



# Diagnostic Value of Ileal Biopsies in Patients with Suspected Crohn's Disease

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**Aim:** to analyze both consultative and current diagnostic biopsy materials to determine the diagnostic value of ileal biopsies and the possible spectrum of morphological changes observed in patients with suspected Crohn's disease.

**Materials and methods.** We analyzed the results of ileal biopsy examinations ( $n = 202$ ) received at the centralized pathological anatomy department of the Clinical Center of Sechenov University from 2022 to 2024, performed both for current diagnostics and for consultations on pre-prepared histological slides. During this period, 568 ileal biopsies from 154 patients were examined, and an additional 200 pre-prepared histological slides from 48 consultative patients were reviewed.

**Results.** Among the examined ileal biopsies, a histological variant of normal tissue was confirmed in 97 patients, focal active ileitis was identified in 42 cases, and chronic active ileitis — in 63 cases. Chronic active ileitis with morphological changes highly suspicious for Crohn's disease was found in 47 (23 %) cases. These findings suggest that the likelihood of diagnosing Crohn's disease based on ileal biopsy results remains low.

**Conclusions.** Diagnosing chronic ileitis requires pathologists to identify reliable signs of chronicity. In addition to structural remodeling and plasma cell infiltration, which are not always easily recognizable in unoriented biopsies and have poor interobserver reproducibility, key indicators include pseudopyloric metaplasia, hypersecretion, microgranulomas, and epithelioid granulomas. The identification of these changes strongly supports the diagnosis of regional ileitis in Crohn's disease. However, the final interpretation of these findings must be made in the context of clinical information and corroborative imaging data.

**Keywords:** inflammatory bowel diseases, Crohn's disease, chronic ileitis, morphological diagnosis

**Conflict of interests:** the authors declare no conflict of interest.

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## Диагностическая ценность биоптатов подвздошной кишки у больных с подозрением на болезнь Крона

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**Цель:** провести анализ наличия и спектра морфологических изменений, обнаруживаемых у больных с подозрением на болезнь Крона, на примере консультативного и текущего диагностического биопсийного материала для определения диагностической ценности биоптатов подвздошной кишки.

**Материал и методы.** Проанализированы все результаты изучения биоптатов подвздошной кишки ( $n = 202$ ), поступившие в централизованное патологоанатомическое отделение Клинического центра Сеченовского Университета за период с 2022 по 2024 г. За это время было изучено 568 биоптатов подвздошной кишки от 154 пациентов и дополнительно пересмотрено 200 готовых гистологических препаратов от 48 консультативных пациентов.

**Результаты.** При изучении биоптатов подвздошной кишки вариант гистологической нормы был верифицирован у 97 пациентов, очаговый активный илеит — в 42 случаях, а хронический активный илеит — в 63 случаях. Хронический активный илеит с морфологическими изменениями, крайне подозрительными в отношении болезни Крона, был установлен в 47 (23 %) наблюдениях. Тем самым можно сделать заключение, что вероят-

ность постановки диагноза болезни Крона по результатам изучения биопсии подвздошной кишки остается невысокой.

**Выводы.** Достоверные признаки хронизации илеита не всегда легко определяются в неориентированных биоптатах и обладают плохой диагностической воспроизводимостью между специалистами. Постановка диагноза хронического илеита требует от патологоанатома умения выявлять достоверные признаки хронизации, к которым, помимо структурной перестройки и плазматизации клеточного инфильтрата, следует отнести обнаружение псевдопилорической метаплазии, гиперкринию, обнаружение микрогранулем и эпителиоидных гранулем. Обнаружение этих изменений является серьезным аргументом в пользу регионарного илеита при болезни Крона, однако окончательно трактовать эти изменения патологоанатом может только в контексте клинической информации и подтверждающих данных визуальных методов диагностики.

**Ключевые слова:** воспалительные заболевания кишечника, болезнь Крона, хронический илеит, морфологическая диагностика

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## Introduction

In routine pathological practice, a large number of ileal biopsies are submitted for morphological diagnosis with a preliminary diagnosis of Crohn's disease (CD), and the number of such biopsies is increasing annually [1]. In some cases, these biopsies are isolated due to the absence of visible lesions in other parts of the gastrointestinal tract, and the accompanying clinical information is limited.

Years ago, there was an attempt to characterize chronic ileitis basing on endoscopic biopsy material [2]. Since then, the volume of submitted material and the spectrum of detected changes have significantly expanded. However, difficulties and challenges in the morphological diagnosis of CD persist, prompting us to revisit this issue.

The aim: to analyze the presence and spectrum of morphological changes detected in patients with suspected Crohn's disease, using the example of consultative and current diagnostic biopsy material to determine the diagnostic value of ileal biopsies.

## Materials and methods

This study analyzed the results of pathological examinations of ileal biopsies submitted to the centralized pathological anatomy department of the Clinical Center of Sechenov University from 2022 to 2024 for routine diagnostics and consultations on pre-prepared histological slides. During this period, 568 ileal biopsies from 154 patients were examined, and an additional 200 pre-prepared histological slides

from 48 consultative patients were reviewed. Additionally, colon biopsies were provided in 102 cases of current diagnostic material and 30 consultative patients. Thus, colon biopsies, in addition to ileal biopsies, were available in over 65 % of all cases.

