antibody-producing cells, per 1 million cells	17±2	11±2* ↓
Nuclear storage cells in the spleen	146±4	121±5* ↓
Thymus cells	57±4	86±6* ↑
Bone marrow cells	11±2	16±1* ↑
Lymph node cells	14±2	21±2* ↑

Note: * is a sign of reliable difference compared to the control group; ↓, ↑ - directions of changes.

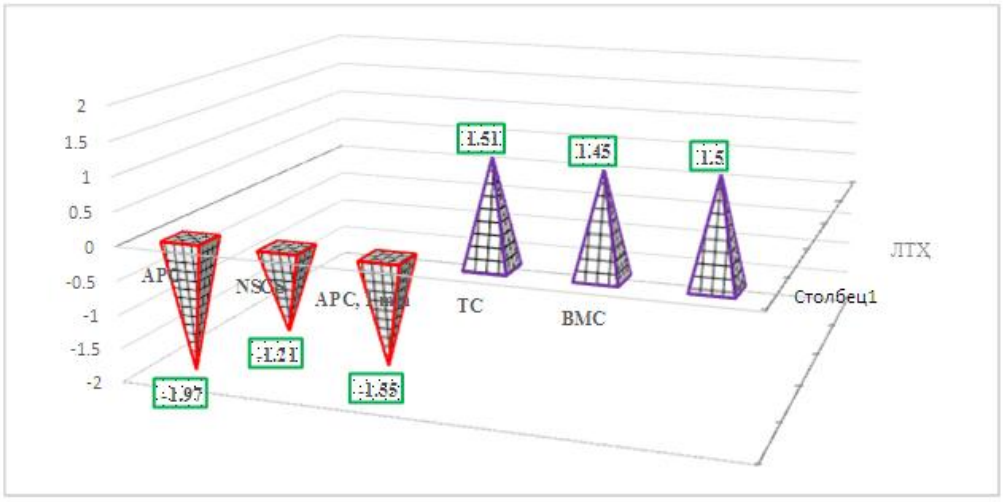
As can be seen from the table, it was noted that the intensity of changes in immune system cells is different. We also observed that the quantitative decline in the quantitative indicators of APC changed more than the other compared indicators.

The number of central organs of the immune system - thymus, bone marrow and peripheral organ - lymph node cells (APC, APC in 1 million cells, NSCS) showed the opposite picture in contrast to these three indicators, that is, the main group, although not in large numbers, was reliably higher than the indicators of the control group was determined (R<0.05).

If the quantitative increase in thymus cells compared to the parameters of the control group was 1.51 times (86±6 versus 57±4 cells, respectively, R<0.05), the increase in bone marrow and lymph nodes showed similar indicators - the control group 1.45 times (16±1 vs. 11±2 cells) and 1.50 times (21±2 vs. 14±2 cells), respectively, were significantly different from each other (R<0.001).

A comparative analysis of the results obtained on the 7th day of the experiment for the 6 studied parameters is presented in Figure 1.1.

Figure 1.1. Indices of stimulation of cells of the immune system on the 7th day of acute osteomyelitis in the experiment



In it, the results of comparative studies of these cells are presented in the form of stimulation indices (SI).

It can be seen from this figure 1.1 that on the 7th day of the development of acute osteomyelitis in the experiment, the quantitative indicators of the immune system cells of white mice changed in opposite directions, three of the studied indicators were in the "positive zone" and the other three were in the "negative zone" shown.

In an experimental study, on the 7th day of the course of acute osteomyelitis, it was found that the parameters of the quantitative indicators of the cells of the immune system of laboratory animals changed in different directions. Against the background of the development of acute osteomyelitis, APC and NSCS (main group) significantly decreased compared to the control group (intact) ($R<0.05$ - $R<0.001$), central organs of the immune system (thymus and bone marrow), as well as cells of intestinal lymph nodes (peripheral organ of the immune system) was found to be significantly increased ($R<0.05$). We believe that this condition is related to the development of purulent-inflammatory process in the body due to acute osteomyelitis. It was explained by the fact that APC and NSCS in the spleen, while APC decreased in 1 million cells under the influence of antigen stimulation, the cells of the thymus, bone marrow, lymph nodes (LNC) increased reliably due to the differentiation and proliferation under the influence of this stimulation. We believe that these specific aspects should be taken into account when evaluating the body's immune status in acute experimental osteomyelitis.

All the above indicators were compared and analyzed on the 14th day of the dynamics of acute osteomyelitis in the experiment. The obtained results are presented in Table 1.2.

