Trace element Limitation of Lymph Nodes Structure according to the X-ray Fluorescent Analysis with Synchrotron Radiation (SR XRF)

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**Abstract.** Unique properties of X-ray fluorescent analysis with synchrotron radiation and classical morphological method allowed to reveal interrelation between the trace elements content and structure of lymph nodes. Belonging of lymph nodes to different regions is defining in formation of a certain microelemental profile and features of the microanatomic organization of lymph nodes. Lymph nodes have different functional specialization with prevalence of immune or drainage function and a certain microelement composition. The inguinal lymph node has active drainage function because of the developed sinus system at the maximum of Mn, Fe, Zn concentration; immune function prevails in a tracheobronchial lymph node at the low content of all trace elements; the mesenteric lymph node has evenly developed drainage and immune functions at the maximum of Cu, Se contents. The content of trace elements is considered as the leading mechanism limitation the structural organization of lymph nodes. Innovative approach helped to reveal formation of lymphoid-microelemental association. Features of this association modify the structural organization of lymph nodes depending on localization (region specifics).

Keywords: morphology, lymph nodes, trace elements, SR XRF

# INTRODUCTION

Emergence of the concept of the lymphatic region is progressive for understanding and development of protective systems of an organism [1, 2]. A hub of the protective system are peripheral lymphoid organs. Lymph nodes are difficult organized structures which carry out at the same time immune and drainage functions, providing a regional homeostasis and protection during the different periods of life [1, 3]. There is an urgent need for morphofunctional evaluation of lymph nodes depending on localization.

At the same time lymph nodes are effector organ of the lymphatic region where chemical elements accumulate. Trace elements function together with immunocompetent cells of a lymphoid tissue and provide an optimum immune response, showing regulatory and structural stabilizing functions [4, 5, 6]. The main functional role of trace elements in immune system is participation as cofactors or catalysts of enzymes of free radical oxidation [7, 8].

Change of activity of enzymes is connected with trace elements. Trace elements and enzymes influence a proliferation and a differentiation of cells of a lymphoid tissue that is reflected in a structure of peripheral lymphoid organs [1, 9]. Differences in concentration of separate elements can be connected with structural originality of lymph nodes. We consider that studying of regional specificity of structure of lymph nodes in interrelation with composition and quantity of microelements is relevant and has theoretical and practical interest.

**The research purpose** – it is to establish causal connection between the content of trace elements and structure of lymph nodes of different localization.

## MATERIALS AND METHODS

60 white rats of Wistar at the age of three months (young) participated in an experiment. Three-months age of rats are a stage of the maximum development of peripheral lymphoid organs [1, 3]. We investigated mesenteric, inguinal and tracheobronchial lymph nodes by a morphological method.

Lymph nodes fixed in 10% neutral formalin. After fixing we adhered to the classical scheme of washing, dehydration, imbibition with a xylol, paraffin and preparation histologic sections on the microtome. Histologic sections of lymph nodes painted hematoxylin and eosine, azure-II-eosine, trichromatic C. Masson’s stain.

The morphometric analysis of structural components of a lymph node was carried out by means of a morphometric grid. We counted quantity of the crossings of a grid falling on all histological section and separately on each of structural compartment of a lymph node with recalculation in percent.

The content of trace elements (Se, Mn, Fe, Cu, Zn) in lymph nodes was defined by the X-ray fluorescent analysis with use of synchrotron radiation (SR XRF). The work was done at the shared research center SSTRC on the basis of the Novosibirsk FEL/VEPP-4 – VEPP-2000 complex at BINP SB RAS, using equipment supported by project RFMEFI62119X0022 [10].

The morphometric analysis of structures of a lymph node was carried out by means of a morphometric grid and the Image-Pro Plus 4.1 program. Statistical data processing was performed with licensed statistical software package StatPlus Pro 2009, AnalystSoft Inc. Data were expressed as average arithmetic with definition of a standard (mean square) error. Belonging to normal distribution was defined when calculating criterion of Kolmogorov-Smirnov and the accompanying indicators. The correlation analysis is used in work. A P-value < 0.05 was considered statistically significant.