It was noted that the consultation samples more often complied with the standards for collecting biopsies from patients with suspected inflammatory bowel disease (IBD), according to which biopsies of the ileum and various parts of the colon are collected in separate containers (the so-called "stepwise biopsy").

Of the 202 patients included in the study, 106 were male. Patient ages ranged from 20 to 71 years, with a mean age of  $34.7 \pm 10.9$  years.

The number of ileal biopsies per patient ranged from 1 to 5, with an average of  $1.6 \pm 0.4$  biopsies.

In 72 (35.6 %) cases, only ileal biopsies were examined. In 54 (26.7 %) cases, ileal and colonic biopsies were submitted without specifying the exact segments. In 78 (38.6 %) cases, ileal and colonic biopsies were provided with segmental breakdown.

In 10 consultative cases and 12 current diagnostic cases, biopsies of the upper gastrointestinal tract, including the esophagus, stomach, and duodenum, were also submitted.

Biopsy processing followed standard protocols. The histological material was fixed in 10 % buffered formalin, processed in a Leica ASP200 tissue processor, and embedded in paraffin. Serial sections of 3–4  $\mu\text{m}$  thickness were stained with hematoxylin and eosin.

**Table 1.** Diagnosed lesions depending on the number of biopsies and the presence of additional biopsies from other gastrointestinal tract locations

**Таблица 1.** Диагностированные поражения в зависимости от количества биоптатов и при наличии дополнительных биоптатов других локализаций желудочно-кишечного тракта

Localization <i>Локализация</i>	Number of patients <i>Количество пациентов</i>	Norm <i>Норма</i>	Ileitis not within the context of IBD <i>Илеит не в рамках ВЗК</i>	Ileitis as part of IBD (CD/UC) <i>Илеит в рамках ВЗК (БК/ЯК)</i>
Peum / <i>Подвздошная кишка</i>				
1 biopsy / <i>1 биоптат</i>	72	36	34	2
2 biopsies / <i>2 биоптата</i>	64	29	8	27
3 biopsies / <i>3 биоптата</i>	53	27	13	13
> 3 biopsies / <i>&gt; 3 биоптатов</i>	13	5	3	5
Total / <i>Всего</i>	202	97	58	47
Peum and colon <i>Подвздошная и толстая кишка</i>				
without division into segments <i>без разделения на отделы</i>	54	23	16	15
divided into segments <i>с разделением на отделы</i>	78	31	29	18
Peum and upper gastrointestinal tract <i>Подвздошная кишка и верхние отделы ЖКТ</i>	22	1	1	6

**Note:** IBD – inflammatory bowel disease, CD – Crohn’s disease, UC – ulcerative colitis.

**Примечание:** ВЗК – воспалительные заболевания кишечника, БК – болезнь Крона, ЯК – язвенный колит, ЖКТ – желудочно-кишечный тракт.

Statistical analysis of the data was performed using SPSS 26 software. Normality was assessed using the Shapiro – Wilk test. Nominal variables in two unrelated groups were compared using the chi-square test or Fisher’s exact test when applicable. A *p*-value of < 0.05 was considered statistically significant.

## Results

In most cases (97 out of 202, which constituted 48 % of observations), no inflammatory changes were detected in the biopsies (Table 2). In younger patients, large aggregates of lymphoid tissue with lymphoid follicles and reactive centers were frequently observed (Fig. 1). In such cases, assessing the structural characteristics of the ileal mucosa was challenging due to flattening and smoothing of the villi in areas of lymphoid tissue (Peyer’s patches and solitary lymphoid follicles), complicating the analysis of mucosal structure and cellular infiltrate in the lamina propria.

In some biopsies without signs of ileitis, an increased number of eosinophilic leukocytes was noted. This infiltration was uneven, not accompanied by eosinophil penetration into the epithelium, and did not show increased

infiltration of the deep lamina propria with eosinophil predominance. Additional criteria characteristic of eosinophilic ileitis were absent.

Simply counting the number of eosinophils per field of view under high magnification proved to be an insufficiently reliable criterion. Normal eosinophil counts demonstrated high variability, their distribution in biopsies was uneven, and depended significantly on the staining characteristics of the histological specimens.

In some cases, isolated increases in intraepithelial lymphocytes were observed without villous shortening or increased cellular infiltrate density, ruling out microscopic ileitis. These cases were described as intraepithelial lymphocytosis, potentially linked to celiac disease. However, duodenal biopsies were unavailable, complicating diagnosis. Patients were advised to undergo immunological testing to exclude celiac disease.

It should be noted that the ileum often contains lymphoid tissue, making it difficult to objectively assess the interepithelial lymphocyte count. In such cases, this indicator cannot be considered a reliable diagnostic criterion.

