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Histological changes in the colon wall in adult patients with chronic slow-transit constipation

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The prevalence of chronic constipation in the population ranges from 3 % to 27 %. Women, senile people, and people of low socio-economic status are in this risk group more often. Many histological studies of the intestinal wall were performed in order to find the causes of slow-transit constipation [6, 4]. The different pathological changes, including myopathies, neuropathy, and pathology of the interstitial cells of Cajal, were established. The specific distribution of the types of histological changes in the colon wall in patients with slow-transit constipation, as presented in the London classification, is currently unknown.

Objective — to determine the specific distribution of the types of histological changes in the colon wall in patients with slow-transit constipation unresponsive to conservative treatment.

Materials and methods. A pathomorphological comparative case-control study was performed. The main group included 105 patients who underwent colectomy as a treatment for chronic slow-transit constipation in the period 2011—2023. The surgical intervention was indicated for patients with chronic slow-transit constipation, resistance to conservative treatment, and a notable decline in quality of life. The comparison group included 27 deceased persons who did not experience constipation during their lifetime. The patient exclusion criteria were Hirschsprung's disease, proctogenic constipation (dysfunction of the rectum and pelvic floor), medication-associated constipation, as well as mental disorders. The histological and immunological examinations of samples were carried out in both groups, in the comparison group — appendix, ileum, cecum, colon and sigmoid colon.

Results. Four main morphological phenotypes of the colon wall structure elements in patients with chronic slow-transit constipation were identified according to the research data: 1) histologically intact type, 2) myopathic type, 3) Cajal type, 4) neuropathic. A combination of different types of histological changes was also registered, but one of them usually dominated.

Conclusions. Four main types of histological changes in the intestinal wall were found in patients with chronic slow-transit constipation resistant to conservative treatment: myopathic changes (56.2 %), Cajal cell pathology (19 %), neuropathic changes (19 %), and a histologically intact variant (5.8 %). The myopathic type is characterised by the heterogeneity of morphological manifestations, which can be referred to as dystrophic changes (dystrophic subtype 79.7 %) and inflammatory changes (inflammatory subtype 20.3 %), p = 0.001.

Keywords
chronic slow-transit constipation, myopathic changes, neuropathic changes, Cajal cell pathology.

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The prevalence of chronic constipation in the population ranges from 3 % to 27 % [10]. The prevalence of constipation among the adult population is about 14 %, according to a meta-analysis based on 45 studies involving 261,040 adults [11]. Women, senile people, and people of low socio-economic status are in this risk group more often [2, 3, 8, 11].

According to the 2018 French National Society of Coloproctology recommendations, chronic constipation should be treated conservatively; surgical treatment should be considered only in cases where all conservative treatment methods are ineffective. The recommended surgical intrusion is a total colectomy [12]. More recent 2022 German guidelines suggest that patients diagnosed with slow-transit constipation who are unresponsive to conservative treatment but still have preserved upper gastrointestinal function and normal rectal evacuation function should undergo a total colectomy (either open or laparoscopic) with ileorectal anastomosis [1].

Many histological investigations were conducted on the intestinal wall with the aim of identifying the underlying factors contributing to slow-transit constipation [4, 6]. The different pathological changes, including myopathies, neuropathy, and pathology of the interstitial cells of Cajal, were established. The London classification encompasses the neuromuscular pathology of the gastrointestinal tract [7]. The histopathological changes observed in patients with chronic slow-transit constipation differ from those observed in cases of hypogangliosis or Hirschsprung's disease [9].

The specific distribution of histological changes in the colon wall among patients with slow-transit constipation, as presented in the London classification, remains uncertain.

Objective — to determine the specific distribution of the types of histological changes in the colon wall in patients with slow-transit constipation unresponsive to conservative treatment.

Materials and methods

General characteristics of research groups

A pathomorphological comparative case-control study was performed. The main group included 105 patients who underwent colectomy as a treatment for chronic slow-transit constipation in the period 2011—2023. The surgical intervention was indicated for patients with chronic slow-transit constipation, resistance to conservative treatment, and a notable decline in quality of life. The comparison group included 27 deceased persons who did not experience constipation during their lifetime.

