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Pathology assessment of inflammatory bowel disease – prospective study from two referral centers

CHRISTOPHER JESSE VLAD PAVEL[1,2], GABRIEL CONSTANTINESCU[1,2], VALENTIN ENACHE[3], GABRIEL BECHEANU[4], MĂDĂLINA STAN-ILIE[1,2], VASILE ŞANDRU[1,2], OANA-MIHAELA PLOTOGEA[1,2], ALEXANDRU CONSTANTINESCU[1], DAN IONUŢ GHEONEA[5], BOGDAN SILVIU UNGUREANU[5], HORIA-DAN LIŞCU[6], MIHAI MIRCEA DICULESCU[1,7], ALEX EMILIAN STEPAN[8]

[1] *5th Department – Gastroenterology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

[2] *Department of Gastroenterology, Emergency Clinical Hospital of Bucharest, Romania*

[3] *Department of Pathology, Emergency Clinical Hospital of Bucharest, Romania*

[4] *Department of Pathology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

[5] *Department of Gastroenterology, Research Center of Gastroenterology and Hepatology, University of Medicine and Pharmacy of Craiova, Romania*

[6] *Discipline of Oncological Radiotherapy and Medical Imaging, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

[7] *Department of Gastroenterology, Fundeni Clinical Institute, Bucharest, Romania*

[8] *Department of Pathology, University of Medicine and Pharmacy of Craiova, Romania*

Abstract

We aimed to perform a comprehensive prospective histological analysis in a cohort of 40 inflammatory bowel disease (IBD) patients, both prior to and during biological therapy. The main objective was to establish the impact of histological activity in assessing treatment response and therapy guidance. Biopsy samples were assessed in two different stages of the disease course – before introduction of biological therapy and during follow-up (6–12 months later). Clinical, biochemical and endoscopic parameters were simultaneously gathered. By univariate analysis, presence of lymphoid aggregates, mucin depletion and ulcerations had a strong association with treatment response. IBD diagnosis >5 years and previous biological therapy were the most susceptible to treatment non-response. Nancy Index (NI) >1 is highly accurate, sensitive and specific in defining treatment responders. In addition to NI, high histological burden (mucin depletion, lymphoid aggregates and ulcerations) seems to predict treatment response in biological therapy-treated patients. Instead of a stringent "histological remission" goal, histological improvement of individual parameters might be a more reasonable goal, avoiding the risk of overtreatment. Further research is needed to assess histological burden in patients with endoscopic remission in order to stratify low- *vs*. high-risk "relapsers" and to avoid under- or over-treatment.

Keywords: inflammatory bowel disease, histopathology assessment, biological therapy.

Introduction

Inflammatory bowel diseases (IBDs) possess a wide spectrum of clinical manifestations and disease severity. The two main entities are Crohn's disease (CD) and ulcerative colitis (UC). IBDs have a relatively unpredictable course regardless of significant scientific struggle aimed at optimizing monitoring and treatment-tailoring parameters [1, 2]. The introduction of biological therapy more than two decades ago represented a quantum leap but the extremely complex pathogenic mechanisms of IBDs have led to high rates of primary or secondary loss of response [3–5].

The absence of a standardized accepted definition and poorly defined cut-off values for inflammatory parameters steers confusion among clinicians and heterogeneity in treatment decisions. It is no longer an accepted paradigm to apply a symptom-based approach to tailor therapy in IBD, regardless of the complexity of the case. The update of *Selecting Therapeutic Targets in Inflammatory Bowel Disease* (STRIDE) consensus has proposed a temporal approach with short, intermediate and long-term targets, with symptomatic response at the beginning and endoscopic healing at the end of the spectrum [6]. Histological healing is only asserted as a formal target, particularly for UC [6]. Mucosal healing has a certain positive impact on relapse rate or hospitalization incidence [7, 8]. Patients with Mayo Endoscopic Score (MES) ≥1 have a considerably higher risk (estimated 3-fold) to present a clinical relapse in comparison to MES=0 [9]. Endoscopic healing is arguably an inaccurate marker of histological activity since mostly 30% of patients do not simultaneously express microscopic healing [10]. Histological remission is usual associated with decreased rates of corticosteroids use, hospitalizations, relapsing episodes and colorectal cancer compared to

mucosal healing alone [11–14]. At the same time, histological grade has the strongest association with clinical relapse risk [13]. Consequently, some leading experts stated that microscopic remission should be considered a critical target in clinical trials [15].