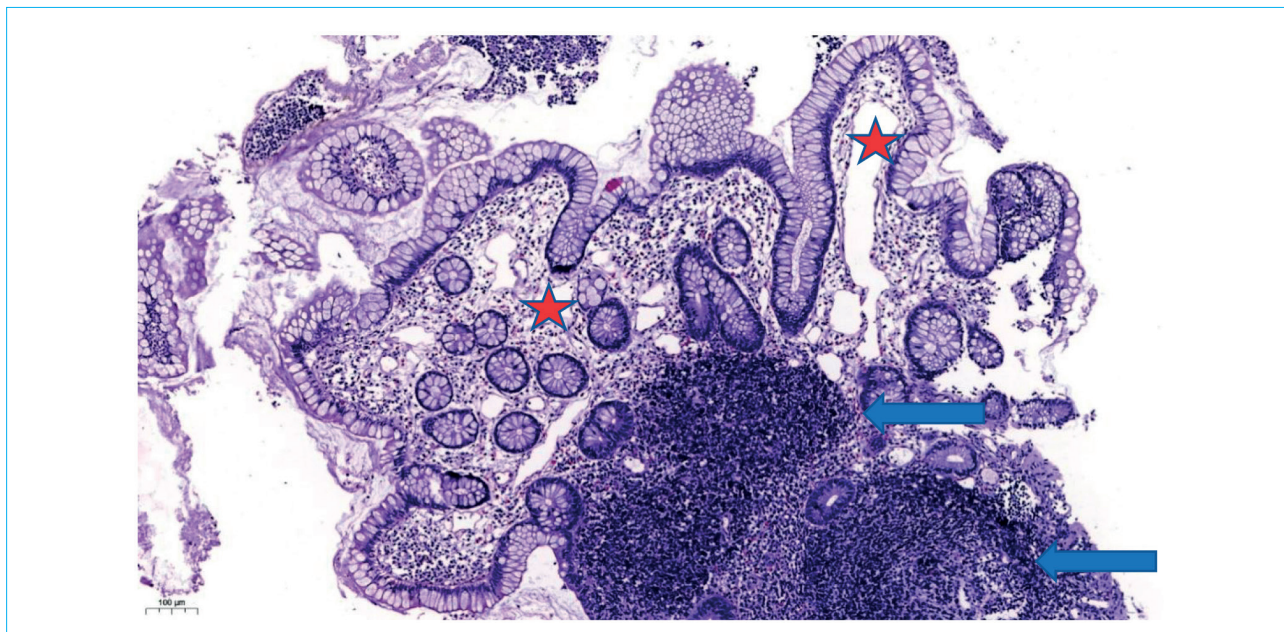
**Table 2.** Morphological changes in ileal mucosal biopsies and their association with the diagnosis of Crohn's disease

**Таблица 2.** Морфологические изменения в биоптатах слизистой оболочки подвздошной кишки и их связь с постановкой диагноза болезни Крона

Morphological diagnosis <i>Морфологический диагноз</i>	Number of patients <i>Количество пациентов</i>	Confirmed CD diagnosis <i>Подтвержден диагноз БК</i>	<i>p</i>
<i>Norm / Норма</i>			
Total / <i>Всего</i>	97	0	<i>p</i> = 1.0
Including follicular hyperplasia <i>В т.ч. фолликулярная гиперплазия</i>	56		
Eosinophilic leukocytes <i>Эозинофильные лейкоциты</i>	23		
Intraepithelial lymphocytes <i>Межэпителиальные лимфоциты</i>	5		
<i>Focal active ileitis / Очаговый активный илеит</i>			
Total / <i>Всего</i>	42	0	<i>p</i> = 1.0
Including follicular hyperplasia <i>В т.ч. фолликулярная гиперплазия</i>	21		
Eosinophilic leukocytes <i>Эозинофильные лейкоциты</i>	15		
Erosions <i>Эрозии</i>	10		
<i>Chronic focal active ileitis / Хронический очаговый активный илеит</i>			
Total / <i>Всего</i>	63	47 (74.6 %)	<i>p</i> < 0.001
Including follicular hyperplasia <i>В т.ч. фолликулярная гиперплазия</i>	31	11	
Eosinophilic leukocytes <i>Эозинофильные лейкоциты</i>	11	7	
Erosions/ulcers <i>Эрозии/язвы</i>	35	28	
Hypercrinia <i>Гиперкриния</i>	40	35	
Pseudopyloric metaplasia <i>Псевдопилорическая метаплазия</i>	18	17	
Histiocytes <i>Гистиоциты</i>	15	14	
Microgranulomas <i>Микрогранулемы</i>	12	12	
Granulomas <i>Гранулемы</i>	18	18	
<i>Chronic focal active ileitis with additional lesions of other parts of the gastrointestinal tract Хронический очаговый активный илеит с дополнительным поражением других отделов ЖКТ</i>			
Chronic focal active colitis <i>Хронический очаговый активный колит</i>	23	21 (91.3 %)	<i>p</i> < 0.001
Chronic diffuse active colitis <i>Хронический диффузный активный колит</i>	8	3	
Upper gastrointestinal tract involvement <i>Поражение верхних отделов ЖКТ</i>	7	7	

