

Immunohistochemical Characteristics of Neoangiogenesis Processes in Regional Lymph Nodes in Alcoholic Pancreatitis

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Abstract In alcoholic pancreatitis, numerous foci of neoangiogenesis and abundant formation of microcirculatory plexuses were detected in simple morphological studies. This, in turn, was used to assess the endothelial proliferative index in lymph nodes using the CD34 marker. In immunohistochemical (IHC) examination of regional lymph nodes and pancreas in alcoholic pancreatitis, each of the above markers is expressed in a specific way, reflecting the processes of inflammation, activation of immune cells, and blood vessel remodeling.

Keywords Immunohistochemical examination, Lymph nodes, Alcoholic pancreatitis, Morphology

1. Relevance of the Topic

In the world, pancreatitis is the third most common acute abdominal disease. An average of 960 million people worldwide are diagnosed with pancreatitis, of which 36.6% (262 million) are alcoholic pancreatitis patients between the ages of 25 and 40 [1,2]. In the United States, more than 25 million people aged 25 to 45 suffer from this disease [3]. In Europe, an average of 66.8 million people suffer from alcoholic pancreatitis [4]. In the Russian Federation, an average of 52.3 million people aged 25 to 55 suffer from this disease [5]. In the CIS countries, a total of more than 12 million people are registered with this indicator. Every year, an average of 12-30 million people die from various forms of alcoholic pancreatitis [6,7,8]. However, no source provides information on the morphological features of regional lymph nodes in acute and chronic alcoholic pancreatitis, however, it is important to develop morphofunctional parameters of regional lymph nodes in acute pancreatitis with edematous and necrotic changes and important determinants of the prevention of severe complications [9,10,12].

Research objective: To study the immunohistochemical features of regional lymph nodes in alcoholic pancreatitis.

2. Materials and Methods

Morphological examination methods: hematoxylin eosin,

histochemical methods Van Gieson, Alcian blue, clinical anamnestic and statistical analysis methods are used. Appropriate immunomarkers were obtained for immunohistochemical examinations. Materials of regional lymph nodes of the pancreas were obtained during autopsy of patients who died from chronic and acute pancreatitis.

3. Results and Discussion

Analysis of the level of expression of the CD34 (T-lymphocyte center) marker in alcoholic pancreatitis and its assessment criteria. CD34 is a membrane protein, an intercellular adhesion molecule, which plays an important role in the early stages of hematopoiesis. CD34 ensures the binding of stem cells to the extracellular matrix of the bone marrow or directly to stromal cells.

It serves as a protein scaffold for binding specific glycans that allow the stem cells to bind to lectins produced by the stroma or other components of the bone marrow. In addition, it provides carbohydrate ligands for highly glycosylated CD34 selectins. Mainly when assessing the level of neoangiogenesis after vascular endothelial damage, intussusceptive angiogenesis (the vessel wall is one, the endothelium is divided into 2, the vascular channel is divided into 2 or continues with the doubling of small-caliber vessels), as a result of a sharp increase in vascular stromal structures in the tissue, which allows for the early assessment of sclerosis and cirrhosis.

In our study, it was revealed that in lymph nodes examined with the CD34 marker, a large number of small vessels with a glomeruloid lattice shape were detected on surfaces of 32,000 μm^2 , 64,000 μm^2 , a large number of lymphoid

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follicles, paracortical areas. These changes indicate that in alcoholic pancreatitis, cytokines and other inflammatory factors are produced in large quantities, bone marrow, and hematogenous cell proliferation is increased.

Therefore, The CD34 marker is the most important and widely used marker of endothelial and hematopoietic stem cells in immunohistochemical (IHC) studies (it stains the endothelium of small blood vessels, especially capillaries and venules, with high sensitivity).

Against the background of inflammation, hypoxia, and compensatory processes observed in regional lymph nodes in alcoholic pancreatitis, the following important immunomorphological changes are detected by CD34 expression. Activation of angiogenesis, expansion of the microcirculatory network, and formation of a vascular stroma in the lymph node.

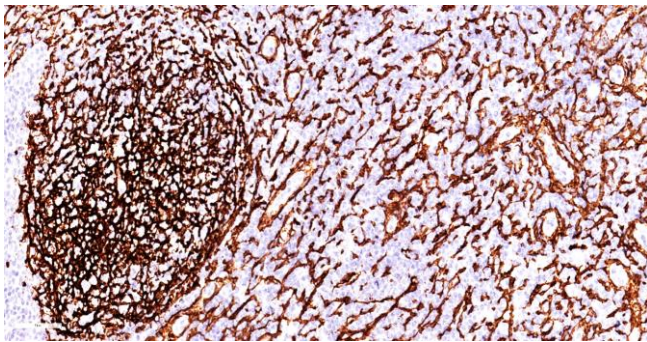


Figure 1. Sample-1. Mesenteric lymph node in alcoholic pancreatitis. High positive expression of the CD34 marker, tortuous vascular trajectory are detected. A tangle consisting of a vascular skeleton is detected in the traces of lymphoid follicles. Staining Dab chromogen. Size 20x10