Histological remission is not invariably defined but presumes complete histological normalization with absence of any neutrophils, inflammatory activity and architectural abnormalities [16]. Although an ideal end-point target, it is considered a strenuous aim to achieve, and histological improvement might be a more pragmatic target with similar benefits in long-term evolution of IBD patients [17].

Aim

This study delves into characterization of histological samples in IBD patients both prior and during biological treatment, aiming to evaluate the impact on clinical evolution, therapy response and management, as well as correlations with biochemical and endoscopic parameters.

Patients, Materials and Methods

The current prospective study was conducted in two referral centers in Romania and had assessed histological parameters of 40 IBD patients in two different stages of the disease, before and after biological therapy treatment:

▪ During index evaluation, tissue samples were examined before biological therapy initiation (both naïve or biological-experienced patients with switch to another therapy due to primary/secondary loss of response);

▪ A second follow-up had been scheduled after 6–12 months of biological therapy to compare and interpret histological findings; clinical, biochemical (inflammatory markers) and endoscopic parameters were simultaneously gathered.

The enrolment period was March 1, 2023–February 29, 2024, and the last follow-up was scheduled in September 2024.

Inclusion criteria

The indication of biological therapy was established as the main criteria of enrollment. Both biological-naïve and biological-experienced patients were included. Medical management was guided according to the currently available recommendations issued by the *European Crohn's and Colitis Organization* (ECCO) and the *British Society Guidelines* (BSG) [18–20].

The histopathology report had been outlined by the Nancy Index (NI), which is recommended in daily clinical practice due to its accuracy and simplicity [21, 22]. NI assesses ulceration, acute and chronic inflammatory infiltrate in a gradual pathway considering the worst characteristic of each parameter. Due to this practical approach, the *ECCO* Guidelines recommend adopting NI in daily clinical routine [23]. NI=0 defines histological remission, while NI≤1 characterizes histological response [24]. It is well-validated for UC [17], while CD lacks any specific score validated in prospective cohorts [25]. A single expert gastrointestinal (GI) pathologist, blinded to clinical or endoscopic data, was responsible for pathological assessment.

The individual parameters that were assessed are listed below:

▪ Chronicity features: crypt architectural distortions, Paneth or pyloric cell metaplasia, basal plasmacytosis, presence of granulomas or lymphoid aggregates;

▪ Activity features: neutrophilic inflammation with cryptitis, crypt abscess, ulceration, mucin depletion;

▪ Dysplasia features: according to Vienna classification (negative/indefinite/low-grade/high-grade/invasive neoplasia) [26].

The Ethics Committee reviewed the protocol and approved the study (Registration No. 45027, October 2022). The written informed consent had been signed by all patients.

Exclusion criteria

We did not include patients under 18 years old, patients who declined to sign the consent and patients with indeterminate colitis or doubtable diagnosis of IBD.

Variables

Our study gathered the following data:

▪ Age at the time of diagnosis and time interval until initiation of biological therapy;

▪ IBD phenotype in accordance with Montreal classification [27];

▪ History of prior biologics or other IBD-related medication;

▪ Surgical history related to IBD and extraintestinal manifestations;

▪ Clinical activity scores [clinical Mayo Score (cMS) and Harvey–Bradshaw Index (HBI) in UC and CD, respectively]:

– HBI proved good correlation with the more complex and rather impractical Crohn's Disease Activity Index (CDAI) [28, 29]: HBI<5 defined remission, while **HBI>5** defined clinical activity;

– cMS is focused on three components of the Mayo Score (MS) (rectal bleeding, stool frequency and physician's global assessment): each component is graded from 0 to 4; clinical activity is defined by **cMS>2**.

▪ Endoscopic activity scores [Simple Endoscopic Score in Crohn's Disease (SES-CD), MES in UC and Rutgeerts score in postoperative cases]:

– SES-CD has been developed in 2004 to address the complexity of the Crohn's Disease Endoscopic Index of Severity (CDEIS), which limited its practical daily use [30, 31]: a strong correlation between SES-CD and CDEIS has been confirmed [32]; SES-CD evaluates the presence of stenoses, the size of ulcers, the percentage of ulcerated and affected surface; each feature is graded 0–3;

– **SES-CD>3** and Rutgeerts score ≥2 confirmed endoscopic activity;

– MES has been the most generally used score in routine practice and clinical trials due to its simplicity; although not formally validated, it proved solid inter- and intra-observer consistency [33]; patients with MES>1 were regarded as having endoscopic activity.