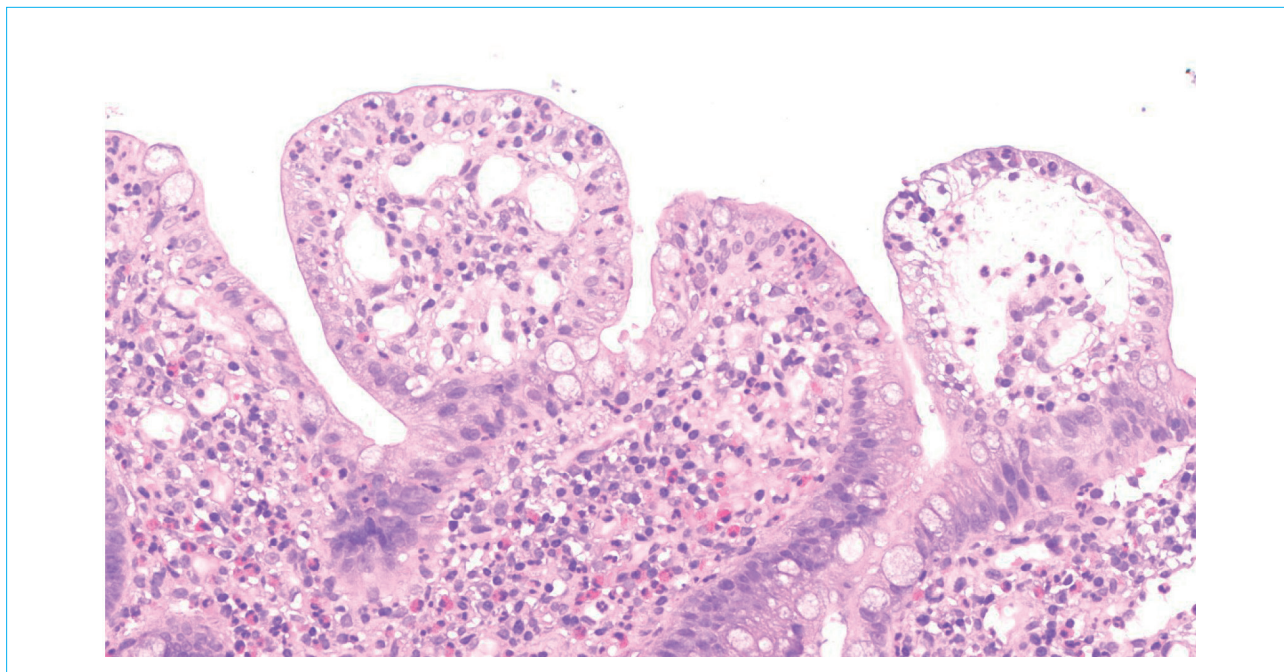
**Note:** CD – Crohn's disease.

**Примечание:** БК – болезнь Крона, ЖКТ – желудочно-кишечный тракт.



**Figure 1.** Ileal mucosal biopsy taken from the area of a Peyer's patch with follicular hyperplasia of lymphoid tissue: large lymphoid follicles with reactive centers are observed (indicated by arrows); villous shortening and deformation, as well as dilation of lymphatic capillaries (marked with red asterisks) are noted; hematoxylin and eosin staining, magnification  $\times 100$

**Рисунок 1.** Биоптат слизистой оболочки подвздошной кишки взят из зоны пейеровой бляшки с фолликулярной гиперплазией лимфоидной ткани: обнаруживаются крупные лимфоидные фолликулы с реактивными центрами (показано стрелками); отмечается укорочение и деформация ворсин, расширение просвета лимфатических капилляров (красные звездочки); окраска гематоксилином и эозином, увеличение  $\times 100$



**Figure 2.** Focal active ileitis: detection of segmented neutrophils in the cellular infiltrate, with their infiltration into the epithelium showing signs of damage; hematoxylin and eosin staining, magnification  $\times 200$

**Рисунок 2.** Очаговый активный илеит: обнаружение сегментоядерных лейкоцитов в клеточном инфильтрате с проникновением их в эпителий с признаками повреждения последнего; окраска гематоксилином и эозином, увеличение  $\times 200$

All the above cases did not meet the criteria for ileitis, effectively excluding IBD.

Focal active ileitis was diagnosed in 42 (20.8 %) cases. Key features included segmented neutrophils infiltrating the surface epithelium (Fig. 2). Villous epithelium showed areas of damage, including reduced height and decreased goblet cells.

Additionally, in 20 cases, areas of erosion were identified, characterized by pronounced flattening and desquamation of the integumentary epithelium, as well as the presence of areas of granulation tissue. In 5 cases, these changes localized to lymphoid structures; in 10 cases, lymphoid hyperplasia with follicle formation was observed. In 10 patients, eosinophilic leukocytes were also increased. These changes were interpreted as focal active ileitis without chronicity, excluding IBD.