## RESULTS

The structural organization and immune response of lymph nodes are optimum at young age. Trace elements are important for a differentiation and proliferation of immunocompetent cells. These cells take part in forming of functional compartment of lymph nodes [1, 9, 11]. The trace element profile of regional lymph nodes has the region-dependent character, reflecting features of functioning of each lymphatic region (Table 1).

The content of trace elements in lymph nodes is considered optimum for young animals. However, concentration of trace elements is different in lymph nodes of various lymphatic regions. Content of manganese (Mn) is 4.12±0.32 mkg/g in an inguinal lymph node at young animals that exceeds by 1.6–1.9 times of an indicator in tracheobronchial (2.54±0.15 mkg/g) and mesenteric (2.15±0.13 mkg/g) lymph nodes; content of iron (Fe) in an inguinal lymph node is 672.55±54.22 mkg/g that exceeds by 2.6–3.0 times of an indicator in mesenteric (254.82±20.66 mkg/g) and tracheobronchial (221.38±12.12 mkg/g) lymph nodes; content of copper (Cu) in an inguinal lymph node is 6.45±0.35 mkg/g that exceeds by 1.2 times of an indicator in tracheobronchial (5.27±0.17 mkg/g) and does not give statistical distinction with an indicator in mesenteric (6.48±0.47 mkg/g) lymph nodes; content of zinc (Zn) in an inguinal lymph node is 75.6±2.81 mkg/g that exceeds by 1.3 times of an indicator in tracheobronchial (58.26±2.30 mkg/g) and does not give statistical distinction with an indicator in mesenteric (68.71±2.52 mkg/g) lymph nodes. At the same time the content of selenium (Se) is the smallest in an inguinal lymph node and is 0.96±0.05 mkg/g that is 1.3-1.4 times less than the content of selenium (Se) in tracheobronchial (1.25±0.06 mkg/g) and mesenteric (1.38±0.05 mkg/g) lymph nodes respectively.

Each lymph node has the certain trace element profile according to SR XRF. So, the mesenteric lymph node has the maximum content for Cu, Se, average content for Fe, Zn, minimum content for Mn; the tracheobronchial lymph node has the smallest content of Fe, Cu, Zn, and average content of Mn, Se; the inguinal lymph node distinguishes the maximum content of Mn, Fe, Zn, average content of Cu, minimum content of Se (Table 1). It is obvious that the distinction of concentration of trace elements is connected with morphological variant of lymph nodes depending on an originality of the drained lymphatic region.

**TABLE 1.** Definition of functional specialization of lymph nodes according to the morphological

 and trace element status

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **LYMPH NODES** | **INGUINAL**  | **MESENTERIC** | **TRACHEO-BRONCHIAL**  | P |
| 1 | 2 | 3 |
| **Lymphoid follicles** | **+** | **++** | **+++** | P1-2 > 0.01P1-3, 2-3 < 0.001 |
| **Cortex plateau** | **+** | **++** | **+++** | P1-2, 1-3 < 0.001P2-3 < 0.01 |
| **Paracortex** | **+++** | **++** | **++** | P1-2, 1-3 < 0.01P2-3 > 0.05 |
| **Medullary cords** | **++** | **+** | **++** | P1-2, 2-3 < 0.01P1-3 > 0.05 |
| **Lymphatic sinus** | **++** | **+++** | **+** | P1-2, 2-3 < 0.001P1-3 < 0.01 |
| **Mn** | **+++** | **+** | **++** | P1-2, 1-3 < 0.001P2-3 > 0.05 |
| **Fe**  | **+++** | **++** | **+** | P1-2, 1-3 < 0.001P2-3 > 0.05 |
| **Zn** | **+++** | **++** | **+** | P1-3 < 0.001P1-2 > 0.05 P2-3 < 0.01 |
| **Cu** | **++** | **+++** | **+** | P1-3 < 0.01P1-2 > 0.05 P2-3 < 0.05 |
| **Se** | **+** | **+++** | **++** | P1-2 < 0.001P1-3 < 0.01P2-3 > 0.05  |
| **Functional specialization** | **drainage** | **drainage and immune** | **immune** |  |