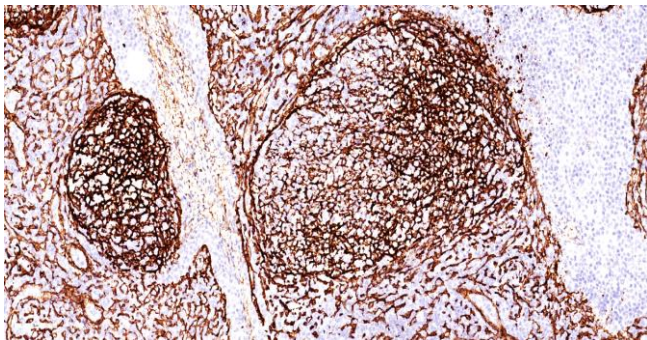


Figure 2. Sample-2. Mesenteric lymph node in alcoholic pancreatitis. High positive expression of the CD34 marker, tortuous vascular trajectory are detected. A tangle consisting of a vascular skeleton is detected in the traces of lymphoid follicles. Staining Dab chromogen. Size 20x10

Toxic inflammation caused by alcohol and its metabolites (acetaldehyde) is characterized by the rapid proliferation of blood vessels in the regional lymph nodes.

CD34 pattern: The number of newly formed small capillaries and venules with a positive reaction for CD34 in the paracortical area of the lymph node and the medulla increases sharply. This creates the necessary conditions for the supply of oxygen and nutrients to the area of inflammation and the influx of immune cells (T and B lymphocytes, macrophages) into the lymph node.

Starting from the subcapsular area of the lymph node, a large number of vascular tangles were detected in the cortical and paracortical areas, and very clear glomerular vascular bundles were detected in place of lymphoid follicles, indicating a sharp increase in vascular proliferation not only in the shadow of the follicles but also in the stroma, which formed a very large area of the lymph node.

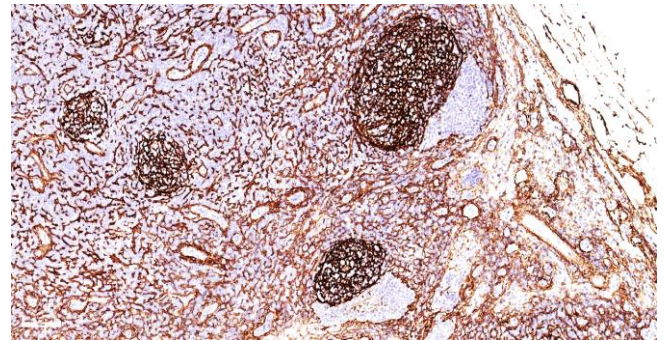


Figure 3. Sample-3. Mesenteric lymph node in alcoholic pancreatitis. High positive expression of the CD34 marker, tortuous vascular trajectory are detected. A tangle consisting of a vascular skeleton is detected in the traces of lymphoid follicles. Staining Dab chromogen. Size 10x10

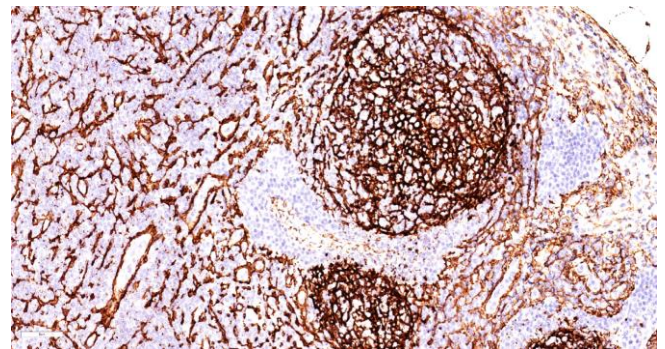


Figure 4. Sample-4. Mesenteric lymph node in alcoholic pancreatitis. High positive expression of the CD34 marker, tortuous vascular trajectory are detected. A tangle consisting of a vascular skeleton is detected in the traces of lymphoid follicles. Staining Dab chromogen. Size 20x10

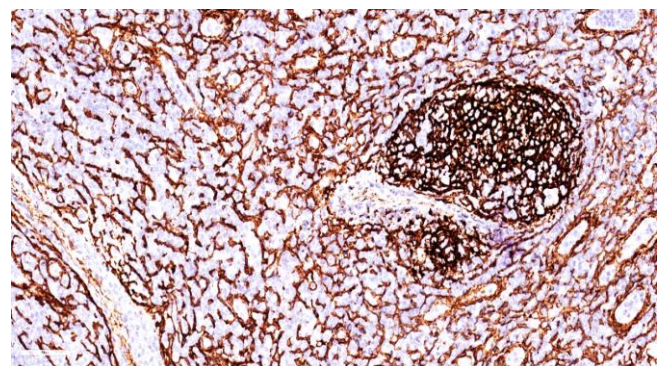


Figure 5. Sample-5. Mesenteric lymph node in alcoholic pancreatitis. High positive expression of the CD34 marker, tortuous vascular trajectory are detected. A tangle consisting of a vascular skeleton is detected in the traces of lymphoid follicles. Staining Dab chromogen. Size 20x10