▪ Laboratory tests [complete blood count (CBC), C-reactive protein (CRP), fecal calprotectin (CLP)];

▪ Histological assessment both prior biological therapy initiation and during follow-up (in cases with complete mucosal healing, biopsies were taken from the same anatomical areas as in the first-phase samples).

Statistical analysis

Statistical analysis was conducted with Statistical Package for the Social Sciences (SPSS) 20.0 v. 20 software (IBM, Armonk, NY, USA). A p-value <0.05 was reckoned as statistically significant. The continuous variables were expressed as the means ± standard deviations and the ranges as medians and minimum–maximum ranges. To assess discrepancies between two categories, scipy.stats library in Python 3.7 [34] was used in order to compute the t-tests. The analysis of variance (ANOVA) unifactorial test was employed using the Statsmodels Library in multiple groups comparison [35]. The categorical variables were evaluated using the χ^2 (*chi*-squared) test, while correlations were assessed using the Pearson's correlation method [36].

Results

Study population and IBD phenotype

The median age of the 40 IBD patients included was 38.5 years old. The median age at the time of the initial IBD diagnosis was 33 years old. In our group, we observed a slight overall female preponderance (52.5%). Approximately one-third of patients were biologically experienced (Table 1).

Table 1 – *Study population data*

Parameter	UC	CD	All IBD
n	20	20	40
Age (median, 5–95%) [years]	45.0 (21.9–69.0)	37.0 (20.9–66.1)	38.5 (20.9–68.0)
Disease duration (median, 5–95%) [years]	7.0 (1.0–16.2)	4.5 (1.0–11.6)	5.0 (1.0–16.2)
Sex			
▪ M	55.0%	40.0%	47.5%
▪ F	45.0%	60.0%	52.5%
Biological therapy			
▪ Vedolizumab	35.0%	30.0%	32.5%
▪ Infliximab	35.0%	30.0%	32.5%
▪ Adalimumab	10.0%	35.0%	22.5%
▪ Tofacitinib	10.0%	/	5.0%
▪ Ustekinumab	5.0%	5.0%	5.0%
▪ Risankizumab	5.0%	/	2.5%

CD: Crohn's disease; F: Female; IBD: Inflammatory bowel disease; M: Male; *n*: No. of cases; UC: Ulcerative colitis.

In terms of CD phenotype, ileocolonic disease was the most frequently encountered (L3, 45%), followed by colonic (L2, 30%) and ileal disease (25%). Stricturing behavior was the most common (B2, 55%), followed by inflammatory (B1) and penetrating pattern (B3), each with similar distribution (25%). In UC patients, pancolitis (E3) was observed in 45% cases, similar to left-sided colitis (E2) while proctitis (E1) was observed in the remainder of 10% cases (Table 2).

The rate of surgical interventions was 30% (according to retrospective analysis of past medical and operation history). All interventions occurred in CD patients with stricturing phenotype requiring either segmental colectomy or right hemicolectomy for ileocecal valve stenosis (Table 2). During the enrolment or follow-up period no surgical interventions interfered with clinical evolution.

Table 2 – *IBD phenotype according to Montreal classification*

Montreal classification in CD – location and behavior		
L1 B1	10.0%	25%
L1 B2	10.0%	
L1 B3	5.0%	
L2 B1	5.0%	30%
L2 B2	20.0%	
L2 B2	5.0%	
L3 B1	15.0%	45%
L3 B2	25.0%	
L3 B3	5.0%	
Montreal classification of extent in UC		
E1	10.0%	
E2	45.0%	
E3	45.0%	

CD: Crohn's disease; IBD: Inflammatory bowel disease; UC: Ulcerative colitis.

Histological assessment

Acute inflammation

All parameters of acute inflammation that were assessed (cryptitis, abscesses and ulcerations) had a lower incidence in the treated-patient group compared to the initial evaluation (Table 3). Thus, the accumulation of neutrophils in the crypt epithelium (cryptitis), or as aggregates in the lumen of the crypts (abscesses), along with the loss of crypts replaced by immature granulation tissue or fibrinopurulent exudate (ulceration) were attenuated in treated patients (Figure 1, A–E). Also, along with the acute phenomena of IBD, mucin depletion and dysplasia were present (Figure 1F).