The patient exclusion criteria were Hirschsprung's disease, proctogenic constipation (dysfunction of the rectum and pelvic floor), medication-associated constipation, as well as mental disorders.

The duration of constipation before surgery in the main group was 1 year to 50 years, on average 21.0 ± 14.0 years (Fig. 1).

The interval between defecation was 3 days to 30 days, on average 9.2 ± 4.83 days (Fig. 2).

The passage of contrast radiopaque markers lasted from 3 days to 30 days, on average 10.0 ± 4.3 days. Colectomy with ileorectoanastomosis without low resection of the rectum was performed in 72 (68.6 %) patients; colectomy with low resection of the rectum in 21 (20 %); and laparoscopic colectomy in 12 (11.4 %).

In the comparison group, the autopsy was performed no later than 8 hours after death, on average 6.2 ± 0.8 days.

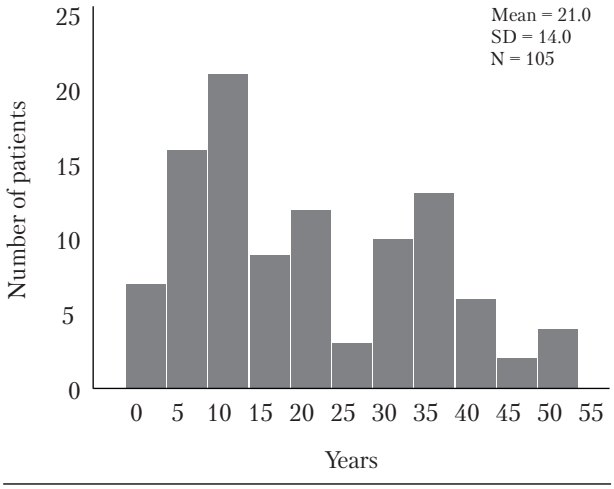

Figure 1. **Distribution of patients by age in the main group**

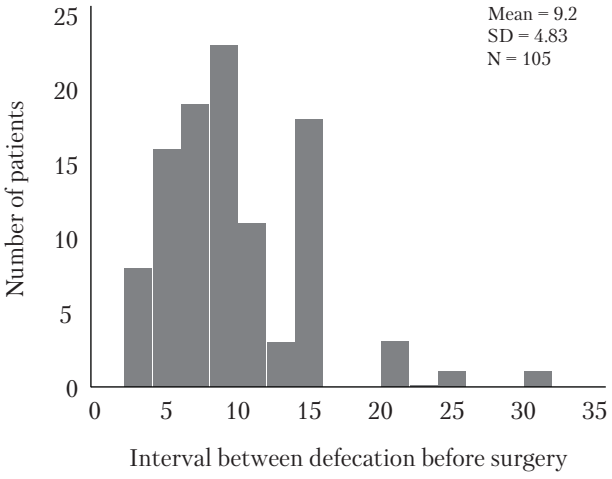

Figure 2. **Distribution of patients in the main group according to the interval between defecations before colectomy**

The main group and the comparison group did not differ statistically by the average age of 43.2 ± 13.7 years versus 45.3 ± 12.2 years ($p > 0.05$) and the ratio of women to men: 95.2 versus 4.8 % and 88.9 against 11.1 %, respectively.

The histological and immunological examinations of samples were carried out in both groups, in the comparison group — appendix, ileum, cecum, colon and sigmoid colon. For our study, samples were taken from all sections of the colon and appendix. Specifically, we obtained samples from at least three sections of the entire thickness of the colon in the transverse and longitudinal projections. Each section was required to be at least 2 cm long. The tissue was fixed in 10 % buffered formalin, wired in alcohol, and embedded in paraffin after collection. Serial sections were stained with the hematoxylin-eosin method. Furthermore, an immunohistochemical analysis was conducted using the Polyclonal Ra a-Hu CD 117, c-kit (Dako, Denmark) antibody on the paraffin blocks. The imaging EnVision FLEX System was chosen to detect Cajal cells.

Pathohistological study

Microscopic examination was carried out on an Olympus CX23 microscope with a nozzle. Morphometric measurements were conducted using the Olympus Stream program. The structure of the entire intestinal wall was evaluated during the histological examination. The architectonics of the glands and the cellular stroma composition in the mucous membrane were described.

