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Regional lymph nodes and hematogenous metastasis of cancer

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Abstract

Aim. Morphological study of the microvasculature of regional lymph nodes in relation to the cancer of the lymph nodes as possible additional or alternative metastasis pathways.

Methods. The lymph nodes of 150 cancer patients (1263 nodes in total), regional to cancer of various localization, were studied. Histological sections staining with hematoxylin and eosin by Van-Gieson's method, pyronine by Brachet's method, toluidine blue and picro-Mallory were prepared. An immunohistochemical study was performed using monoclonal antibodies to pan-cytokeratins, CD31, type IV collagen, CD3, CD20, and CD68. The area of metastases to the lymph nodes was determined by using a morphometric grid and used to identify the four study groups. In addition, the immunomorphological reactions of the lymph nodes were taken into account in each group.

Results. It was identified that the microvasculature of the lymph nodes can be involved in the metastatic process along with the lymphatic pathways. At the same time, there is a decrease in vascular wall function and violation of the rheological properties of blood, accompanied by the deposition of intra- and extravascular fibrin. Hematogenous metastasis is largely influenced by the state of lymph node sinuses, in which blood is found, and in some observations — by the expression of CD31 (a marker of blood endothelium). Hematogenous dissemination of cancer often begins after the appearance of lymph node metastases. The greater the anatomical extent of lymph node metastases, the more often tumor cells are present in the blood vessels. In addition, an isolated lesion of the microvasculature with the presence of tumor cells in the extranodal vessels without metastases in the lymph node itself was revealed. It was observed that the invasion of tumor cells into the microvasculature depended on the immunomorphological reactions of the lymph nodes.

Conclusion. The microvasculature of regional lymph nodes can be both an additional and an alternative lymphogenous metastasis pathway of cancer; at the same time, vascular invasion is accompanied by microcirculation disorders and depends on the volume of metastases and the immunomorphological reactions of the lymph nodes.

Keywords: cancer, regional lymph nodes, hematogenous metastasis, morphological examination.

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The state of the lymph nodes (LN) regional to a cancerous tumor is the most important criterion in determining the approach of surgical intervention, particularly in determining the volume of lymph node dissection [1]. In addition, the disease prognosis is largely determined by the absence or presence of metastases, their size, and the nature of the LN immunomorphological reactions [2].

The lymphogenous pathway of metastasis has been studied in sufficient detail. Tumor cells enter the LN through the afferent lymph vessels, penetrate the marginal sinus, and spread along the intermediate sinuses to the cortex and the medulla [3]. Subsequently, metastases appear in the efferent lymphatic vessels and spread further along the lymphatic system. Moreover, the presence of a well-developed microcirculatory system in the LN, which communicates with the lymphatic pathways through the marginal sinus and intranodal veins, cannot be ignored.

The close relationship between the lymphatic and circulatory systems of the LN is proven by certain conditions, in which vascular transformation of the sinuses occurs with the formation of anastomosing canals lined with endothelium with signs characteristic of blood vessels [4]. This phenomenon can also be noted in regional LNs owing to a cancerous tumor. In addition, pronounced microcirculatory disorders develop in the LN during cancer development, which increases during metastasis

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Table 1. Characterization of the first antibodies

Antigen	Clone	Specificity	Working dilution	Manufacturer
Pan-cytokeratins	AE1/AE3	Epithelial cells	1:300	Lab Vision
CD31	9611	Endothelium of blood vessels	1:20	BioGenex
Collagen IV	PHM-12 + CIV22	Basement membranes	1:150	Lab Vision
CD3	SP7	T-lymphocytes	1:150	Lab Vision
CD20	L26	B-lymphocytes	1:250	Lab Vision
CD68	PGM1	Macrophages	1:200	BioGenex

[5]. Thus, the possibility of hematogenous spread of the tumor from regional LNs cannot be excluded.

