



## To Study Morphometric Parameters of Lymphoid Tissue of the Spleen of White Healthy Rats in Postnatal Ontogenesis from three Months to Six Months of Age

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**Abstract:** The new approach makes it possible to objectively and accurately assess the structural and functional state of organs both in normal conditions and in conditions of pathology, which reflects the immune status and the general level of the organism's adaptive potential. In humans, one of the main functions of the spleen is the formation of a generalized immune response to the effects of various damaging factors, that is, direct participation in maintaining immune homeostasis and, consequently, the necessary level of the body's adaptive potential. However, the relationship of immunological processes with the structural organization of the spleen has not been studied enough.

**Keywords:** immunocompetent compartment lymph nodes, functional immunomorphology, morphometry, new research algorithm.

However, in the studies of different authors, different and sometimes overlapping morphometric criteria were used to assess morphofunctional status of the spleen, based on various measurements of the tissue and cellular components of the organ. In addition, it is often not possible to trace the relationship between the dynamics of the quantitative parameters of various structural components of the spleen tissue using the presented "bare" numbers.

This state of affairs makes it difficult to interpret the results obtained, especially in a comparative sense, and does not allow a sufficiently convincing judgment of the morphofunctional changes occurring in the organ. Therefore, it is urgently necessary to unify the methods of morphometric study of the spleen, develop a unified algorithm for obtaining quantitative data and an unambiguous approach to assessing their dynamics both in the aspect of ontogenesis and in various pathological conditions of the organism's existence. This was the aim of the present work. Based on the analysis of literature data concerning morphometric methods for studying the functional morphology of the spleen, as well as our own developments, we propose, in our opinion, the optimal research algorithm based on the determination of five parameters at the tissue level and the calculation of the same coefficients (indices).

An important role in the adaptation of the organism to the effects of unfavorable endo- and exogenous factors is played by the immune system (IS), the importance of which in maintaining immune homeostasis and, consequently, the necessary level of the organism's adaptive potential (APO) [4], can hardly be overestimated [1]. The specified function of the IS is carried out during the interaction of various immunocompetent organs, one of which is the lymph nodes (LN), visceral and peripheral (somatic) [3].

The immunocompetent compartment of the spleen parenchyma is represented by its white pulp (BP), where two main B- and T-dependent zones are formed, that is, areas of localization mainly of B- and T-lymphocytes.

These are, respectively, lymphoid follicles (LF) and periarterial lymphoid cuffs (PALM) [9].

The study of reactive changes in the spleen, in particular the structural elements of PD, is the subject of a large number of scientific studies based on the study of this organ both in humans [19] and various agricultural and laboratory animals [10]. Usually, many of them are descriptive in nature, but in recent works [19] morphometric methods are often used, which make it possible to objectify the results and conclusions drawn, since the final data are quantitative and easily amenable to statistical analysis [1; 12].

In connection with the development of immunology, the interest of morphologists in the study of both primary and secondary organs of the immune system has increased, the main function of which is the production of lymphocytes, which, together with other cells of the immune system, participate in the defense reactions of the body. To date, numerous studies of the structure of the peripheral organs of immunogenesis have been carried out, which have provided detailed information on the structure of the palatine (2), etc.

At present, it is known that the function of adaptive immunity, which determines the cellular and humoral homeostasis of the body, is performed by the immune system [15]. Its morphological basis is lymphoid tissue organized into functional formations [17], the largest of which is the spleen.

Considering that the spleen plays an important role in maintaining the immune status in the body, the study of the relationship and dynamics of the development of lymphoid tissue under the influence of anti-inflammatory drugs of high concentration is of great theoretical and practical importance.