The total muscle layer thickness was evaluated, and the ratio of thickness to the layer's number, the presence of lymphoid cell infiltration, the presence or absence of cytoplasmic inclusions in smooth myocytes, and their relative size were estimated.

Meissner's plexuses were identified between the outer and inner muscle layers according to typical histological features; glial cells were determined by their size, the presence of large vesicular nuclei, and Nissl substance; their approximate number was calculated; and additional characteristics were assessed: the presence or absence of dystrophic changes and lymphoid infiltrates.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics, V 22. Descriptive statistics were calculated. The data were assessed for normality using the Shapiro-Wilk test. Mean values are presented as M ± SD. Categorical data were marked as numbers (%). A comparison of quantitative variable mean values was performed using the Mann-Whitney U-test. Relative values were compared using the Pearson xi-square test. The null hypothesis of variable equality was rejected at $p < 0.05$.

Results

Four main morphological phenotypes of the colon wall structure elements in patients with chronic slow-transit constipation were identified according to the research data:

1) histologically intact type,
2) myopathic type,
3) Cajal type,
4) neuropathic.

A combination of different types of histological changes was also registered, but one of them usually dominated.

Morphological criteria of myopathic type

Changes in the structure of the muscle layer can be divided into two variants depending on the predominance of the inflammatory component, or the so-called dystrophic changes:

A) myopathic dystrophic type,
B) myopathic inflammatory type.

The myopathic dystrophic type is characterised by hyaline and cytoplasmic inclusions in the cytoplasm of smooth myocytes, (17 % of cases) hypertrophy of smooth myocytes with thickening of the muscle layer (Fig. 3*A*; 8.5 % of cases), or vice versa, atrophy of myocytes with thinning of the muscle layer (Fig. 3*B*; 85.1 % cases). In most cases, there is an irregular thinning of the muscle layer in different sections of the large intestine. A combination of various changes was also observed, for example, thinning of the muscle layer in the cecum and thickening in the sigmoid. The sigmoid colon and cecum are more prone to thinning. Morphological changes in patients with the myopathic type of constipation and their frequency are shown in Table 1.

The inflammatory myopathic type is characterised by the presence of inflammatory, often lymphocytic, infiltration of the muscle layer (Fig. 3*C*, Table 1).

In our study, the myopathic type was found in 59 (56.2 %) patients; among them, the dystrophic subtype was established in 47 (79.7 %), and the inflammatory subtype was observed in 12 (20.3 %) ($p = 0.001$).

Morphological criteria of neuropathic type

Colonic nervous system changes may include a change in the neuronal number in nerve clusters: hypogangliosis (90 % of cases) (Fig. 4A), hypergangliosis (10 % of cases) (Fig. 4*B*), degenerative changes in neuronal bodies, and glial cell changes in nerve plexuses. It is possible to observe cytoplasmic