This study aimed to analyze the morphology of the circulatory microvasculature of regional LNs to a cancerous tumor as a possible pathway of additional or alternative metastasis.

LNs of 150 patients with cancer (1,263 LNs in total), regional to the cancer of the stomach, lung, breast, colon, and esophagus were studied. We used both the surgical material and the material obtained from the archive of paraffin blocks of the Kazan Republican Cancer Center. LNs of corresponding localizations of 10 healthy humans who died from accidental causes (forensic autopsies) were used as a control.

Fresh material was fixed in 10% neutral Lilly formalin or Bouin's fluid. According to the generally accepted technique [6], after the appropriate processing of the samples, dehydration, and embedding in paraffin, histological sections were made, which were stained with hematoxylin and eosin according to Van Gieson, pyronin according to Brachet, and toluidine blue. We used a modified Picro-Mallory staining for fibrin [7], which enables to differentiate it into "young," "mature," and "old." Immunohistochemical study [8] was performed using a set of monoclonal antibodies (MCAB), which characteristics are presented in Table 1.

Binding of the first antibodies to cellular and structural elements was performed using the standard biotin-streptavidin-peroxidase method (DAKO: LSAB® + System-HRP, code K0690) with diaminobenzidine as a chromogen and additional staining with Mayer's hematoxylin. A morphometric grid of random step [9] was used to determine the area of metastases in the LN.

The following groups were identified for the study:

1) intact LNs

2) LNs with isolated tumor cells or their clusters

3) LNs with micrometastases (<10% of the LN area)

4) LNs with metastases of various sizes (more than 10% of the LN area)

5) total replacement of LNs by metastases

In addition, the nature of the LN immunomorphological reactions was taken into account in each group:

1) paracortical hyperplasia with a high T-cell count

2) follicular hyperplasia with a high content of B cells and plasmatization

3) sinus histiocytosis with a high count of macrophages

4) unstimulated LN without morphological manifestations of the immune response

As a result of the production of serial sections with the reaction of MCAB against pan-cytokeratins, 551 intact LNs were revealed, 38 LNs had isolated tumor cells or their clusters (Fig. 1), 122 LNs had micrometastases (Fig. 2), 479 LNs had metastases of various volumes, and 73 LNs were completely replaced by tumor tissue. In the last two cases, metastases were well defined without immunohistochemical study. The network of the circulatory microvasculature of the LN was detected by the expression of MCAB against CD31 in the endothelium, and against type IV collagen in the basement membranes of the vessels (Fig. 3).

In all cases, microcirculatory disorders were expressed to varying degrees, which progressed depending on the degree of metastatic lesion of the LN. The vessels were plethorical, and their walls were edematous, often with signs of mucoid swelling. The normal structure of the endothelial lining and basement membranes was impaired, as determined by MCAB against CD31 and type IV collagen. In 289 LNs (22.88% of all cases), the sinuses contained blood, which was not found in the norm (Fig. 4). Erythrocyte masses were often located in the lymphoid tissue outside the vessels or sinuses, especially in the presence of large metastases in the LN. In the areas of such hemorrhages, there were conglomerates of tumor cells (Fig. 5). Moreover, in some cases, the endothelium of the sinuses gave a positive reaction with MCAB against CD31, which was an endothelial marker of blood vessels (Fig. 6).

Fibrin was deposited inside and outside the lumen of blood vessels, and its volume increased in accordance with the stages of metastasis in the LN.

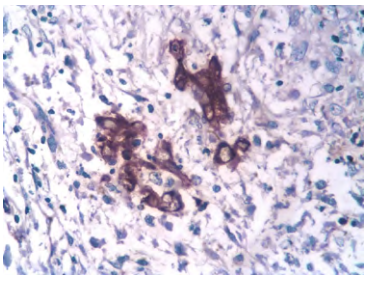

Fig. 1. Isolated tumor cells and their clusters. Reaction with monoclonal antibodies against pan-cytokeratins. LSAB-method with additional hematoxylin staining (magnification × 400).