Anti-inflammatory drugs are one of the most commonly used drug groups in medicine. Their advantage is their complex action (antipyretic, anti-inflammatory, thinning and analgesic), as well as a wide range of indications for which they can be used, especially during a coronavirus pandemic. Analysis of the literature data on the clinical experience of managing patients with SARS associated with SARSCoV and MERSCoV coronaviruses makes it possible to distinguish several groups of drugs that were used by patients in combination, such as antibiotics, antiviral, anti-inflammatory, antiplatelet agents, anticoagulants, and cytostatics. However, the information available today about the results of therapy with these drugs does not allow making an unambiguous conclusion about their effectiveness or ineffectiveness, as well as about the development of side effects, not to mention polypharmacy with these drugs. At the same time, it is known that damage can be localized in almost any part of the spleen, despite the relatively rare discussion in the literature, it occurs much more often than is commonly believed. So, it is not uncommon for situations when it is the damage to the spleen induced by the intake of anti-inflammatory drugs that is the cause of the development of life-threatening conditions, a decrease in the body's immune response. (M.A. Evseev. 2015)

Lymph nodes are the most numerous organs of immunogenesis [11]. Their number in an adult is about 460, and the total weight is about 1% of the body weight (500-1000 g) [6]. This is three to five times the mass of the largest solitary organ of the IS - the spleen.

Lymph nodes carry out two main functions - immune and drainage-detoxification [12], which allows these organs to be attributed to both IS and the lymphatic system [10]. The drainage function is performed mainly by the medulla lymph nodes, the immune one belongs to the cortex, where three separate structural and functional units are distinguished: 1) lymphoid follicles (LF), 2) interfollicular zone, or cortical plateau (CP) and 3) inner cortex, or paracortical zone, paracortex (PC) [15].

In the cellular composition of Lf, B-lymphocytes predominate, which, upon antigenic stimulation, undergo blast transformation and subsequent differentiation into plasma cells, forming light (germinal) centers (HC) of Lf [17]. In this case, the primary Lf turns into a secondary one, which documents the presence of an immune response of the humoral type [4].

On the contrary, the population of T-lymphocytes is localized in the CP and PC [18], the expansion of which indicates an increase in the immune response of the cell type [4; eighteen; nineteen; 38]. A mixed type of immune response is observed with a reactive change in all immunocompetent structures of the LN [4; nineteen]. Therefore, the morphological development of these components of the parenchyma lymph nodes reflects the level of functional immune activity of these IS organs [4].

A huge number of publications are devoted to the study of the functional immunomorphology of lymph nodes in various conditions of the organism's existence and under the influence of numerous endo- and exogenous factors. Among them, an important place is occupied by works based on the use of morphometric research methods. At the same time, numerous authors use various approaches to quantitatively assessing the state of the structural components of the lymph nodes at the tissue level. It is of some interest to consider these approaches in more detail and try to critically assess their information content and appropriateness of their use.

Some authors made a direct measurement of various tissue structures using an eyepiece micrometer, or measurements were carried out on images of lymph nodes (sketches or photographs). Based on the obtained linear dimensions, the areas of the objects under study and even their volumes were calculated. The obtained planimetric and volumetric results were presented as a percentage of the total cut area of the lymph nodes or the total volume of the organ [2].

In our opinion, such a determination of quantitative parameters, associated with rather laborious calculations, is inappropriate and unnecessary. Indeed, all calculations by the corresponding formulas are based on the linear dimensions of certain structures under study. Therefore, the derived quantities (area and volume) do not carry any new information, and their dynamics fully corresponds to that of the primary (linear) quantitative quantities. It should be emphasized that this position is true not only in relation to the morphometry of the lymph nodes, but in general for morphometric studies in general [1].

A widespread and logically quite reasonable technique for the quantitative study of the morphology of lymph nodes is the determination by the method of point counting of the areas occupied by certain tissue components [10]. This uses a variety of eyepiece reticle. Of these, the most common are the models proposed by G.G. Avtandilov [2] and S. B. Stefanov [5].

The ratio of the number of points per each structure to the total number of points on the cut, expressed as a percentage, characterizes the proportion of these structural elements in the LN tissue as a whole. The magnification of the microscope during the subfrontal examination is selected in such a way that at least one point of the eyepiece grid falls on each structure [1; 2].

In addition, some researchers calculated the number of Lf in the cortical layer as a whole, as well as separately the number of primary and secondary Lf [17].

The morphometric characteristics of the functional morphology of the lymph nodes are substantially supplemented by quantitative indices and coefficients used by different authors [3; 6; 14]. In particular, the cortical-cerebral (C / M) index has a certain value, based on the values of which three types of lymph nodes are distinguished - fragmented, compact and intermediate [20], which is of serious importance in assessing the drainage function of the LN.