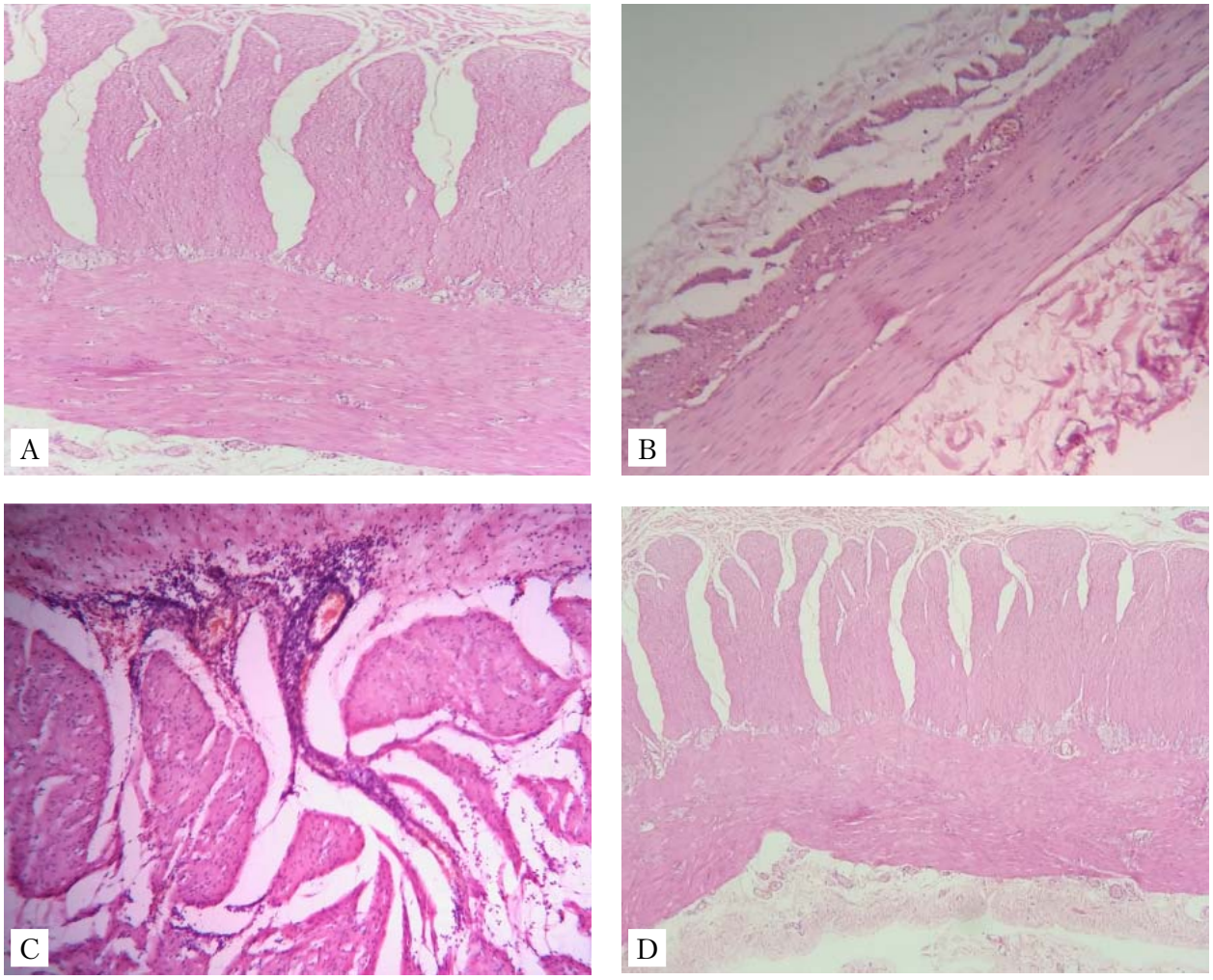

Figure 3. **Histological examination of colon wall specimens in patients with myopathic constipation. Staining: hematoxylin/eosin. Equal thickening of the inner and outer layers of the muscle layer,** ×100 **(A); atrophy of the muscle layer in the right parts of the intestine and its thickening in the left parts,** ×100 **(B); chronic, mainly perivascular lymphoid cell inflammatory infiltration of the muscle layer,** ×100 **(C); the structure of the muscle wall of the large intestine is normal,** ×400 **(D)**

Table 1. **Morphological changes in the myopathic type of constipation**

Pathological changes	Myopathic type (n = 59)		Comparison group (n = 27)
	Dystrophic (n = 47)	Inflammatory (n = 12)	
Changes in the thickness of the muscle layer			
Thinning in all parts	40 (85.1 %)*	0#	3 (11.1 %)
Thickening in all parts	4 (8.5 %)	10 (83.3 %)*#	1 (3.7 %)
Combination of cecal wall thinning and sigmoid wall thickening	3 (6.3 %)	2 (16.7 %)	0
Presence of hyaline and cytoplasmic inclusions in myocytes	8 (17.0 %)*	0#	0
Inflammatory lymphocytic infiltration of the muscle layer	0	12 (100 %)*#	0

* Significant differences with the comparison group ($p < 0.05$).
# Significant differences with the dystrophic subtype ($p < 0.05$)