Table 3 – *Acute inflammatory parameters, before and after treatment*

Disease	*p*-value	No before	No after	Yes before	Yes after
Cryptitis					
CD	0.23	85%	100%	15%	0%
UC	0.5	60%	75%	40%	25%
IBD	0.162	72.5%	87.5%	*27.5%*	*12.5%*
Abscesses					
CD	1	80%	80%	20%	20%
UC	0.34	45%	65%	55%	35%
IBD	0.474	62.5%	72.5%	*37.5%*	*27.5%*
Ulcerations					
CD	0.333	50%	70%	50%	30%
UC	0.479	65%	80%	35%	20%
IBD	0.156	57.5%	75%	*42.5%*	*25%*
Mucin depletion					
CD	0.405	75%	90%	25%	10%
UC	*0.027*	30%	70%	*70%*	*30%*
IBD	*0.018*	52.5%%	80%	*47.5%*	*20%*

CD: Crohn's disease; IBD: Inflammatory bowel disease; UC: Ulcerative colitis.

The most significant improvement was observed for mucin depletion, with a 57.8% downgrade (p-value 0.018) in the second-phase evaluation. It was followed by cryptitis (54.5% downgrade), ulcerations (41.1%) and abscesses (26.6%). Despite the notable improvement in histological evaluation, the only parameter that reached statistical significance was mucin depletion (p-value 0.018).