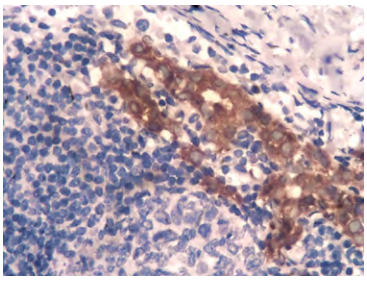

Fig. 2. Micrometastasis in the subcapsular sinus. Reaction with monoclonal antibodies against pan-cytokeratins. LSAB-method with additional hematoxylin staining (magnification × 400)

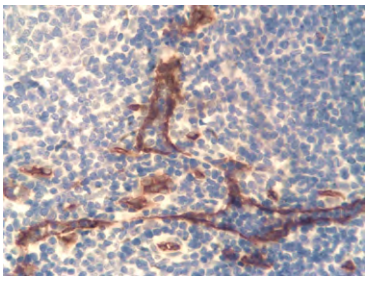

Fig. 3. Expression of CD31 in the endothelium of blood vessels. LSAB-method with additional hematoxylin staining (magnification × 400)

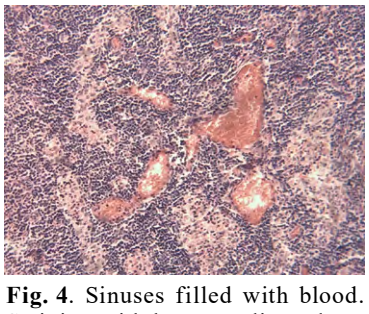

Fig. 4. Sinuses filled with blood. Staining with hematoxylin and eosin (magnification × 200)

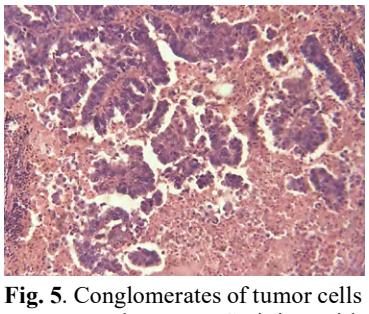

Fig. 5. Conglomerates of tumor cells among erythrocytes. Staining with hematoxylin and eosin (magnification × 200)

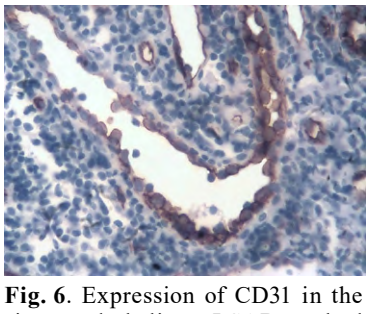

Fig. 6. Expression of CD31 in the sinus endothelium. LSAB-method with additional staining with hematoxylin (magnification × 400)

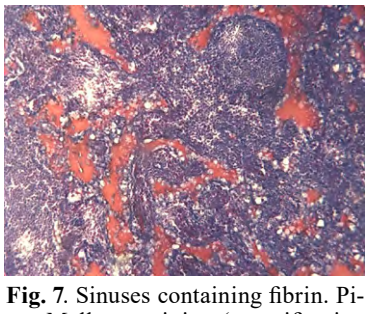

Fig. 7. Sinuses containing fibrin. Picro-Mallory staining (magnification × 200)

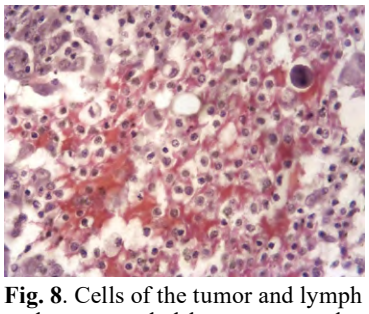

Fig. 8. Cells of the tumor and lymph node surrounded by extravascular fibrin. Picro-Mallory staining (magnification × 200)