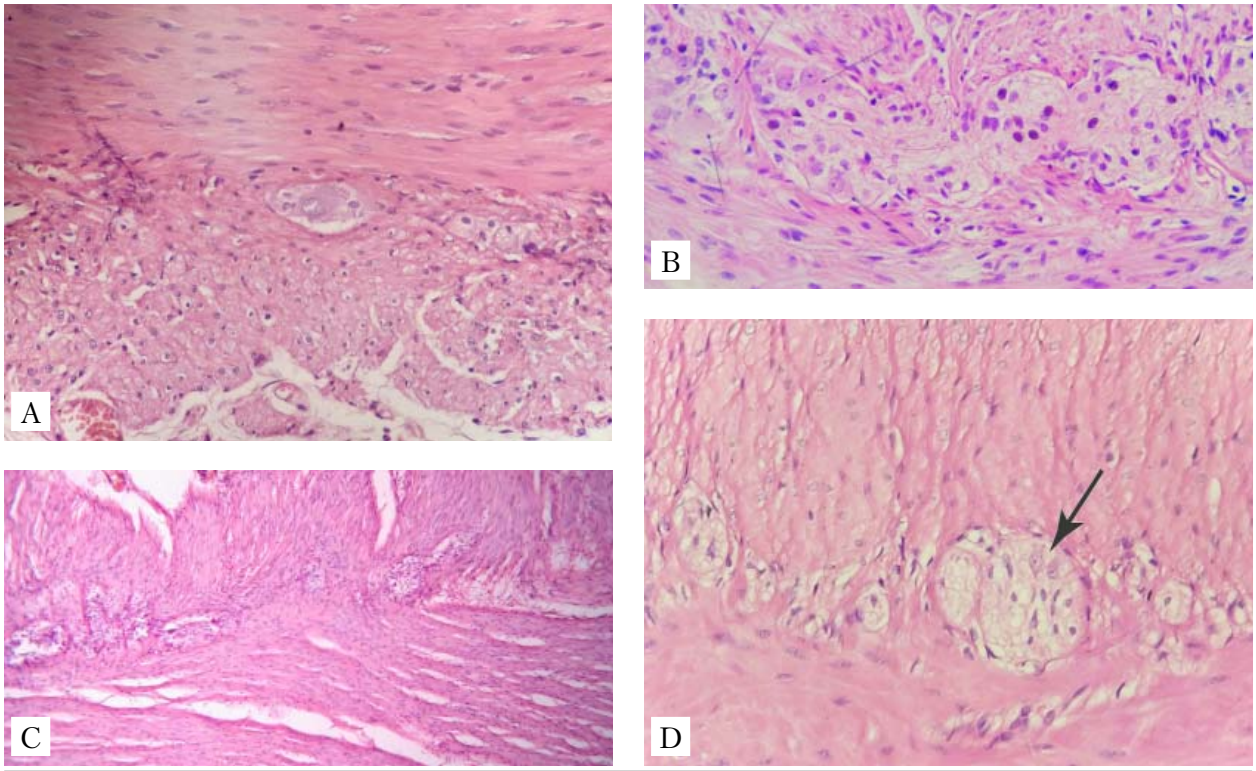

Figure 4. **Histological examination of colon wall specimens in patients with neuropathic constipation. Staining: hematoxylin/eosin. A single neuron body in the intermuscular plexus with significantly increased size, pale cytoplasm, and luminescence in the nucleus,** ×100 **(A); multiple neurons are seen in the intermuscular plexus, marked by arrows,** ×400 **(B); lymphocytic infiltration of nerve plexuses,** ×100 **(C); neurons in the intermuscular nerve plexuses are normal. They are determined by the presence of vesicular nuclei and light, voluminous cytoplasm,** ×400 **(D)**

Table 2. **Morphological changes of the neuropathic type**

Pathological changes	Neuropathic type (n = 20)	Comparison group (n = 27)
Change in the number of neurons in nerve clusters		
Hypogangliosis	18 (90.0 %)*	0
Hypergangliosis	2 (10.0 %)	1 (3.7 %)
Degenerative changes in neurons		
Cytoplasmic swelling	19 (95.0 %)*	2 (7.4 %)
Presence of cytoplasmic inclusions	8 (40.0 %)*	0
Inflammatory infiltration of nerve plexuses	8 (40.0 %)*	3 (11.1 %)
Glial cell changes in nerve plexuses	15 (75.0 %)*	8 (29.6 %)

*Significant differences with the comparison group ($p < 0.05$).

swelling of the neuron bodies (Fig. 4A) and the presence of cytoplasmic inclusions (Fig. 4A). Furthermore, chronic inflammatory infiltration of nerve plexuses was observed in one case (Fig. 4*C*). Morphological changes in the neuropathic type of constipation and their frequency are shown in Table 2.