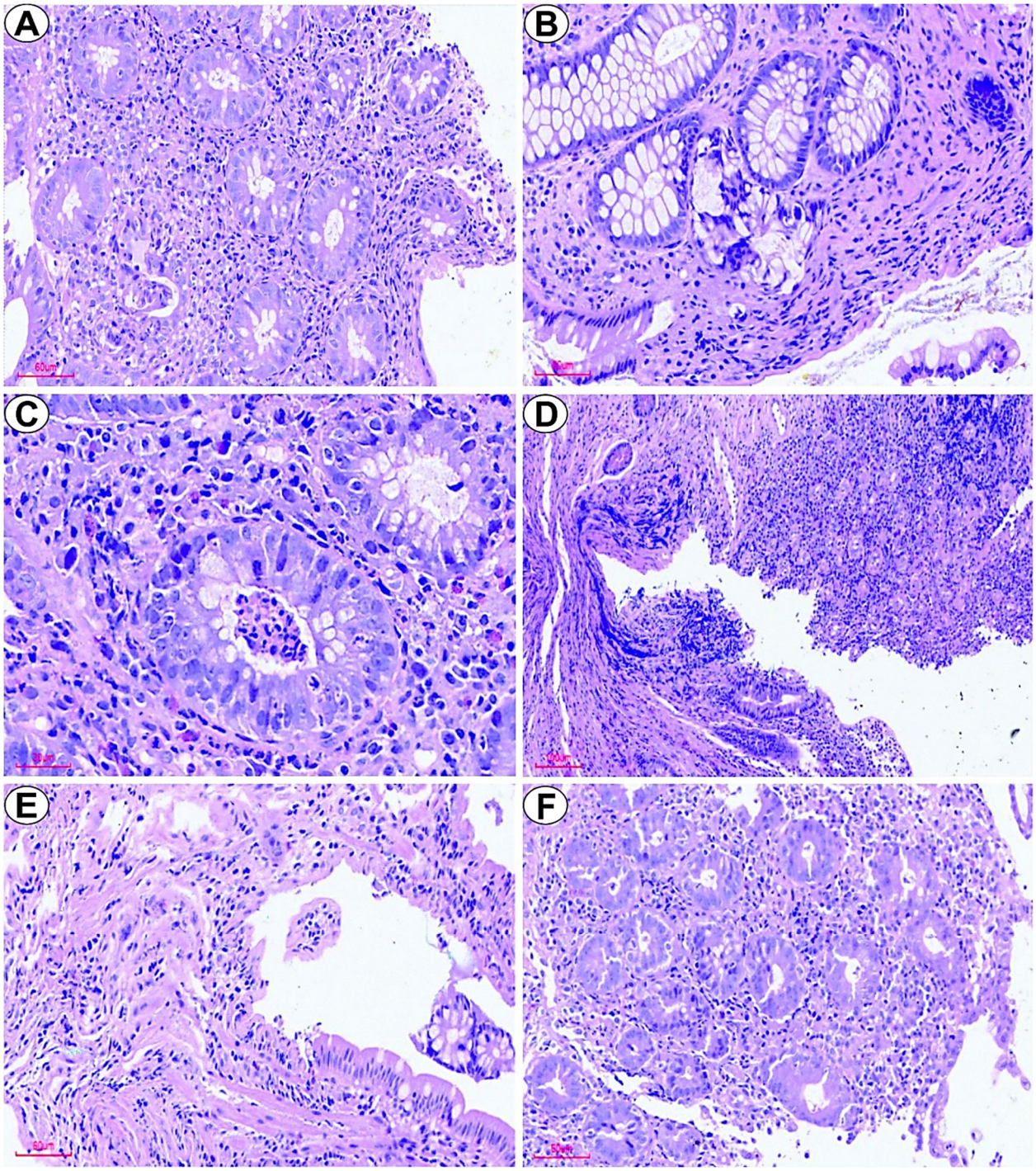

Figure 1 – ***(A) UC, before treatment – cryptitis and abscesses; (B) UC, same patient after treatment (six months) – significant histological improvement (resolution of abscesses and cryptitis); (C) UC, crypt abscess; (D) CD, before treatment – ulceration; (E) CD, same patient after treatment (seven months) – fibrosis; (F) UC, mucin depletion and low-grade dysplasia – before treatment. HE staining: (A, B and F) ×200; (C) ×400; (D and E) ×100. CD: Crohn's disease; HE: Hematoxylin–Eosin; UC: Ulcerative colitis.***

Chronic inflammation

Evaluation of IBD cases indicated the presence of histopathological aspects of chronicity represented by plasmacytic infiltration of deep mucosal areas such that the crypts did not reach the muscularis mucosae (basal plasmacytosis, Figure 2A), accumulations of lymphocytic mononuclear cells, with formation of lymphoid aggregates, sometimes with germinal centers (Figure 2, B and C), Paneth cell metaplasia observed in both CD and UC (Figure 2, D and E), as well as the presence of granulomas in CD (Figure 2F). In all cases, there were cryptoepithelial architectural distortions.

Basal plasmacytosis and metaplastic changes improved considerably during follow-up evaluation, with a decrease of 83.3% and 64.2%, respectively. Both parameters reached statistical significance. A similar significant decline (27.5%) was not observed in the assessment of lymphoid aggregates. Granulomas were detected in the same number of CD patients (Table 4).