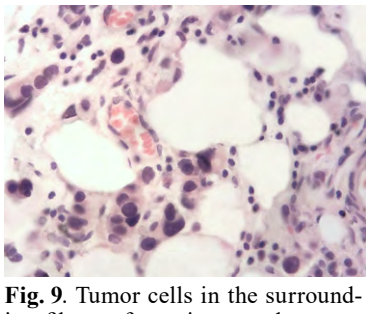

Fig. 9. Tumor cells in the surrounding fibrous-fatty tissue and extranodal blood vessels of the lymph node, free from metastases. Staining with hematoxylin and eosin (magnification × 400)

Significant amounts of fibrin were often found in the sinuses before the appearance of metastases in the LN (Fig. 7). In the presence of large metastases and total lesions of the LN, fibrin surrounded the cells of both the tumor and LN, often delimiting them from each other (Fig. 8).

The study of metastatic lesions of the blood microvasculature revealed two (0.36%) cases showing tumor cells in the surrounding fibrous fat and extranodal vessels of the LN, free of metastases (Fig. 9, Diagram 1). The histological presentation of the LN had no morphological manifestations of immune responses, such as paracortical and follicular hyperplasia or sinus histiocytosis (Diagram 2).

Isolated tumor cells in the LNs were detected in the lumen of an intranodal blood vessel in one case. Moreover, LN did not show any signs of antigenic stimulation.

Due to micrometastases in the LN, the lesion of the circulatory microvasculature was determined in four (3.28%) cases, and intranodal vessels of the cortex were observed in all cases (Fig. 10). The immunomorphological presentation in one case corresponded to an unstimulated LN, whereas follicular hyperplasia with high levels of B cells and plasmatization was registered in the other three cases. This was despite the fact that in the LN without metastases, paracortical hyperplasia and sinus

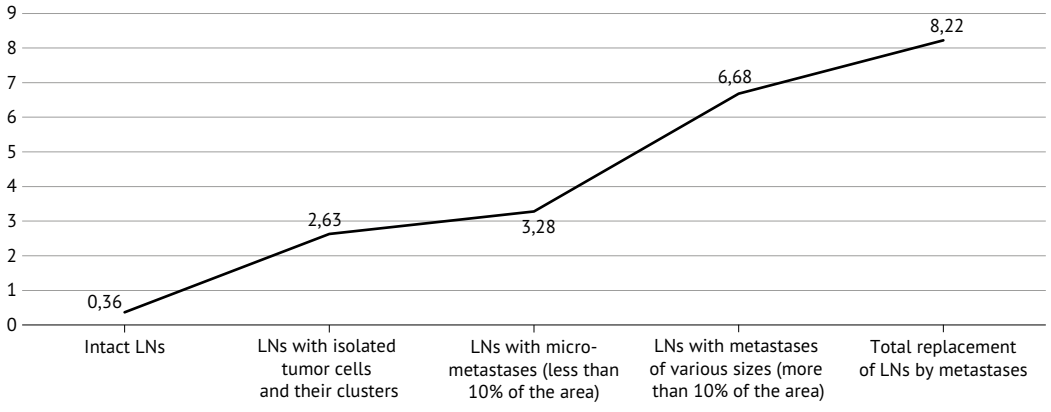

Diagram 1. Incidence of lesions of the circulatory microvasculature of lymph nodes (LNs) at different stages of metastasis (%)

