

# Morphological and Immunohistochemical Changes in the Large Intestine in White Rats with Experimental Pneumonic Fibrosis

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**Abstract** This article reveals morphological changes in the lymphoid structures of the colon wall of purebred rats with experimental pneumosclerosis. When studying the immune status of rats against the background of prolonged hypoxia in experimental pneumosclerosis, significant disturbances were revealed in the form of a sharp decrease in the number of lymphocytes. This served as the basis for a comparative analysis of various morphological changes in lymphoid tissue and dynamics in rats, and allowed us to determine structural changes in intestinal tissues.

**Keywords** Colon, Pneumosclerosis, Experiment, Hypoxia, Morphology, Immunohistochemistry, Lung

## 1. Introduction

According to the World Health Organization, lung diseases are one of the world's greatest challenges, causing 1/6 of global deaths. The term "pneumosclerosis" defines a pathological condition in which the lung parenchyma undergoes an irreversible process of excessive growth, sclerosis and / or scarring, which is associated with excessive deposition of extracellular matrix components, including collagen. Fibrosis of lung tissue is an irreversible process that can only be prevented or stopped in the early stages [1,2].

Interstitial lung diseases (ILDs) are a heterogeneous group characterized by a variety of clinical, radiological and pathological patterns that widely affect the lung parenchyma. Some IPFs are characterized by varying degrees of pneumosclerosis, of which idiopathic pneumosclerosis (idiopathic pulmonary fibrosis, IPF) is the most representative. IPF has the worst prognosis, with a median survival of 2–5 years after diagnosis, making it a major medical problem that has not yet been resolved. IPF is more common in older age, in men than in women, and in the absence of any specific provocation. Pneumosclerosis also represents the final stage of IPF [3,4,5]. Fibroblasts play an important role in tissue repair by proliferating and differentiating into myofibroblasts and by modulating the volume of the extracellular matrix [7,9]. Myofibroblasts produce a denser extracellular matrix than fibroblasts, and the presence of smooth muscle actin

leads to spatial reorganization of collagen fibrils. Thickening and densification of lung tissue impedes gas exchange and ultimately leads to decreased lung function [8,10,11].

**The aim of the research.** To determine the dynamics of the condition and characteristics of lymphoid tissue in various parts of the colon wall in experimental pneumosclerosis.

## 2. Materials and Methods

The experiment involved 120 mature white mongrel rats weighing 220–240 g and aged 5–6 months. All animals were divided into experimental and control groups. Before the experiment, the rats were acclimatized for 7 days in standard conditions corresponding to the sanitary standards of Uzbekistan. The conditions included a temperature of 20–24°C, humidity of 50–70%, and a 12-hour light cycle (day/night). The animals were fed standard granulated feed and had free access to water. The experimental group included 96 animals, the control group consisted of 24. The method of inhalation exposure to nitric oxide (NO) was used to reproduce pneumosclerosis. The concentration of NO in the air was 10 ppm for a long range. The exposure was carried out daily for 1 hour in a sealed chamber with a controlled gas composition. The exposure period was 6 days. This contributed to the induction of a chronic inflammatory process in the lung tissue, which is a key pathogenetic mechanism for the development of pneumosclerosis. After the end of the experiment, the animals were euthanized with the collection of lung tissue samples for histological and immunohistochemical examination.

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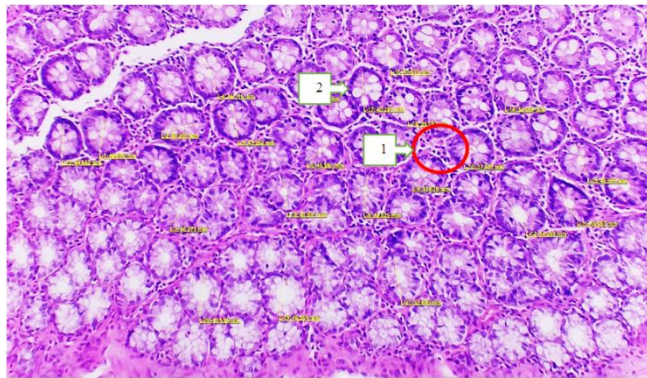
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3. Results and Discussion

Based on the morphological parameters, the following morphometric data of the large intestine were obtained. In the morphological and morphometric study of the large intestine tissue obtained after dissection of white poor rats of the control group, the wall of the large intestine consists of 4 layers, the mucous layer is covered with a single-layer prismatic epithelium. In the field of view, high columnar epithelial cells, gocaloid cells and a large number of undifferentiated cells were observed. The mucous membrane is represented by a thin layer of connective tissue with a crypt, white fibrous tissue of the control group is on average 3.1-5.3 μm. The crypts were deep, slightly widened apical part, ix and medium in diameter, and the control squirrel crystallized 9.4-9.7 μm. Under the microscope, it was found that the crypts are in a state of weakly oxyphilic cytoplasm and in a state of gocaloid cells. The number of goblet cells in the crypts is 7–19 (Fig. 1).