Table 1.2. Comparative indicators of quantitative changes of immune system cells in laboratory animals during the course of acute osteomyelitis on the 14th day of the experimental study

Indicators	Control group, n=12	1b main group, n=12
Antibody-producing cells	2083±127	1196±139* ↓
Antibody producing cells, per 1 million cells	15±1	13±2 ↔
Nuclear storage cells in the spleen	134±5	122±4* ↓
Thymus cells	69±4	74±3 ↔
Bone marrow cells	13±1	15±2 ↔
Lymph node cells	23±3	27±3 ↔

Note: * is a sign of reliable difference compared to the control group; ↓, ↑ - directions of changes; ↔ - no reliable difference.

After the induction of acute osteomyelitis, the trend of changes on the 14th day was close to the parameters after the 7th day, but the changes in the obtained numbers differed from each other by the low intensity of the changes.

APC indicators were 1196±139 cells in the main group, while in the control group this indicator was equal to 2083±12.7 cells, the difference between them was 1.74 times ($R<0.001$).

Quantitative indicators of NSCS also differed from each other as above - 122±4 versus 134±5 cells, respectively (difference up to 1.10 times, $R<0.05$). Believable differences between parameters of both indicators remained, but the intensity of changes decreased compared to the 7th day - 1.74 and 1.10 times after the 14th day, and 1.97 and 1.21 times after the 7th day, respectively). It was also observed in the quantitative indicators of APC in 1 million cells, practically no significant difference was observed between the main (subgroup 1b) and control groups - 13±2 versus 15±1, respectively ($R>0.05$).

The numbers of thymus cells (TC), bone marrow cells (BMC) and lymph node cells (LNC) did not show a significant difference ($R>0.05$), but the increasing trend remained similar to the above,

indicating that the obtained numbers were very close to each other showed his lack of confidence. This gave rise to the impression that the results were practically the same [**11**].

The results obtained for TC (74±3 cells vs. 69±4 cells) were close to the numbers obtained for LNC (27±3 cells vs. 23±3 cells) and BMC (15±2 cells vs. 13±1 cells) (R> 0.05).

In the experiment, on the 14th day of the course of acute osteomyelitis, the differences between the parameters of the main and control groups disappeared, only 2 of the 6 immunological parameters (33.33%) showed reliable differences, and among the remaining parameters (66.67%), practically no reliable changes were observed.. After the induction of acute experimental osteomyelitis, on the 7th day, all 6 parameters (100.0%) had reliable differences. On the 14th day of the course of acute osteomyelitis, all the cells of the immune system (TC, BMC, LNC) showed signs of stimulation, i.e. quantitative increase, disappeared on the 7th day, all indicators remained equal to the parameters of the control group [**12**].

The results of the 21st day of acute osteomyelitis induced in the experiment were also studied and analyzed and the results are presented in the form of table 1.3. It has been shown that these obtained and comparatively analyzed quantitative parameters are reliably different from the parameters of the previously obtained periods (7th and 14th day).

Table 1.3. Comparative indicators of quantitative changes of immune system cells in laboratory animals during the course of acute osteomyelitis on the 14th day of the experimental study

Indicators	Control group, n=12	1c main group, n=12
Antibody-producing cells	2104±119	1410±122* ↓
Antibody producing cells, per 1 million cells	18±1	17±2 ↔
Nuclear storage cells in the spleen	148±5	137±3* ↓
Thymus cells	67±3	71±3 ↔
Lymph node cells	19±2	23±2 ↔
Bone marrow cells	16±1	14±1 ↔

Note: *-symbol of a reliable difference compared to the control group; ↓, ↑-directions of change, ↔ - there is no convincing difference.

It can be seen from this table 1.3 that in 2 out of all 6 studied indicators, APC and NSCS (33.33%) showed convincing changes, in other cells the changes were close to each other, no clear differences were observed, therefore, no convincing differences were taken into account (R>0, 05).

Significant difference scores were obtained for APC (1410±122 vs. 2104±119 cells, R<0.05) and NSCS (137±3 vs. 148±5 cells, R<0.05), the difference being 1.49 and 1.49, respectively. It was 1.08 times. TC and LNC maintained a non-significant (R>0.05) increase trend, but BMC decreased (R>0.05) compared to the control group by the same time period [**9**, **13**].

The changes in the cells of the members of the immune system differed in different directions and showed that antigen stimulation started to affect them differently. It was shown that while the thymus and lymph nodes maintain the level of resistance to the increasing external influence (test-microorganism influence), this "reserve" is not left in the bone marrow.

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