The structure of lymph nodes is not static. The histologic picture of lymph nodes with different type of an immune response is described rather in detail [1], but researchers did not connect the structural organization of lymph nodes with regional accessory and trace element composition. The dimension of internal structures (compartment) differs in lymph nodes because of an originality of lymphatic regions, showing regional specifics (Table 1).

The area occupied by the cortical plateau progressively increases row: inguinal (6.20±0.47%) – mesenteric (12.53±0.50%) – tracheobronchial (15.2±0.70%) lymph nodes. The paracortical area of an inguinal lymph node has the big area equal of 36.9±1.72%. It is 1.3-1.4 times more than the similar area of visceral lymph nodes (p<0.01). The cortical plateau and paracortical area are considered as a thymic-dependent zone, and dominance of this zone indicates the created immune response on cellular type in a lymph node.

Changes affect a thymic-independent area (B-zone) – lymphoid follicles and medullary cords, responsible for an immune response on humoral type. Lymphoid follicles are provided in all studied groups of lymph nodes. The area of lymphoid follicles with the germinative center increases progressively: inguinal (8.32±0.43%), mesenteric (9.21±0.27%), tracheobronchial (11.8±0.46%) lymph nodes. It is indicated to the active lymphopoiesis in all lymph nodes which are most expressed in a tracheobronchial lymph node. Medullary cords occupy the different area in medullary substance of lymph nodes of different localization. There is the largest area in inguinal (26.87±1.65%) and tracheobronchial (25.56±0.86%) lymph nodes, the smallest area in mesenteric (17.08±0.52%) lymph node.

The ratio of T- and B-zones makes the size which is a little exceeding unit (an interval 1,03–1,28) at three-months age of young rats. This size demonstrates uniform development a thymic-dependent and a thymic-independent zones with easy dominance of the T-zone in the mesenteric lymph node.

Lymph nodes possess transport function, being a component of a lymphatic bed. We can estimate transport function of lymph nodes by the size of a lymphatic sine. There is the smallest area of sinus system in the tracheobronchial lymph node (4.38±0.32%). There is the largest area of lymphatic sinus in the mesenteric lymph node (11.8±0.19%). The inguinal lymph node has intermediate value of the area of a lymphatic sine (6.71±0.62%). It is obvious that the active drainage of a lymph is noted more in inguinal and mesenteric lymph nodes because of features of lymphatic regions.

The structure of lymph nodes is comparable to the content of trace elements. Belonging of lymph nodes to different regions is defining in forming of a microelement profile and features of the microanatomic organization. It allows to allocate lymph nodes of different functional specialization with dominance of immune or drainage function. So, drainage function prevails in an inguinal lymph node because of the developed sinus system; immune function prevails in the tracheobronchial lymph node, proceeding from the size of peripheral and deep cortex; drainage and immune functions are evenly developed in a mesenteric lymph node because of development of sinus system and immune compartment.

Lymphoid follicles can be considered as a system factor in a lymph node. Lymphoid follicles are the reactive structures which are responsible for proliferation of lymphoid cells (lymphopoiesis) and forming of compartments. Trace elements are necessary for proliferation and functioning of lymphoid (immune) cells [4, 5]. Trace elements have average and strong correlation with lymphoid follicles of lymph nodes of different localization at young animal (Fig. 1).