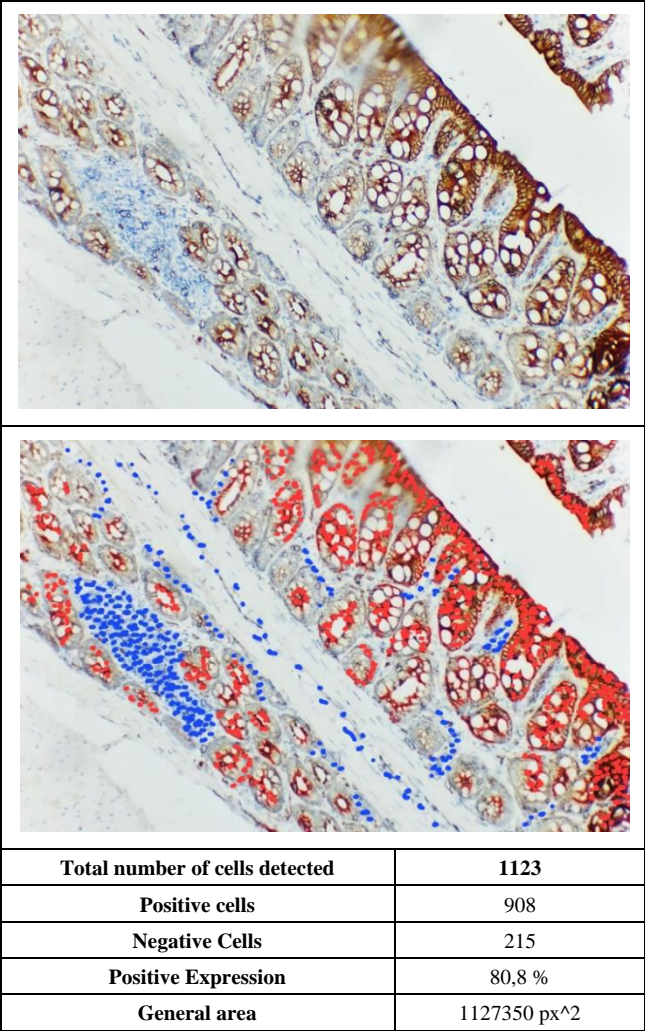
**Figure 1.** Microscopic picture of the large intestine of white outbred rats of the main group. Hematoxylin and eosin staining. 40x20. 1-Cross-section of crypts; 2-goblet cells

There are few lymphocytes and macrophages in the field of view. An average of 57 lymphocytes were counted per field of view. The muscular layer of the mucosa has a 2-layer structure: the outer layer consists of longitudinal (muscle cells) and the inner part - circular muscles. The cellular composition of the submucosal layer is represented mainly by loose fibrous connective tissue cells, a small amount of fat and adipose cells. The muscular membrane has a normal morphological structure. The outer layer is represented by bundled longitudinal, three-band-shaped fibers.

Between these bands, a small number of clusters of smooth myocytes were found, and the inner layer was represented by circularly oriented fibers. Between them, loose fibrous connective tissue, blood vessels, and a small amount of lymphatic and nerve fibers were found to be located in the muscular-intestinal tangle. The combined thickness of the submucosa and muscularis mucosa of the colon of the control group of white outbred rats was 492.43 mkm.

Increased Ki-67 expression was observed in the colonic mucosa after exposure to (NO<sub>2</sub>) nitrogen dioxide. Ki-67 was most localized, i.e. its expression was observed more in the mucosal walls and colonic crypts (parts of the colon with a large number of dividing processes). This indicated an attempt

to repair the damaged mucosa and activate the immune system.



