STUDY OF THE THYMUS OF RATS AFTER VARIOUS DEGREES OF TRAUMATIC BRAIN INJURY WITH IMMUNOHISTOCHEMICAL MARKERS (CD 68)

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Abstract: *Overall, the results obtained using CD68 showed a significant increase in inflammation, phagocytic activity, and macrophage count in the thymus with increasing severity of brain injury. This allows us to evaluate CD68 as a reliable indicator as one of the main biomarkers of destructive changes in the thymus, an organ of the immune system, under the influence of stress and inflammation.*

Key words: *thymus, brain injury, expression, immune response reaction.*

Relevance. Traumatic brain injury (TBI) remains one of the leading causes of disability and mortality worldwide, with long-term consequences not only limited to the central nervous system but also extending to peripheral organs, including those of the immune system [2]. The thymus, as a central lymphoid organ, plays a crucial role in maintaining immune homeostasis and T-lymphocyte maturation. Experimental and clinical data indicate that severe brain injury can trigger systemic immune dysregulation, leading to immunosuppression, increased susceptibility to infections, and delayed recovery [1,5,8,9].

The use of immunohistochemical markers, particularly CD68, which reflects macrophage and monocyte activity, provides an opportunity to study the cellular mechanisms underlying thymic alterations following different degrees of TBI. Such investigations are highly relevant, as they can clarify the link between neurotrauma and peripheral immune dysfunction, contribute to understanding of post-traumatic immunodeficiency, and potentially identify new diagnostic and therapeutic targets [3,4,6].

Given the growing incidence of TBI in both civilian and military populations, as well as its socioeconomic burden, comprehensive study of the thymic response using CD68 immunohistochemistry is of significant scientific and practical interest.

Was the general macroscopic specimen of the thymic tissue of experimental white outbred rats, as well as histological materials obtained from various parts of the thymus of experimental animals [1,7,10.

Aim of the Study. The aim of the present study is to evaluate structural and cellular changes in the thymus of rats following various degrees of traumatic brain injury, with a particular focus on the expression of the immunohistochemical marker CD68, in order to assess the role of macrophage activity in post-traumatic immune alterations.

Materials and methods. The study was conducted on the thymus of a 3-month-old white rat (*Rattus norvegicus*) following the induction of mild traumatic brain injury (TBI). After the experimental procedure, the animal was euthanized according to standard ethical guidelines, and the thymus gland was carefully removed for histological and immunohistochemical analysis.

Tissue samples were fixed in 10% neutral buffered formalin for 24 hours, dehydrated in graded ethanol series, cleared in xylene, and embedded in paraffin blocks. Serial sections of 4–5 μm thickness were prepared using a rotary microtome and mounted on glass slides.

For immunohistochemical detection of CD68 expression, the sections were deparaffinized, rehydrated, and subjected to antigen retrieval by heating in citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide, and nonspecific binding was reduced by incubation with normal serum. The slides were then incubated overnight at 4 °C with a primary monoclonal antibody against CD68 (macrophage marker), followed by incubation with a secondary

antibody conjugated to horseradish peroxidase. Immunoreactivity was visualized using a DAB (3,3′-diaminobenzidine) chromogen solution, and the sections were counterstained with hematoxylin.

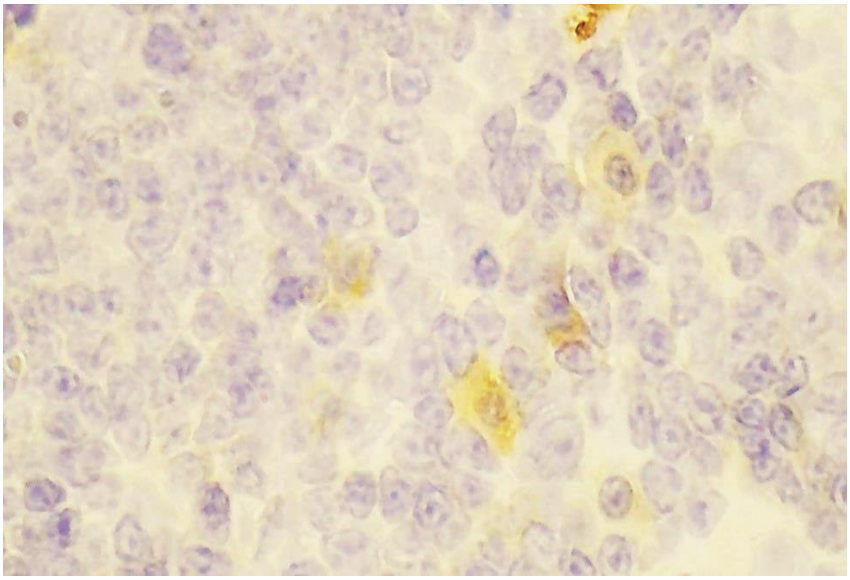
The stained sections were examined under a light microscope at ×400 magnification. For each micropreparation, the number of CD68-positive and CD68-negative cells was counted within a defined field of view. In the analyzed section, a total of 223 cells were counted on a surface area of 1764 μm². Among these, 45 cells were CD68-positive and 178 were negative. The percentage of CD68 expression was calculated as:

$$\text{Expression level (\%)} = \frac{\text{Number of positive cells}}{\text{Total number of cells}} \times 100$$