In D.E. Batushenko (2004) [3], in addition to the K / M index, such derived quantitative indicators as the T / B ratio and the parenchymal-stromal index of the cortex and medulla of the LN were calculated.

The so-called follicular coefficient [9; 14], which is the ratio of the number of secondary LFs to the number of primary ones, reflects the severity of the immune response of the humoral type, carried out due to the development of HC, where the processes of plasmacytization of B-lymphocytes and antitelopoiesis take place.

At present, digital photography and various computer programs have become quite widespread [16], allowing to automate the process of morphometric study of LN and significantly reduce the complexity and duration of research.

Using the considered morphometric methods, the state of various tissue structures of the lymph nodes was studied. Their list is different for different authors, depending on the goals of the study. In many works, the morphometric characteristics of all components of the parenchyma and stroma of the lymph nodes are given [13]. Other researchers limited themselves to several parameters in various combinations [3].

Critically evaluating the presented literary material concerning the morphometric study of lymph nodes, we should, in our opinion, recognize the most attractive algorithm used in the work of employees of the Research Institute of Clinical and Experimental Lymphology of the Siberian Branch of the Russian Academy of Medical Sciences (Novosibirsk) [8]. In this case, the point counting method determines the specific areas (as a percentage of the total cut area of the lymph nodes) of structures such as the capsule, subcapsular sinus, CP, primary LF (F1), secondary LF (F2), PC, pulp cords, cerebral sinus. If necessary, the K / M index and the follicular coefficient (F2 / F1 index) are calculated. The specified algorithm allows one to fairly objectively judge the level of both main functions of the lymph nodes - immune and drainage.

However, all of the listed methods of morphometric study of lymph nodes, each of which has its own pros and cons, still insufficiently clearly reflect the more subtle nuances of changes in the morphological substrate of the immune function of the lymph nodes in different conditions of the organism's existence. We are talking about the severity and ratio of immune responses of the humoral and cellular types, as well as about the processes due to which the level of functional immune activity of the lymph nodes of the humoral type changes - hyper- / hypoplasia of Lf in general or hyper- / hypotrophy of individual Lf.

To avoid the noted disadvantages, in our opinion, the proposed new approach to the problem, based both on the experience of other researchers, in particular, given in the review part of the article, and on our own developments, allows us.

It should be noted here that the proposed method is intended to study at the tissue level the morphological basis of only the immune function of the lymph nodes, leaving aside the drainage function of the organ. Therefore, the number of structural objects of tissue lymph nodes, subjected to morphometry, is significantly reduced. This also has a certain plus, since it saves research time and reduces its labor intensity.

The new algorithm assumes determination of five morphometric parameters, on the basis of which three coefficients (indices) are calculated. Let's consider the research procedure in sequence.

Six histological sections from each object are studied, measurements are carried out in six fields of view of each section, followed by finding the average values of these parameters, as recommended by S.A. Kashchenko and O. N. Petizina [16]. As the experience of a similar study of the spleen shows [7; 15], this number of observations is quite enough to obtain representative results.

If both initial parameters (Dra, and Df) change simultaneously, then two variants of the GPI fluctuations are possible here. First, with a unidirectional process, that is, a simultaneous increase or decrease in Dra and Dlph, the GPI value may differ little from UN. Everything will depend on the severity of the change in each parameter. With a uniform (proportional) nature of changes in both of these indicators, the value of GPI is approximately equal to UN. The predominance of the rate of shift of any of the initial parameters causes a deviation of the GPI from the UN in one direction or another, depending on which of the indicators will change to a greater extent. If this is the numerator (Dra.), then the GPI will exceed UN (with a simultaneous increase in Dra, and Dlph) or, conversely, will be less (if both parameters decrease).

The use of such an approach to a series of studies on the immunomorphology of the spleen in the aspect of ontogenesis and with damaging exogenous effects on the body, in particular, due to the side effects of antipsychotic drugs, allows us to obtain comparable quantitative results and objectively assess the nature and degree of reactive changes occurring in the spleen as in the process of aging of the individual, as well as in pathological conditions.

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