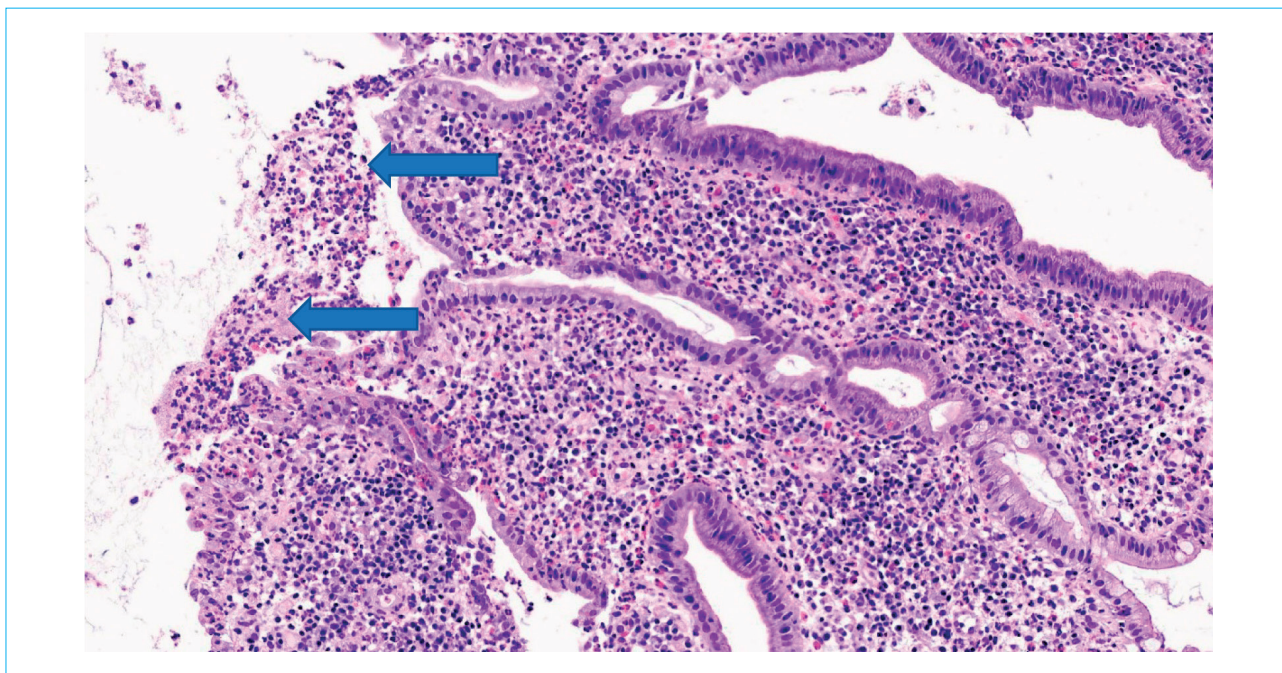
Most cases were isolated, but in 15 cases, colonic biopsies were also available, showing either no pathology or focal active colitis. In 5 patients with pancolitis, ileal changes were interpreted as retrograde ileitis associated with severe ulcerative colitis.

Chronic ileitis was diagnosed in 63 (31.2 %) cases. This pathology featured significant mucosal remodeling, including villous deformation, crypt hyperplasia, and distortion. Villous epithelium showed damage, with areas of flattening. Crypt epithelium exhibited increased Paneth cells migrating upward, while goblet cell hyperplasia formed a single cell type at villous tips (so-called “hypercrinia”).

Erosions were found in most cases (48 of 63) (Fig. 3, 4). Pseudopyloric metaplasia, represented by individual or groups of mucous glands, was noted in over half of the cases (39 of 63) (Fig. 5). Granulomas, microgranulomas, and histiocyte aggregates were detected in 23 (36.5 %) cases (Fig. 6).

Colonic biopsies from 45 patients showed chronic active colitis. In 22 cases, upper gastrointestinal biopsies (esophagus, stomach, duodenum) were available: 1 case of active esophagitis, 5 cases of gastritis, and 6 cases of duodenitis. These findings strongly supported CD diagnosis.

If we arrange the diagnoses in a sequence, the likelihood of a CD diagnosis increases in



**Figure 3.** Focal active erosive ileitis: identification of an erosion site with epithelial damage and detachment, an accumulation of leukocytic exudate on the surface (indicated by arrows), and an increased density of inflammatory cell infiltrate with a relative increase in eosinophilic leukocytes; hematoxylin and eosin staining, magnification  $\times 120$

**Рисунок 3.** Очаговый активный эрозивный илеит: обнаружение участка эрозии с повреждением и отслойкой покровного эпителия, участком скопления лейкоцитарного экссудата на поверхности (показано стрелками) и повышением плотности воспалительного клеточного инфильтрата с относительным повышением содержания эозинофильных лейкоцитов; окраска гематоксилином и эозином, увеличение  $\times 120$

the following order: acute focal superficial ( $p = 1.0$ ), chronic focal active ( $p < 0.001$ ), chronic focal active erosive ( $p < 0.001$ ), with pseudopyloric metaplasia ( $p < 0.001$ ), and with granulomas ( $p < 0.001$ ).

This indicator increases with the simultaneous detection of chronic focal active colitis and involvement of the upper gastrointestinal tract.

When examining biopsies, the probability of a CD diagnosis rises with the detection of mucosal structural reorganization accompanied by hypercrinia ( $p < 0.001$ ), pseudopyloric metaplasia ( $p < 0.001$ ), and the presence of granulomas ( $p < 0.001$ ).