For this specimen, the calculated expression level was 20.17%, which corresponds to a moderate degree of expression. The presence of CD68-positive cells in the thymus was interpreted as an indicator of phagocytic activity associated with inflammatory or stress responses following brain injury.

The subject of the study was the general macroscopic specimen of the thymic tissue of experimental white outbred rats, as well as histological materials obtained from various parts of the thymus of experimental animals.To conduct the study and achieve the objectives, experimental, morphological, morphometric, immunohistochemical, and statistical research methods were used

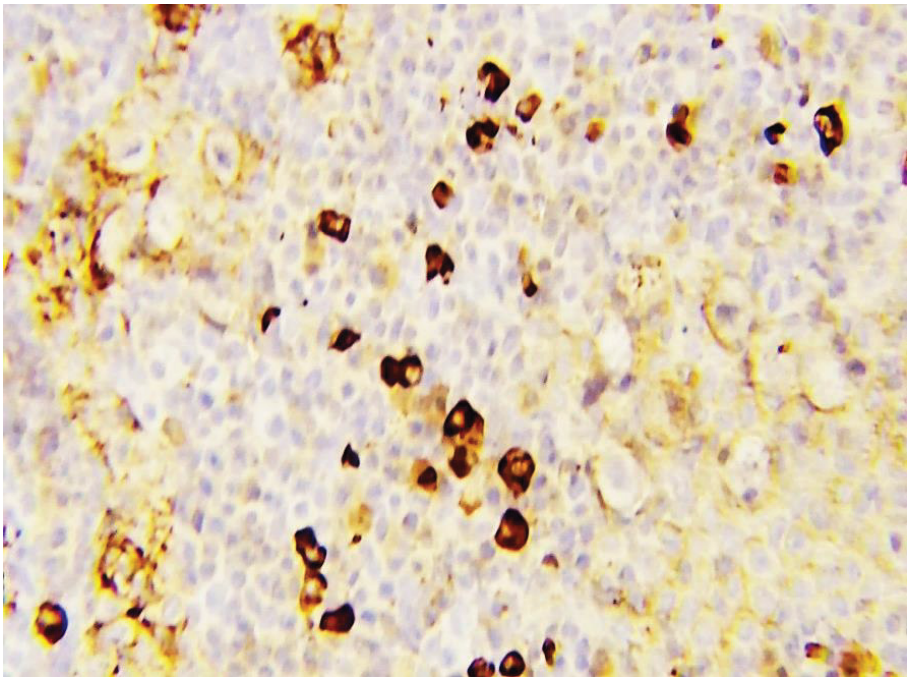
Results. When the level of CD68 expression was determined in the thymus of a 3-month-old white rat after mild brain injury, a total of 223 cells were counted on the surface of the tissue, which occupied a total area of 1764 11px^, of which 45 were positive and 178 were negative. Thus, the level of expression in this micropreparation was 20.17%. This indicator means that the expression is moderate.Its expression in the thymus indicates phagocytic activity, such as a response to inflammation, stress or injury



Total number of cells detected	223
Positive cells	45
Negative Cells	178
Positive Expression	20,17 %
Total area	1764 11px^2

Fig. 1. Expression of immunohistochemical CD68 marker in thymus tissue of 3-month-old white mongrel rats after mild brain injury.