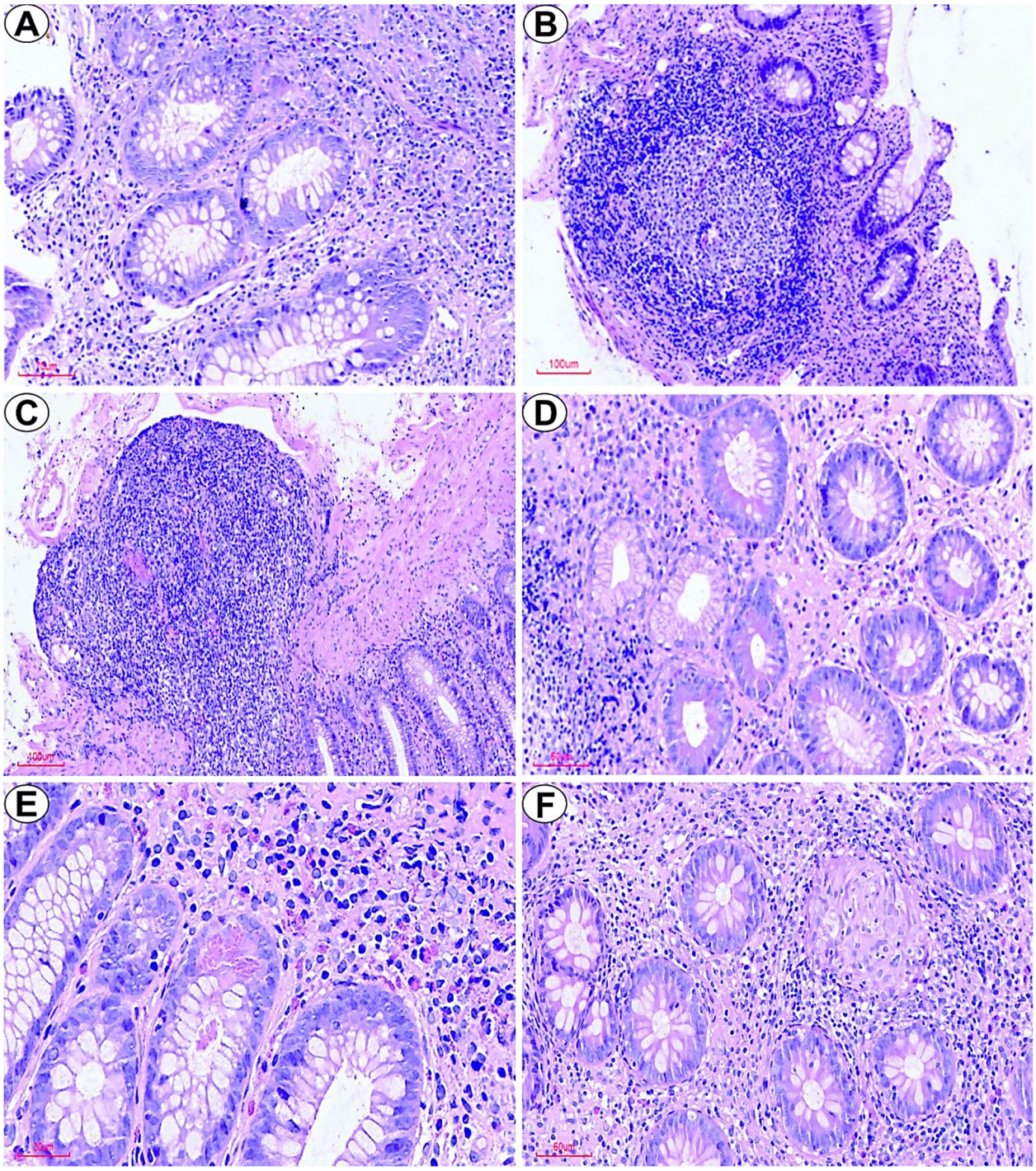

Figure 2 – *(A) UC, basal plasmacytosis; (B and C) UC patient, lymphoid aggregates; (D) CD, post-operative status – Paneth metaplasia (peri-ileocolic anastomosis); (E) UC, Paneth metaplasia; (F) CD patient – presence of granuloma and architectural distortion. HE staining: (A, D and F) ×200; (B and C) ×100; (E) ×400. CD: Crohn's disease; HE: Hematoxylin–Eosin; UC: Ulcerative colitis.*

Table 4 – *Chronic inflammatory parameters, before and after treatment*

Disease	*p*-value	No before	No after	Yes before	Yes after	Paneth before	Paneth after	Pyloric before	Pyloric after
Basal plasmacytosis									
CD	1	95%	100%	5%	0%				
UC	*0.007*	45%	90%	*55%*	*10%*				
IBD	*0.008*	70%	95%	*30%*	*5%*				
Lymphoid aggregates									
CD	0.333	30%	50%	70%	50%				
UC	0.32	25%	45%	75%	55%				
IBD	0.106	27.5%	47.5%	*72.5%*	*52.5%*				

Disease	*p*-value	No before	No after	Yes before	Yes after	Paneth before	Paneth after	Pyloric before	Pyloric after
Paneth/pyloric cell metaplasia									
CD	0.105	70%	90%			10%	10%	20%	0%
UC	0.157	60%	85%			40%	15%	0%	0%
IBD	*0.03*	65%	87.5%			*25%*	*12.5%*	*10%*	*0%*
Granuloma									
CD	0.57	75%	85%	*10%*	*10%*				

CD: Crohn's disease; IBD: Inflammatory bowel disease; UC: Ulcerative colitis.

By univariate analysis, the presence of lymphoid aggregates, mucin depletion, and ulcerations indicates a strong positive association with treatment response [odds ratio (OR) >4]. On the opposite side, patients with a diagnosis of IBD >5 years and previous biological therapy are the most susceptible to treatment non-response (OR 0.1 and 0.23, respectively) (Figure 3, A–C; Figures 4 and 5; Table 5).