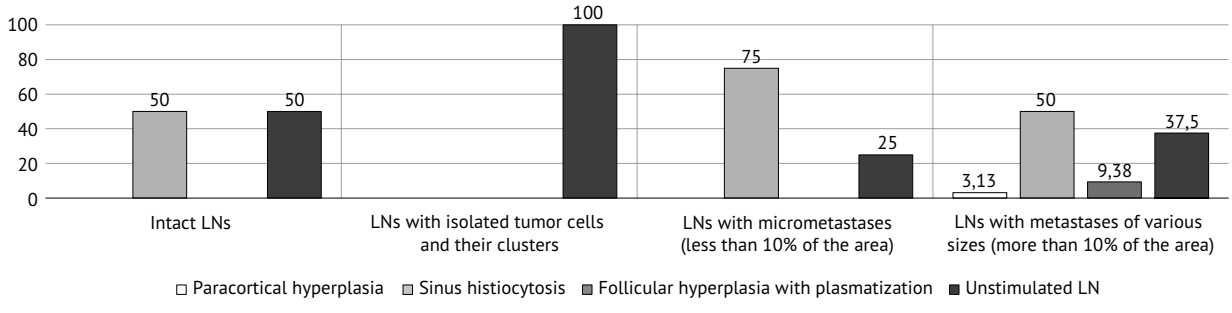

Diagram 2. Incidence of lesions of the circulatory microvasculature of the lymph nodes (LN) depending on the nature of immunomorphological reactions (%)

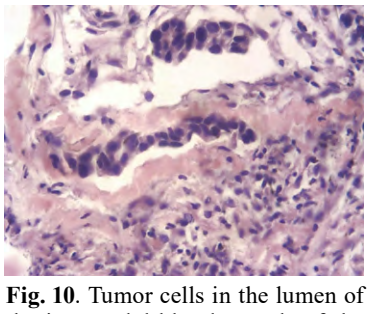

Fig. 10. Tumor cells in the lumen of the intranodal blood vessels of the lymph node with micrometastases. Staining with hematoxylin and eosin (magnification × 400).

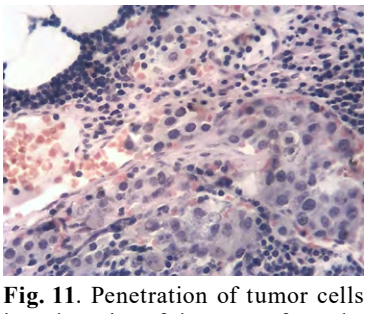

Fig. 11. Penetration of tumor cells into the veins of the cortex from the subcapsular sinus. Staining with hematoxylin and eosin (magnification × 400).

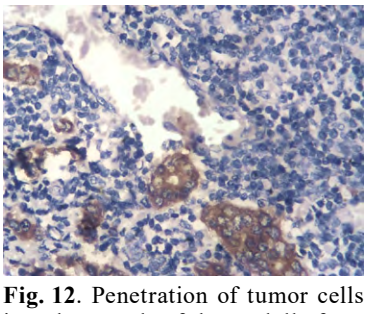

Fig. 12. Penetration of tumor cells into the vessels of the medulla from the intermediate sinuses. Reaction with monoclonal antibodies against pan-cytokeratins. LSAB-method with additional staining with hematoxylin (magnification × 400).

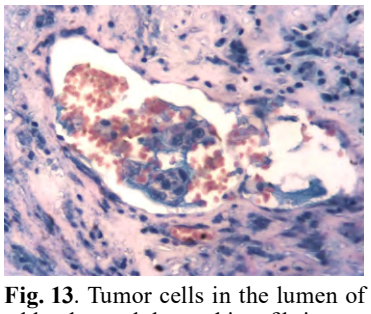

Fig. 13. Tumor cells in the lumen of a blood vessel, located in a fibrin network. Picro-Mallory staining (magnification × 400).

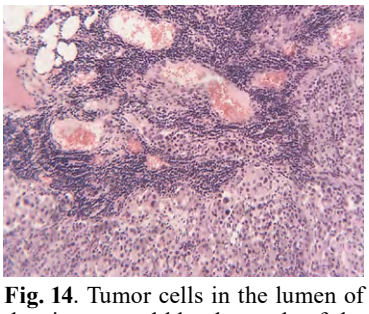

Fig. 14. Tumor cells in the lumen of the sinuses and blood vessels of the lymph node replaced by metastases. Staining with hematoxylin and eosin (magnification × 200).

histiocytosis occurred with the presence of isolated tumor cells and their clusters, as well as micrometastases in most cases, with no invasion of tumor cells into the blood vessels in any case of such a presentation of LN.