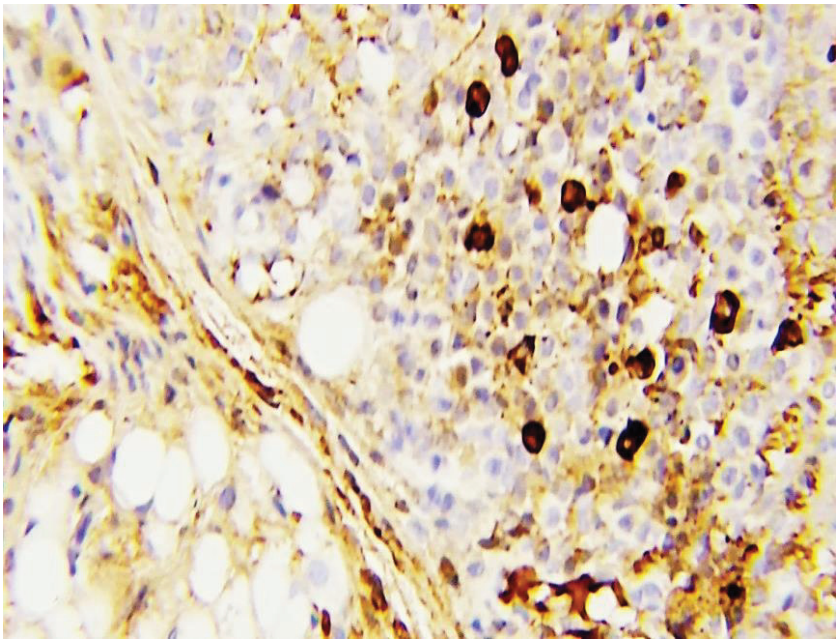
When determining the level of CD68 expression in the thymus of a 3-month-old white mongrel rat after moderate brain injury, a total of 111 cells were counted on the surface of the tissue with a total area of 1834 11px^2, of which 32 were positive and 79 were negative. Thus, the expression level in this micropreparation was 28.82%. Increased expression of CD68 in the thymus indicates increased phagocytosis and immunological activity. This expression can be considered as an average level, and in some cases, it can also be considered as an average-high level.



Total number of cells detected	111
Positive cells	32
Negative Cells	79
Positive Expression	28,82 %
Total area	1834 11px^2

Fig. 2. Expression of immunohistochemical CD68 marker in thymus tissue after moderate brain injury in 3-month-old white outbred rats. Expression 28.82%.

In the thymus, CD68 cells are more abundant in the cortical substance, and types III and IV cells, identified as monocytes, predominate. Large cells of types I and II, corresponding to macrophages, are rarely found in the cortical substance of the thymus lobules, which is consistent with scientific data. In the thymus medulla, CD68 types III and IV cells are most often detected. The results of our studies allowed us to draw the following conclusions: the increase in CD68+ cells in the cortical substance of the thymus lobules led to



Total number of cells detected	220
Positive cells	90
Negative Cells	130
Positive Expression	40,02 %
Total area	1824 11px^2

Fig. 3 Expression of immunohistochemical CD68 marker in thymus tissue of 3-month-old white mongrel rats after severe brain injury. 40.02% expression.

When determining the level of CD68 expression in the thymus of 3-month-old white mongrel rats after severe brain injury, a total of 220 cells were counted on the surface of the tissue with a total area of 1824 11px^2, of which 90 were positive and 130 were negative. Thus, the expression level in this micropreparation was 40.02%. An expression level of 40% is considered a high level of expression. Severe brain injury causes a severe stress reaction in the body. As a result, phagocytic elements (macrophages) in the thymus are sharply activated. An increase in CD68 expression to 40% indicated that the immune system organ was responding strongly to the injury, i.e., activating inflammation, autophagy, or cellular remodeling processes.

In the thymus, this level of activity was above the physiological norm and led to pathological processes or dystrophic changes.

In the study, the expression of the immunohistochemical marker CD68 in thymus tissue after 3-month-old white rats with traumatic brain injury was the highest, reaching 20.17% (moderate expression).

A slight increase in the number of CD68-positive cells was observed in the thymus after mild traumatic brain injury. This is interpreted as a physiological response, indicating the activation of macrophages in response to mild stress in the tissue. CD68 expression was mainly observed in the medullary part of the thymus, and no significant disorganization of lymphoid cells was observed.

In the study, the expression of the immunohistochemical marker CD68 in thymus tissue from moderately severe brain injuries in 3-month-old white outbred rats gave the highest index, which was 28.82% (moderate expression).

In moderate injury, CD68 expression was significantly increased, which was manifested by increased phagocytic activity in the tissue and the formation of inflammatory markers. Macrophages

were mainly concentrated in the perivascular zones and medullary part of the thymus, and a decrease in the number of lymphoid thymocytes was observed. This indicates a weakening of the immune response to injury. In the study, the expression of the immunohistochemical marker CD68 in thymus tissue after severe brain injuries in 3-month-old white rats gave the highest index, which was 40.02% (moderate expression).

Conclusion. A significant increase and hyperexpression of CD68-positive cells was observed after severe traumatic brain injury. A clear disorganization of the lymphoid tissue in the thymus, a decrease in the volume of the thymus, an increase in the number of dead cells, and activation of phagocytosis by macrophages were noted. CD68 expression was also increased in the medullary and cortical parts, which indicates a severe course of the inflammatory process in the thymus. Overall, the results obtained using CD68 showed a significant increase in inflammation, phagocytic activity, and the number of macrophages in the thymus with increasing severity of traumatic brain injury. This allows us to evaluate CD68 as one of the main biomarkers of destructive changes in the thymus, an organ of the immune system, under the influence of stress and inflammation.

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