**Figure 1.** Correlation coefficient between microelements and lymphoid follicles with the germinative center (on the left) and without the germinative center (on the right) in inguinal (the blue line), mesenteric (the red line) and tracheobronchial (the black line) lymph nodes

Lymphoid follicles without the germinative center have positive correlation for Fe and Se (r=0.51-0.52, p <0.01) in the tracheobronchial lymph node. There are a positive correlation between lymphoid follicles without the germinative center and microelements – Mn (r=0.48, p <0.01), Cu (r=0.78, p <0.001) and negative correlation for Zn (r=-0.51, p <0.01), Se (r=-0.37, p <0.05) in the inguinal lymph node. There is a positive correlation between lymphoid follicles without the germinative center and Fe (r=0.48, p <0.01) in the mesenteric lymph node.

There is a positive correlation of lymphoid follicles with the germinative center for Mn (r=0.37, p <0.05), Cu (r=0.70, p <0.001) in the tracheobronchial lymph node. There is a positive correlation between lymphoid follicles with the germinative center and Cu (r=0.42, p <0.01) and negative correlation with Zn (r=-0.50, p <0.01) and Se (r=-0.39, p <0.05) in the inguinal lymph node. There is a positive correlation between lymphoid follicles with the germinative center and Cu (r=0.52, p <0.01), Mn (r=0.43, p <0.05), Fe (r=0.69, p <0.01) and negative correlation with Zn (r=-0.38, p <0.05) in the mesenteric lymph node. Availability of correlation between trace elements and lymphoid follicles indicates emergence of the lymphoid-microelement association having the region-dependent character.

## DISCUSSION

Concepts of the lymphatic region were strengthened by attention to lymph nodes as to important bodies of protective system of an organism [1, 2]. The morphological option of a structure of a lymph node depends on localization and an originality of contact with external environment. This fact causes features of accumulation of trace elements. It is obviously important to discuss interrelation of trace elements with structure of lymph nodes.

Firstly, immune function of lymph nodes is connected with development of compartment during the process of proliferation and a differentiation of immunocompetent cells [3, 8, 9, 11]; secondly, availability of direct or indirect relationship between the microelements and enzymes participating in proliferation of lymphoid cells [4, 6, 8], thirdly, a possibility of purposeful use of trace elements for correction of an immune response [11, 12].

Trace elements have the modifying properties concerning a design of lymph nodes, defining degree of cellular and humoral immunity. Influence of trace elements on an immune response has ambiguous character and depends on localization of lymph nodes. The size of concentration of trace elements affects interstructural changes in a lymph node. Interaction of compartment with trace elements is a distinguishing sign of effectively operating structures of a lymph node over all life.

Forming of lymphoid-microelement association has the region-dependent character and leads to forming of a certain morphological type of a structure of a lymph node, integrated with functional specialization in the lymphatic region. It does the importance of lymphoid-microelement association as structural, functional and metabolic complex in ensuring efficiency of lymph nodes. Trace elements positively influence a condition of an immunological homeostasis [8]. We assume a protective role of trace elements in preservation the region-dependent morphological option of structure of lymph nodes.

## CONCLUSION

The received results revealed patterns of structural modification of lymph nodes of different localization, proceeding from features of a microelement profile of microelements. Features of a design of lymph nodes are combined with the high content of Cu, Se in the mesenteric lymph node and Mn, Fe, Zn in the inguinal lymph node and the smallest content of Fe, Cu, Zn in the tracheobronchial lymph node. Each lymph node has a certain functional specialization in the lymphatic region. Sufficient providing with microelements is a condition of functioning of lymph nodes. Trace elements enter cofactors of enzymes and carry out the regulatory and structural stabilizing function on the relation of structure of lymph nodes.

We obtained the evidence of forming of lymphoid-microelement association. This association is active and defines proliferation of lymphoid cells and development of an immune response according to morphological variant of lymph nodes structure. The content of trace elements is considered as the leading mechanism in limitation of the structural organization of the lymph nodes belonging to different lymphatic regions.

SR XRF is an informative method for understanding the region-dependent content of trace elements for modification of structure of lymph nodes. This method is of essential interest from the theoretical and practical point of view.

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