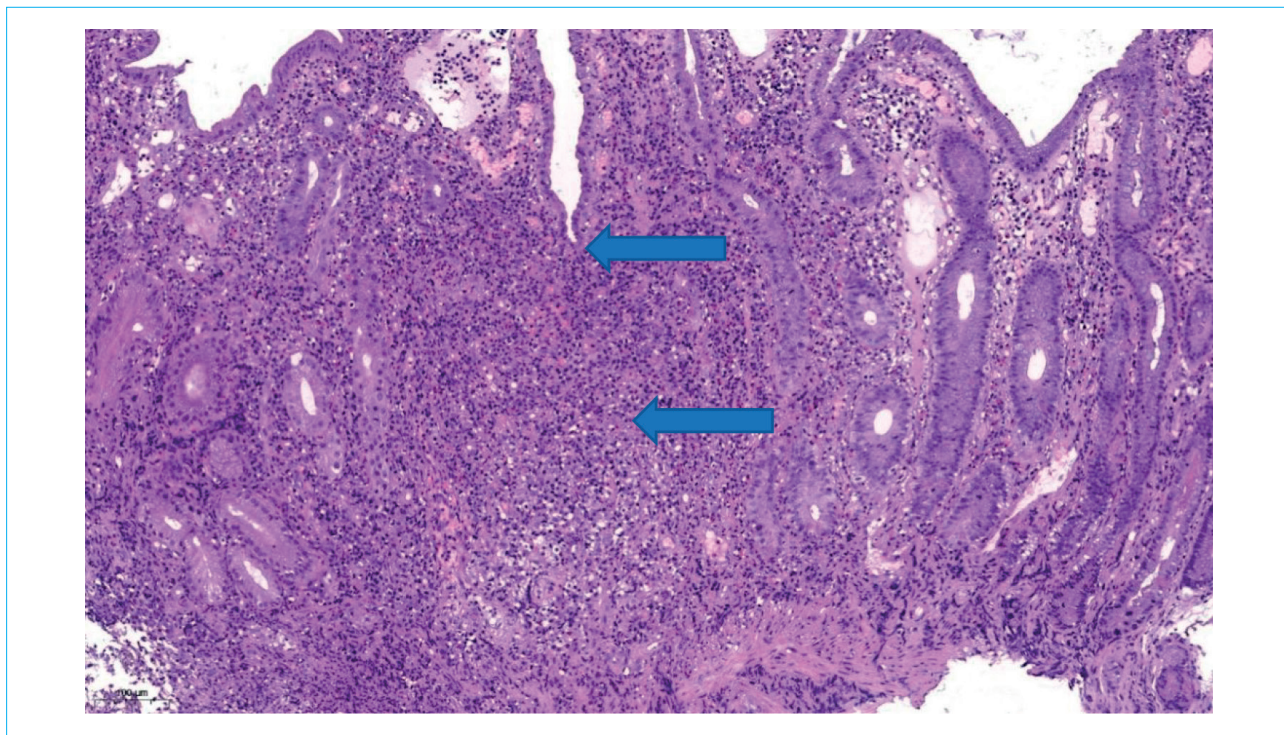
Mucosal biopsies represent only the inner layers of the intestinal wall, while CD is most often, by definition, a transmural lesion. The likelihood of a CD diagnosis increases when considering data from imaging methods if the affected segment exceeds 10 cm and if there is characteristic deformation and wall thickening with the formation of a narrowed lumen in the ileum.

## Discussion

Crohn's disease (CD) can be diagnosed in asymptomatic patients undergoing colonoscopy for reasons unrelated to IBD [3]. Most patients diagnosed with asymptomatic CD can likely be managed without treatment. Although in the majority of cases the disease remains asymptomatic and does not cause complications during follow-up, careful monitoring is advisable to rule out disease progression [4, 5].