The presence of metastases of various sizes, occupying an area >10% of the LN section, significantly increased the frequency of the involvement of the blood microvasculature in the metastatic process. Thus, in the lumen of vessels, mainly intranodal tumor cells were found in 32 (6.68%) of 479 LNs. Metastases most often penetrated into the veins of the cortical substance from the subcapsular sinus (Fig. 11) and less often into the vessels of the medulla from the intermediate sinuses (Fig. 12), which occurred in case of deepening of the microcirculatory disorders, accompanied by the deposition of intra- and extravascular fibrin.

Notably, the fibrin network often contained tumor cells in the lumen of blood vessels (Fig. 13).

Evaluation of the immunomorphological reactions of LNs showed that the involvement of the circulatory microvasculature in the metastatic process in half of all cases (50.00%) occurred in follicular hyperplasia with a high content of B cells and plasmatization or in unstimulated LN (37.50%). However, paracortical hyperplasia with a high content of T cells (3.13%) and sinus histiocytosis with a high content of macrophages (9.38%) were less common. A similar morphological presentation was typical for all LNs of this group, regardless of the vascular involvement. In the presence of metastases, the manifestations of humoral immune reactions prevailed more often. Thus, hyperplastic follicles with large reactive centers were preserved even in a dense environment of the tumor tissue.

With the total replacement of LN by metastases, blood vessels were affected in 8.22% of cases (Fig. 14). Moreover, they were often difficult to distinguish from sinuses because they contained erythrocytes and expression of anti-CD31 MCAB in some cases.

Based on the results of our study, the microvasculature of the LN is involved in the metastatic process along the lymphatic pathways. The vessels in the LNs regional to the cancer are sharply plethorical, their lumen is dilated, and the walls are edematous, with low phosphatase activity and symptoms of mucoid swelling [10]. Moreover, the structures of reticulin fibers and elastic membranes are impaired.

Along with these changes that characterize a decrease in the functional activity of the vascular walls, the rheological properties of blood are impaired, with a tendency for intravascular coagulation, as demonstrated by the deposition of fibrin in the lumen of the vessels. In addition, fibrin masses are also located extravascularly in the lymphoid tissue.

These processes progress depending on the stage of the disease, and fibrin promotes the fixation of metastases in the LN, providing consolidation and nutrition of tumor cells and protecting them from the cytotoxic action of immunocompetent LN cells [11]. However, in previous studies, such microcirculatory disorders were not considered in terms of hematogenous metastasis. We established that the described changes in the circulatory microvasculature of the LN accompany the appearance of tumor cells in the blood vessels.

Hematogenous metastasis is largely influenced by the state of the LN sinuses. The microvasculature of the LN is connected through the intranodal veins with the subcapsular sinus. In addition, with the development of cancer, the sinuses undergo a vascular transformation and contain masses of red blood cells. Yin et al. [12] demonstrated in their study of 1322 LNs in gastric cancer of 809 cases that sinuses contained blood, which was absent in the LNs of healthy people. Moreover, the sinuses expressed CD31, a marker of the blood endothelium in several cases. In our study, we also established the presence of erythrocyte masses in the sinuses, although in fewer cases (289 LN out of 1,263). Vascular transformation of sinuses with the expression of anti-CD31 MCAB was also established. The presence of tumor cells in blood vessels was accompanied by similar changes in the sinuses. We agree with Yin et al. that the lymphogenous and hematogenous pathways of metastasis are interrelated and inseparable.