In our study, all 26 cases were found to have high positive expression of the CD34 marker, which was characterized by increased proliferative activity of endothelial cells in all

anatomical layers of the lymph node, including the perimeter of the cerebral striations, cortical and paracortical areas, and massive intussusceptive angiogenesis over an area of 32,000 μm^2 [12,13,14,15].

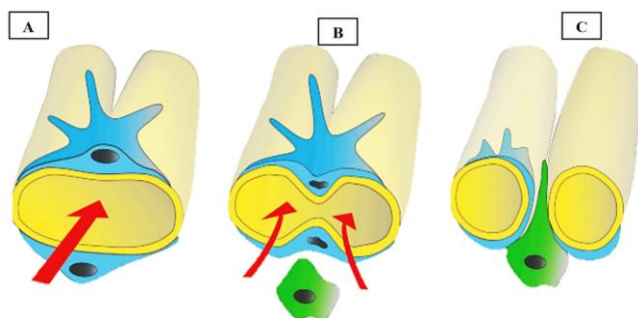


Figure 6. The process of intussusceptive angiogenesis of blood vessels under the influence of the CD 34 marker. In the blood vessel, endothelial proliferation initially leads to the division of the lumen into two and the subsequent formation of two separate vascular lumens.

The proliferation and swelling of endothelial cells, activation of the inner layer of blood vessels under the influence of inflammatory mediators (cytokines, VEGF and other growth factors) are detected. In the blood vessel, endothelial proliferation initially divides the lumen into 2 and subsequently forms 2 separate vascular lumens, which confirms the increase in vascular density without changing the angioarchitecture [16,17,18].

This process was evaluated as an immunomorphological substrate, proving that alcoholic pancreatitis is not acute, but chronic. At the same time, in our study, it was proven that chronic hypoxia progressively progresses clinically morphologically, increasing by 2.5 times in the 35-43-year-old contingent compared to the 44-52-year-old contingent. Analysis of microscopic images by immunohistochemical staining revealed swelling of the vascular endothelium, invagination, narrowing of the vascular lumen, thickening of the vascular wall and clearly visible signs of cell division (mitosis) [19,20]. This, microscopically, manifests itself in the form of stasis and erythrocyte sludge phenomena in the microcirculatory vessels in the proliferation phase, which proves that a massive intussusceptive angiogenesis process is taking place in the vessels with these changes [21]. Endothelial dysfunction and microcirculatory disorders (increased stasis and permeability) The toxic effect of alcohol sharply increases the permeability of the vascular wall (perivascular edema occurs). As a result, the density of the vascular arrangement is disturbed, capillary interweaving occurs, and in areas of erythrocyte stasis, fragmentation or hyperdense accumulation of the endothelial network is observed [22].

The CD34 marker is of decisive importance in assessing the adaptive and destructive changes occurring in the vascular system of regional lymph nodes in alcoholic pancreatitis. It accurately indicates the intensity of angiogenesis during the inflammatory period and the effect of long-term intoxication on microcirculation.

The results of immunohistochemical analysis of regional

lymph nodes in the control group (those who died of sudden myocardial infarction or hemorrhagic stroke) and the study group were compared and the severity indicators were evaluated in a scoring system and presented in the table. The criteria for assessing the severity of expression in regional lymph nodes based on the following ICH markers (CD3, CD20, CD68, CD34) were developed in the form of the following table and evaluated using a scoring scale.

Authors' Contribution

Conceptualization, Y.A.M. and A.E.L.; methodology, Y.A.M.; validation, Y.A.M. and A.E.L.; formal analysis, A.E.L.; investigation, A.E.L.; resources, Y.A.M.; data curation, A.E.L.; writing—original draft preparation, A.E.L.; writing—review and editing, Y.A.M.; visualization, A.E.L.; supervision, Y.A.M.; project administration, Y.A.M. All authors have read and agreed to the published version of the manuscript.

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Ethics Approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Fergana Medical Institute of Public Health (protocol code K29, approval date 08.26.2025). Only archival, de-identified post-mortem material was used.

Consent for Publication

Patient consent was waived because the study used only de-identified archival autopsy material and no individual can be identified from the data or images presented.

Data Availability Statement

The histological slides, digital micrographs and anonymised grading datasets that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly archived because of institutional restrictions on post-mortem material.

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Conflict of Interest

The authors declare no conflicts of interest. No funders had any role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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