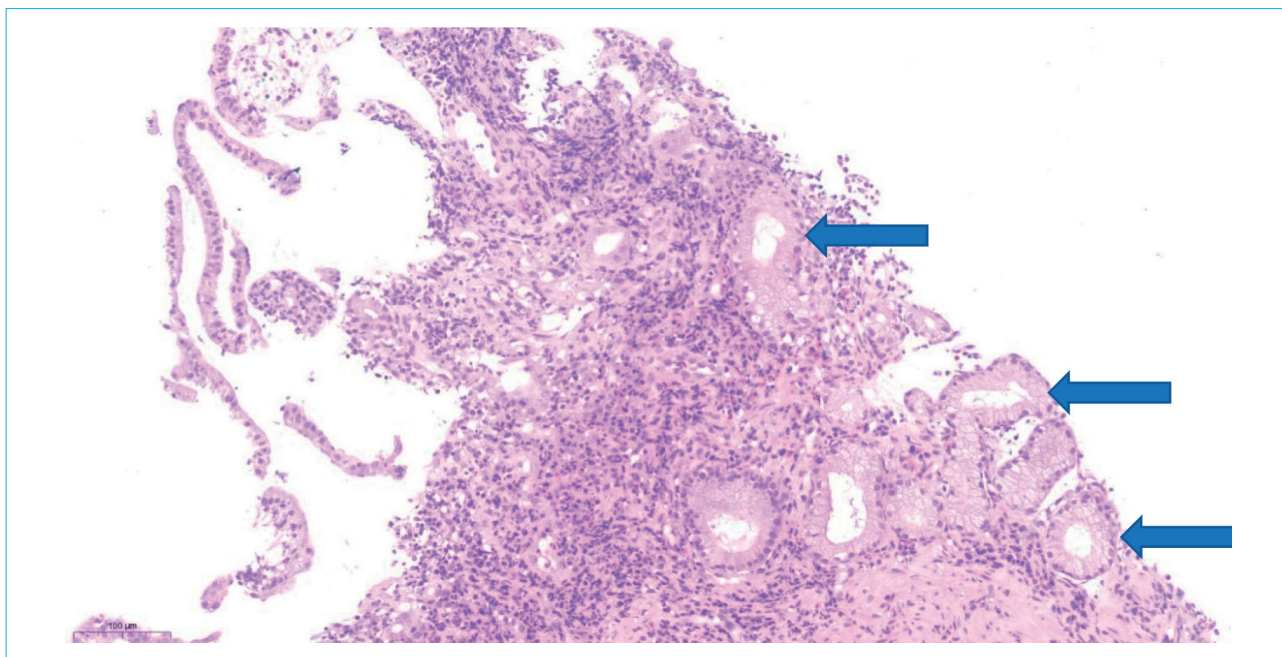
The term "incidentally diagnosed terminal ileitis" has been proposed in the literature to describe ileal lesions detected in asymptomatic patients undergoing colonoscopy for other indications. The cumulative prevalence of this condition is 1.6 %. Progression to clinically overt CD is rare, and dynamic monitoring is a justified management strategy for such patients [6].

Nonspecific terminal ileitis was diagnosed in 92 out of 5,353 patients undergoing colonoscopy (prevalence – 1.7 %). Among these patients, 56 (61 %) were followed for  $\geq 6$  months after



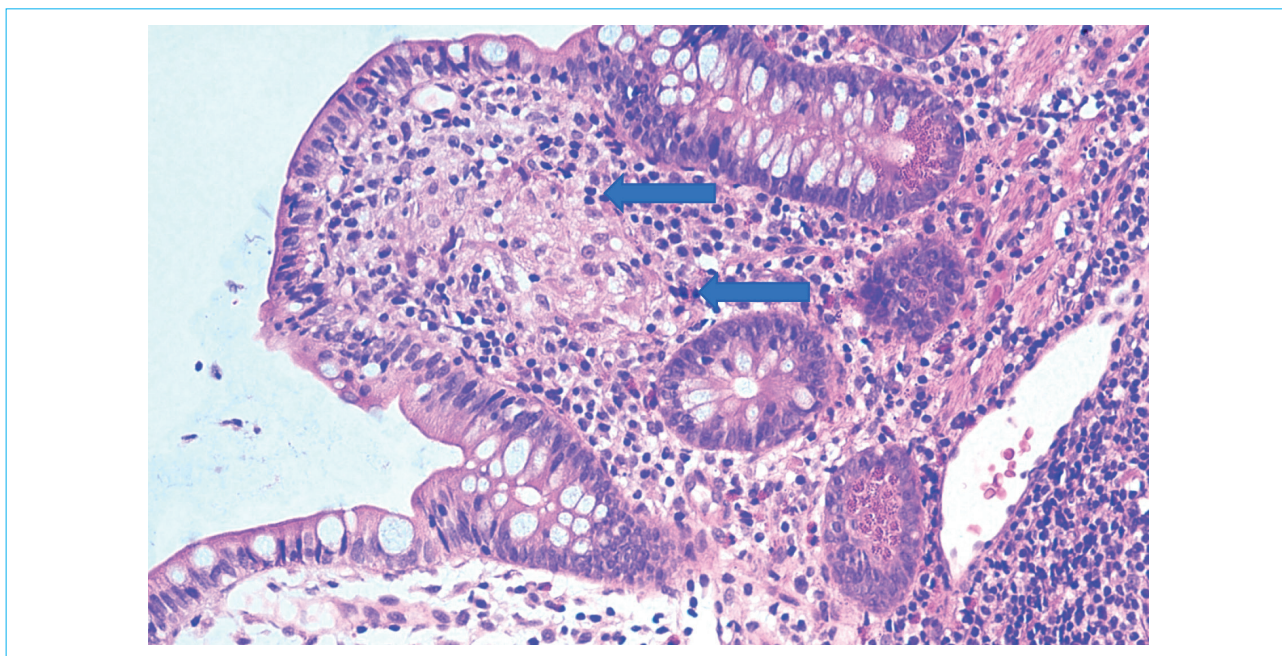
**Figure 4.** Chronic focal active ileitis: villous deformation, crypt hyperplasia and deformation, and focal increased cellular infiltrate density in the area of an epithelialized slit-like erosion (indicated by arrows) are observed; the infiltrate contains a high proportion of plasma cells and segmented neutrophils; hematoxylin and eosin staining, magnification  $\times 120$