The possibility of alternative hematogenous metastasis through the LN requires special discussion. According to experimental data, there are two possible options:

1) tumor cells, without lingering in the LN, penetrate the circulatory system and form distant metastases;

2) first, metastasis forms in the LN, and hematogenous dissemination occurs through the extranodal veins.

Most researchers are inclined toward Option 2. Thus, Coste et al. [13] proved that LNs are capable of hematogenous dissemination of cancer cells to distant sites in their study on the metastasis of breast cancer in mice. In their study on the same localization of cancer in mice, E.R. Pereira et al. [14] revealed that tumor cells could enter local blood vessels inside the LN, leave it, enter the circulatory system, and colonize the lung. Such metastasis occurs because extranodal veins communicate with the intranodal veins through the branches penetra-

ting into the LN capsule, and tumor cells from the marginal sinus enter these communicating veins and spread hematogenously.

Based on the studies performed, we also believe that the hematogenous dissemination of cancer often starts after the emergence of metastases in the LN. Moreover, the larger the volume of the LN lesion, the more often tumor cells are present in the blood vessels. Thus, the invasion of the circulatory microvasculature occurred in 2.63% of cases in the presence of single tumor cells in the LN, in 3.28% of cases in the presence of micrometastases, in 6.68% of cases in the presence of large metastases, and in 8.22% of cases with total replacement of the LNs.

The possibility of tumor cells penetrating into the circulatory system without fixing them in the LN cannot be excluded. We identified an isolated lesion of the microvasculature with the presence of tumor cells in the extranodal vessels in the absence of metastases in the LN only in two (0.36%) cases. However, this fact cannot be ignored, especially when examining the sentinel or signal LN, which state influences the volume of lymphadenectomy [15]. Thus, the data obtained by T. Kodama et al. [16] indicate the possibility of hematogenous metastasis from the LN at an early stage of the disease, when it is not possible to detect tumor cells in the LN by standard diagnostic imaging or aspiration biopsy methods. The authors conclude that LN, estimated falsely as N0, could be a source of hematogenous metastasis.

The study of the nature of the immunomorphological reactions of LN showed that the invasion of tumor cells into the circulatory microvasculature occurred either with unstimulated LN (37.50%) or in cases of follicular hyperplasia with a high count of B cells and plasmatization (50.0%). Paracortical hyperplasia with a high count of T cells (3.13%) and sinus histiocytosis with a high count of macrophages (9.38%) were less common because T-cell and macrophage reactions can prevent or restrain the process of metastasis, caused by the high activity of B-lymphocytes with plasmatization due to the blocking action of humoral antibodies on the cytotoxicity of effector cells [17]. Thus, when assessing the lesion of the circulatory microvasculature of the LN, it is necessary to assess the response of the surrounding lymphoid tissue.

CONCLUSIONS

1. The blood microvasculature of regional LN can be both an additional and an alternative way of lymphogenous metastasis of cancer.

2. The incidence of vascular invasion increases with an increase in the volume of metastases in the lymph node.

3. The appearance of tumor cells in blood vessels is accompanied by microcirculation disorders and depends on the nature of the immunomorphological reactions of the lymph node.

4. The presence of tumor cells in the blood vessels is possible if they are absent in the lymphatic vessels and the lymph node sinuses.

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Conflict of interest. The authors declare no conflict of interest.

REFERENCES

1. Kim H.J., Choi G.S. Clinical implications of lymph node metastasis in colorectal cancer: Current status and future perspectives. *Ann. Coloproctol.* 2019; 35 (3): 109–117. DOI: 10.3393/ac.2019.06.122019.

2. Bazhanov A.B., Tsyplakov D.E. State of regional lymph nodes and prognosis in stomach cancer. *Kazan Medical Journal.* 2017; 98 (3): 328–333. (In Russ.) DOI: 10.17750/KMJ2017-328.