Morphological criteria of the Cajal type

Morphological changes in Cajal cells can be visualised exclusively using an immunohistochemical study. One of the most specific immunohistochemical markers expressed by Cajal cells is the C-kit protein (CD117). A decrease in expression

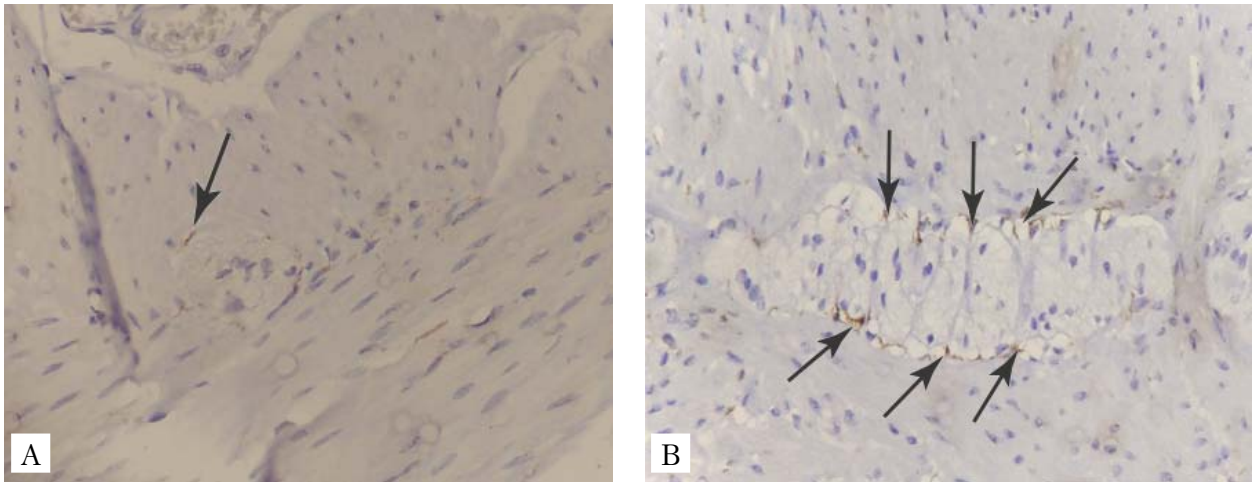

Figure 5. **Immunohistochemical study of C-Kit expression by Cajal cells in the colon wall.**
Background staining: hematoxylin. Magnification ×400.
Expression of the C-kit protein (CD117) in a single cell (indicated by an arrow) in the nerve plexus (A); C-kit (CD117) protein expression by Cajal cells is normal, indicated by arrows (B)

strength and a decrease in cell number expressing this protein were found in some cases of slow-transit constipation (Fig. 5).

In the histologically intact variant, no differences between the studied parameters in the comparison group were found.

Most often, the myopathic type of histological changes was detected in 59 (56.2 %) patients, and the myopathic dystrophic type was dominant. The neuropathic type of constipation and constipation associated with Cajal cell pathology occurred with almost the same frequency in 19 % of cases. Histological or immunohistochemical changes were not observed in 6 patients («histologically intact type») in contrast to the control group.

The youngest patients were diagnosed with Cajal and myopathic inflammatory types of constipation, and the oldest with neuropathic. The detection frequency for various types of histological changes is shown in Table 3.

The age of patients with the Cajal type was significantly lower than with the myopathic ($p = 0.006$) and neuropathic types ($p < 0.001$). Patients with the neuropathic type were significantly older compared to those with the myopathic type ($p = 0.003$).