**Рисунок 4.** Хронический очаговый активный илеит: отмечается деформация ворсин и гиперплазия и деформация крипт, очаговое повышение плотности клеточного инфильтрата в зоне эпителизированной щелевидной эрозии (показано стрелками) с высоким содержанием в составе клеточного инфильтрата плазматических клеток и сегментоядерных лейкоцитов; окраска гематоксилином и эозином, увеличение  $\times 120$



**Figure 5.** Chronic focal active erosive ileitis with focal pseudopyloric metaplasia in Crohn's disease: the biopsy shows an area of granulation tissue and clusters of mucous glands (indicated by arrows); hematoxylin and eosin staining, magnification  $\times 160$

**Рисунок 5.** Хронический очаговый активный эрозивный илеит с очаговой псевдопилорической метаплазией при болезни Крона: в биоптате обнаруживается участок грануляционной ткани и группы слизистых желез (показано стрелками); окраска гематоксилином и эозином, увеличение  $\times 160$



**Figure 6.** Chronic focal active granulomatous ileitis in Crohn's disease: the biopsy reveals a sarcoid granuloma consisting of a focal accumulation of epithelioid cells (indicated by arrows); hematoxylin and eosin staining, magnification  $\times 100$

**Рисунок 6.** Хронический очаговый активный гранулематозный илеит при болезни Крона: наличие в биоптате саркоидной гранулемы, состоящей из очагового скопления эпителиоидных клеток (показано стрелками); окраска гематоксилином и эозином, увеличение  $\times 100$



the initial endoscopy. Repeat endoscopy was performed in 23 (41 %) of the 56 patients, and persistent endoscopic changes were observed in 15 (65.2 %) cases. Eleven (19.6 %) of the 56 patients were eventually diagnosed with CD. The likelihood of a CD diagnosis was significantly higher in patients with persistent symptoms ( $p = 0.002$ ) and endoscopic changes on follow-up ( $p = 0.038$ ) [7].

The authors attempted to evaluate the diagnostic value of biopsy in macroscopic lesions of the terminal ileum and to identify the association between endoscopic indications and the presence of what they termed “significant disease”, where biopsy results suggested or confirmed a specific condition requiring further investigation or treatment. Among 551 biopsies of terminal ileum lesions, 44 (8.0 %) had significant disease. The rate of significant disease was high in patients with clinically suspected IBD (50.0 %) [8].

The diagnostic value of terminal ileum biopsy varies depending on the indications and endoscopic findings. Biopsy is most valuable in patients undergoing endoscopy for known or highly suspected Crohn’s disease [9]. Biopsy of endoscopically normal mucosa is unlikely to provide diagnostically useful information and is not routinely recommended. However, when ileitis, ulcers, or erosions are detected, biopsy can be highly useful [10, 11].

## Conclusion

The study results showed that in the examination of ileal biopsies, histological normality was confirmed in 97 patients, focal active ileitis was diagnosed in 42 cases, and chronic active ileitis was found in 63 patients. In 47 (23 %) observed cases of chronic active ileitis, morphological changes highly suspicious for Crohn’s disease were identified, including mucosal structural reorganization and metaplastic changes.

The probability of establishing a Crohn’s disease diagnosis based on ileal biopsy remains limited and does not exceed a quarter of cases. Isolated ileal involvement without other gastrointestinal tract involvement is insufficient for a definitive diagnosis. In 28 % of cases, inflammatory changes lacked morphological features specific to Crohn’s disease.

With the advancement of endoscopic imaging techniques, the number of ileal biopsies has increased. However, endoscopically detected changes are not always confirmed by morphological examination. Similar to the diagnosis of gastritis, the diagnosis of ileitis requires morphological verification [12]. The detection of erosions in the ileal mucosa is not specific to Crohn’s disease and is confirmed in only a limited number of cases — in our study, this frequency was 23 %. More often, inflammatory changes are acute and lack specific diagnostic features. In some cases, signs of eosinophilia and intraepithelial lymphocytosis may indicate immune disorders or allergic/drug-induced pathology, but these assumptions require clinical and laboratory confirmation.

The diagnosis of chronic ileitis requires the identification of reliable signs of chronicity, such as mucosal structural reorganization, plasmacytosis of the cellular infiltrate, pseudopyloric metaplasia, hypercrinia, as well as the detection of microgranulomas and epithelioid granulomas [3, 13]. The presence of these morphological changes significantly increases the likelihood of a diagnosis of regional ileitis associated with Crohn’s disease. However, the final interpretation of results should be based on a combination of clinical data and instrumental diagnostic findings. The detection of colonic and upper gastrointestinal tract involvement makes the Crohn’s disease diagnosis more substantiated.

An important morphological sign of chronicity is the presence of pseudopyloric metaplasia foci, although its diagnostic significance in adult patients is lower than in children. Combined involvement of the ileum and colon may again suggest Crohn’s disease, but to be conclusive, structural reorganization and plasmacytosis should be present, which may not always be evident in early disease stages.

It is essential to emphasize the necessity of performing biopsies not only of the ileum but also of the colonic mucosa and upper gastrointestinal tract, especially when Crohn’s disease is suspected, during initial diagnosis, and when morphological confirmation is required. Comprehensive analysis of biopsies from different segments improves diagnostic accuracy, and the availability of clinical-laboratory and endoscopic data makes the diagnosis more reliable.

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