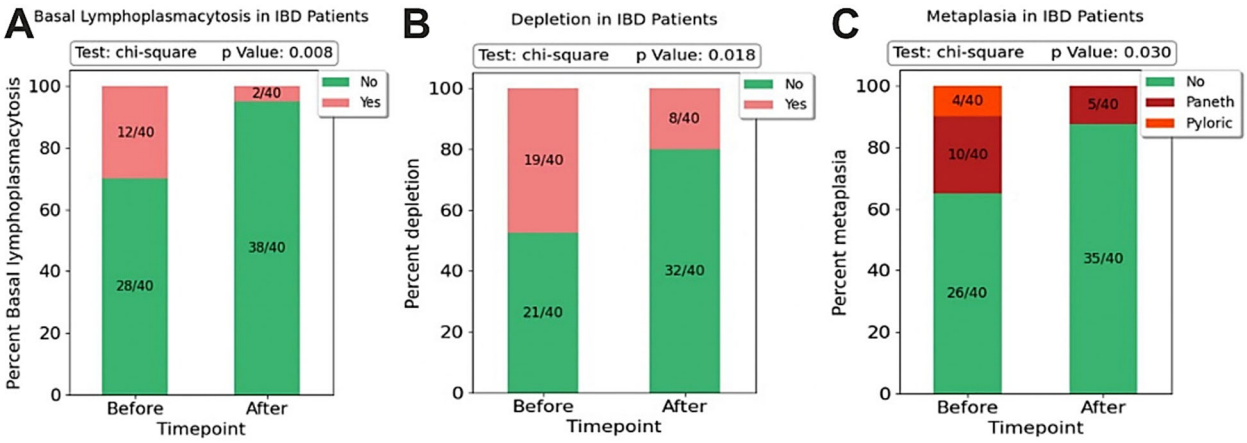

Figure 3 – *(A–C) Histopathological features with statistical significance (p<0.05): basal plasmacytosis, mucin depletion and metaplastic changes. IBD: Inflammatory bowel disease.*

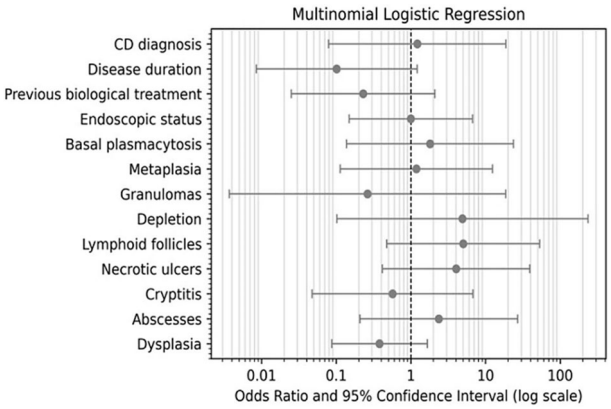

Figure 4 – *Forest plot expressing odds ratio for biological therapy response. CD: Crohn's disease.*