The age at which Cajal-type manifestation occurred was found to be significantly lower compared to both myopathic ($p = 0.038$) and neuropathic ($p < 0.006$) types. The onset of symptoms in patients with the neuropathic type did not differ significantly compared to the myopathic type ($p = 0.442$).

Discussion

The decision to perform colectomy was based purely on clinical and instrumental criteria and

Table 3. **Detection frequency for various types of histological changes in patients with chronic slow-transit constipation**

The main variant of histological changes	Number of patients	Age. years	
		Mean	Mediana
Myopathic variant			
Inflammatory	12 (11.4 %)	36.9 ± 8.5	35
Dystrophic	47 (44.8 %)	43.7 ± 12.8	44
Total	59 (56.2 %)	43.1 ± 12.7	43
Pathology of Cajal cells	20 (19.0 %)	33.9 ± 10.3	35
Neuropathic	20 (19.0 %)	52.5 ± 13.5	54
Histologically intact	6 (5.8 %)	49.2 ± 14.8	44

Table 4. **The age of manifestation for different variants of histological changes in patients with chronic slow-transit constipation**

The main variant of histological changes	Number of patients	Manifestation age. years
Myopathic type	59 (56.2 %)	21.38 ± 14.8
Inflammatory	12 (11.4 %)	21.0 ± 12.6
Dystrophic	47 (44.8 %)	21.5 ± 15.5
Pathology of Cajal cells	20 (19.0 %)	11.9 ± 9.2
Neuropathic	20 (19.0 %)	27.2 ± 20.4
Histologically intact	6 (5.8 %)	48.75 ± 15.0

the ineffectiveness of conservative treatment over a period of 6 months to 1 year for the majority of patients in this study. The possibility of a preoperative histological diagnosis was considered, and a biopsy during colonoscopy was performed in some patients. However, we refused biopsy as the material primarily consisted of the mucous membrane with a small amount of the muscle layer, so it made the procedure uninformative and dangerous due to the risk of perforation (Table 4).

The main cohort of patients with chronic constipation in our study were women [2, 3, 8, 11].

The available literature does not provide information regarding the frequency of detecting different forms of histopathological changes in patients with chronic constipation. Some authors [6] pay attention to the pathology of interstitial cells of Cajal in the development of constipation [4], although these changes were detected in only 19 % of cases according to our materials. Myopathic changes were detected in most patients (56.2 %) with constipation, and it is not clear whether they were the primary cause of constipation or its consequence. This requires further analysis.

Gonçalves found that severe neuropathic changes were observed in young people, appeared in early childhood, and, accordingly, were treated by paediatricians [5].

The oldest patients who underwent surgery in our study were those diagnosed with neuropathic constipation. This indicates a slow progression of severe constipation with these types of histological changes. Compared to the Cajal cell pathology, the myopathic dystrophic type of constipation was more common in older people. This difference in prevalence may be attributed to its slower development and acquired pathogenesis, which result from the accumulation of dystrophic changes in the intestinal muscular layer with age.

In contrast, the myopathic inflammatory type was found in younger people, which can be related to inflammatory processes in the intestinal wall, which later led to muscle layer dystrophy. On the contrary, the Cajal cell pathology is most likely congenital [4] and related to the youngest operated patients with this type of change in our study. The latter also corresponds to the data we received regarding the manifestation of various types of constipation in patients.

Limitations of the study. The histological data were obtained only from patients resistant to conservative treatment, so there is no data from the entire population of patients with constipation. This study did not include a large group of patients with severe constipation since birth or early age who were treated before the age of 18.

The histologically intact type probably includes patients with dysfunction of the neuromuscular apparatus, which cannot be identified by the available histological or immunohistochemical examination. Therefore, it requires further study.

Myopathic constipation subtypes, as well as immunohistochemical variants associated with abnormalities in neuropeptide expression, which are important in the development of chronic slow-transit constipation, were not studied in this work. Changes in cells described in other works [7], such as the pathology of mitochondria or other organelles, were also not studied because we did not use electron microscopy.