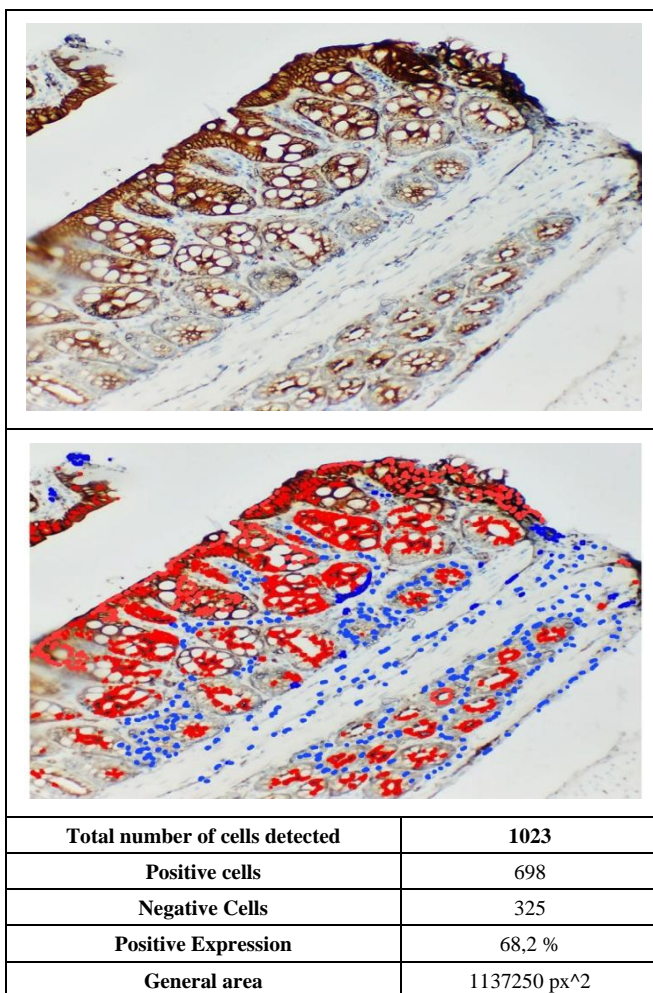
Total number of cells detected	1123
Positive cells	908
Negative Cells	215
Positive Expression	80,8 %
General area	1127350 px^2

**Figure 2.** In white rats of the experimental group, a high level of expression of the Ki-67 marker was observed in the tissue of the colon mucosa. The smear was stained with the chromogenic method

The image was enlarged 400 times. QuPath-0.4.0.ink. was scanned in the program and the expression level was determined. Expressed cells are highlighted in red. Morphometric studies using QuPhat 4.4.0 and 5 polyax zreniya pri 200-400 times magnification are carried out for immunohistochemical results. Positively expressed cells in the selected region are calculated as a percentage of the total number of cells and the total area. The expression level is about 20% (low level expression), 20-60% (medium level expression) and more than 60% (high level expression) (Fig 2). The expression of the immunohistochemical marker Ki-67 is a vital indicator of the proliferative activity of cells, which allows the state of cell division and regeneration of tissues. The expression of Ki-67 in the result of the production of nitrogen dioxide (NO<sub>2</sub>) in the mucous membrane of the thick lining of the intestine, as it affects the proliferation of cells and the development of pathological cells.

Expressed cells are highlighted in red. Immunohistochemical examination of the small intestine allows us to evaluate morphological and functional changes in tissues that occur

against the background of systemic pathologies, such as pulmonary pneumosclerosis. For this purpose, we determined the features of morphological restructuring of the intestine during chronic hypoxia caused by pulmonary pneumosclerosis using immunohistochemical markers of inflammation, apoptosis and regeneration. Red-colored areas indicate the epithelial structures of intestinal crypts, which indicates changes in regenerative processes. Connective tissue is predominantly colored in blue, indicating the accumulation of cellular elements, such as immune cells. Destruction of the mucous membrane, a possible increase in the number of inflammatory infiltrates and structural changes in the crypts were observed. The Ki-67 marker determined the proliferative activity of crypt cells.



**Figure 3.** In white rats of the experimental group, a high level of expression of the Ki-67 marker was observed in the tissue of the colon mucosa. The smear was stained with the chromogenic method. The image was enlarged 400 times. QuPath-0.4.0.ink. was scanned in the program and the expression level was determined

## 4. Conclusions

The study found high expression of the Ki-67 marker in

the colonic mucosa and submucosa, especially in the crypt walls and goblet cells (86.6%, 80.8%, and 68.2%). This showed that cell proliferative activity increased in response to (NO<sub>2</sub>) nitrogen dioxide, a substance used to induce experimental pneumosclerosis. Changes in the morphological structure of the intestine against the background of pneumosclerosis may include mucosal atrophy, impaired epithelial regeneration and an increased inflammatory response, which is confirmed by immunohistochemical studies.

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