3. Carr I. Lymphatic metastasis. *Cancer Metastasis Rev.* 1983; 2 (3): 307–317. DOI: 10.1007/BF00048483.

4. Basha B.M., Hsi E.D. Immunohistochemical analysis of endothelial cells in vascular transformation of lymph node sinuses: Vascular or lymphatic differentiation? *Appl. Immunohistochem. Mol. Morphol.* 2019; 27 (6): 482–489. DOI: 10.1097/PAI.0000000000000661.

5. Tsyplakov D.E. Changes in the microvasculature of regional lymph nodes in the process of cancer metastasis. *Kazan Medical Journal.* 1987; (1): 29–31. (In Russ.)

6. Sarkisov D.S., Perov Yu.L. *Mikroskopicheskaya tekhnika.* (Microscopic technique.) M.: Meditsina. 1996; 544 p. (In Russ.)

7. Zerbino D.D., Lukasevich L.L. Method for determining the "age" of fibrin in the syndrome of disseminated intravascular blood coagulation. *Arkhiv patologii.* 1984; (8): 72–75. (In Russ.)

8. Petrov S.V., Raykhlin N.T. *Rukovodstvo po immunogistokhimicheskoy diagnostike opukholey cheloveka.* (Guidelines for the immunohistochemical diagnosis of human tumors.) Kazan: DESIGNstudio "RED". 2012; 624 p. (In Russ.)

9. Stefanov S.B. Morphometric grid of random step as a means of accelerated measurement of morphogenetic elements. *Tsitologiya.* 1974; (6): 785–787. (In Russ.)

10. Tsyplakov D.E., Ziganshina L.E. The role of microcirculatory factors in cancer metastasis. *Voprosy onkologii.* 1988; (8): 932–936. (In Russ.)

11. Tsyplakov D.E., Petrov S.V. Effect of the microvasculature on the cellular immune response in the lymph nodes regional to the cancer (immunohistochemical and ultrastructural study). *Gematologiâ i transfuziologiâ.* 1995; (6): 3–7. (In Russ.)

12. Yin T., Ji X.L., Shen M.S. Relationship between lymph node sinuses with blood and lymphatic metastasis of gastric cancer. *World J. Gastroenterol.* 2003; 9 (1): 40–43. DOI: 10.3748/wjg.v9.i1.40.

13. Coste A., Karagiannis G.S., Wang Y., Xue E.A., Lin Y., Skobe M., Jones J.G., Oktay M.H., Condeelis J.S., Entenberg D. Hematogenous dissemination of breast cancer cells from lymph nodes is mediated by tumor microenvironment of metastasis doorways. *Front. Oncol.* 2020; 10: 571100. DOI: 10.3389/fonc.2020.571100.

14. Pereira E.R., Kedrin D., Seano G., Gautier O., Meijer E., Jones D., Chin S.M., Kitahara S., Bouta E.M., Chang J., Beech E., Jeong H.S., Carroll M.C., Taghian A.G., Padera T.P. Lymph node metastases can invade local blood vessels, exit the node, and colonize distant organs in mice. *Science.* 2018; 359 (6382): 1403–1407. DOI: 10.1126/science.aal3622.

15. Okur O., Sagiroglu J., Kir G., Bulut N., Alimoglu O. Diagnostic accuracy of sentinel lymph node biopsy in determining the axillary lymph node metastasis. *J. Can. Res. Ther.* 2020; 16 (6): 1265–1268. DOI: 10.4103/jcrt.JCRT_1122_19.

16. Kodama T., Mori S., Nose M. Tumor cell invasion from the marginal sinus into extranodal veins during early-stage lymph node metastasis can be a starting point for hematogenous metastasis. *J. Cancer Metastasis Treat*. 2018; 4: 56. DOI: 10.20517/2394-4722.2018.61.

17. Tsyplakov D.E., Bazhanov A.B. Morphometric and immunohistochemical study of regional lymph nodes in gastric cancer. *Kazan Medical Journal.* 2015; 96 (6): 971–978. (In Russ.) DOI: 10.17750/KMJ2015-971.