Serotonin receptor changes that can be referred to as histologically intact constipation were also not studied. A preoperative histological diagnosis remains unresolved because the main changes occur in the deep layers of the intestinal wall, and an entire intestinal wall biopsy is a technically difficult and dangerous procedure due to the risk of perforation. Therefore, laparoscopic appendectomy should be considered as an option for taking material for preoperative histological diagnosis.

Conclusions

Four main types of histological changes in the intestinal wall were found in patients with chronic slow-transit constipation resistant to conservative treatment: myopathic changes (56.2 %), Cajal cell pathology (19 %), neuropathic changes (19 %), and a histologically intact variant (5.8 %).

The myopathic type is characterised by the heterogeneity of morphological manifestations, which can be referred to as dystrophic changes (dystrophic subtype 79.7 %) and inflammatory changes (inflammatory subtype 20.3 %), p = 0.001.

Declaration of interests

The authors declare that they have no conflicts of interest.

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Authors contributions

I. M. Leshchyshyn: idea of work, recruitment of patients, surgical operations; P. L. Byk, writing the text of the article, performing surgical operations, analyzing the obtained data; M. M. Plodienko: conducting histological and immunohistochemical studies.; O. I. Okhot'ka: literature review, translation, work on the text of the article.; L. Y. Markulan: work on the text of the article, analysis and statistical calculations.; N. S. Martyniuk: literature review, data analysis; K. M. Dmytriieva, work with a literature review

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Гістологічні зміни в стінці товстої кишки при хронічних повільнотранзитних запорах у дорослих хворих

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Поширеність хронічних запорів у популяції становить від 3 до 27 %. Частіше хворіють жінки, особи старшого віку та низького соціоекономічного стану. Для визначення причин повільнотранзитних запорів багато авторів провели гістологічні дослідження стінки кишки. Вони встановили різні варіанти змін: міопатії, нейропатії та патологію інтерстиційних клітин Кахаля. Частота варіантів (типів) гістологічних змін у стінці товстої кишки у хворих із повільнотранзитними запорами згідно з Лондонською класифікацією невідома.

Мета — визначити частоту типів гістологічних змін у стінці товстої кишки у пацієнтів із повільнотранзитними запорами, резистентними до консервативного лікування.

Матеріали та методи. Проведено патоморфологічне порівняльне дослідження за типом випадок-контроль. В основну групу залучено 105 хворих, яким виконано колектомію з приводу хронічного повільнотранзитного запору. Показанням до операції був хронічний повільнотранзитний запор, резистентний до консервативного лікування, який суттєво погіршував якість життя пацієнтів. Групу порівняння утворено із 27 померлих, у яких за життя не зафіксовано запорів. Критеріями вилучення з дослідження були наявність хвороби Гіршпрунга, проктогенних запорів (дисфункція прямої кишки і тазового дна), медикаментозно-асоційованих запорів, психічних порушень. В обох групах проводили гістологічне та імунологічне дослідження зразків відділів видаленого препарату, у групі порівняння — апендиксу, клубової, сліпої, ободової та сигмоподібної кишки.

Результати. За даними дослідження ідентифіковано 4 основних морфологічних фенотипи структурних елементів стінки товстої кишки пацієнтів із хронічними повільнотранзитними запорами: 1) гістологічно інтактний тип (5,8 %), 2) міопатичний тип (56,2 %), 3) Кахальний тип (19,0 %), 4) нейропатичний (19,0 %). Також виявлено комбінацію різних типів гістологічних змін, але з домінуванням одного з них.

Висновки. У пацієнтів із хронічними повільнотранзитними запорами, що є резистентними до консервативної терапії, виявлено 4 основні типи гістологічних змін в стінці кишечника: міопатичні зміни (56,2 %), патологію клітин Кахаля (19,0 %), нейропатичні зміни (19,0 %) та гістологічно-інтактний варіант (5,8 %). Міопатичний тип характеризується гетерогенністю морфологічних виявів, які можна віднести до дистрофічних (дистрофічний підтип — 79,7 %) та запальних (запальний підтип — 20,3 %) змін ($p = 0{,}001$).

Ключові слова: хронічний повільнотранзитний запор, міопатичні зміни, нейропатичні зміни, патологія клітин Кахаля.

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