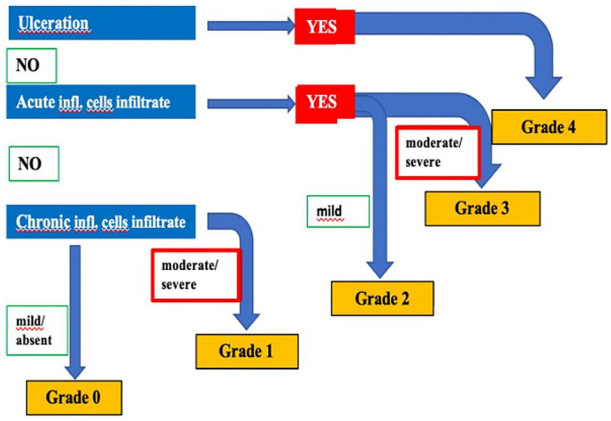

Figure 5 – *Nancy Index, algorithm.*

Table 5 – *OR for biological therapy response (CI 2.5–97.5%)*

Biological therapy response	OR (2.5–97.5%)	*p*-value
CD diagnosis	1.22 (0.08–18.68)	0.88
Disease duration >5 years	0.1 (0.01–1.21)	0
Previous biological treatment	0.23 (0.03–2.09)	0.19
Endoscopic activity	1 (0.15–6.71)	0.99
Basal plasmacytosis	1.8 (0.14–23.7)	0.65
Metaplasia	1.18 (0.11–12.37)	0.89
Granulomas	0.26 (0–18.61)	0.53
Depletion	4.9 (0.1–235.37)	0.42
Lymphoid aggregates	5.02 (0.48–53.07)	0.18
Ulcerations	4.03 (0.41–39.09)	0.23
Cryptitis	0.57 (0.05–6.77)	0.65
Abscesses	2.37 (0.21–26.94)	0.48
Dysplasia	0.38 (0.09–1.66)	0.19

CD: Crohn's disease; CI: Confidence interval; OR: Odds ratio.

Blinded to clinical or endoscopic data, the GI pathologist observed two groups of patients, "responders" and "non-responders". The "responders" were represented by the cases of improvement of at least two histological parameters. Statistical analysis highlights the accuracy of NI≥1 in defining treatment-responders, while Nancy grade 3 and 4 was associated with lack of response in IBD (Figure 6).

In addition to NI, we assessed clinical, biochemical and endoscopic data aiming to identify the accuracy and sensibility of each parameter in predicting treatment responders (Table 6).

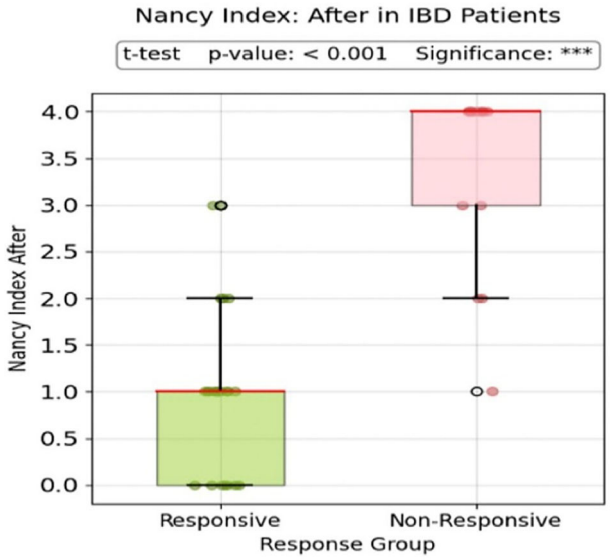

Figure 6 – ***Nancy Index, statistical comparison by t-test between responsive and non-responsive status after biological therapy. IBD: Inflammatory bowel disease.***

A logistic regression analysis was used, and model performance was assessed using confusion matrices, classification metrics and receiver operating characteristic (ROC) curves, as detailed in Table 6 and Figure 7 (A and B).

NI is the most accurate (88%), sensitive (92%) and specific (88%) parameter in identifying treatment responders. Endoscopic activity has a reasonable sensitivity (86%) but lacks specificity (60%). On the other side of the spectrum, clinical activity (HBI in CD patients and cMS in UC, respectively) lacks both sensibility and specificity (36% and 66.6%, respectively). Quite surprisingly, fecal CLP had a less-than-expected sensibility (60%) (Figure 7, A and B).

Table 6 – ***Accuracy, sensitivity, specificity of clinical, biochemical, endoscopic and histopathological scores***

Index assessment	Acc [%]	Se [%]	Sp [%]
Clinical activity			
▪ CD: HBI >5	48.0	36.0	66.67
▪ UC: cMS >2			
WBC count >10 000/μL	55.0	36.0	76.67
CRP >5 mg/L	57.5	64.0	46.67
CLP >200 μg/mg	67.5	60.0	80.0
Endoscopic status			
▪ CD: SES-CD >3 / RS ≥i2	77.5	86.0	60.0
▪ UC: MES >1			
NI ≥2	88.0	92.0	80.0

Acc: Accuracy; CD: Crohn's disease; CLP: Calprotectin; cMS: Clinical Mayo Score; CRP: C-reactive protein; HBI: Harvey–Bradshaw Index; MES: Mayo Endoscopic Score; NI: Nancy Index; RS: Rutgeerts Score; Se: Sensitivity; SES: Simple Endoscopic Score; Sp: Specificity; UC: Ulcerative colitis; WBC: White blood cell.

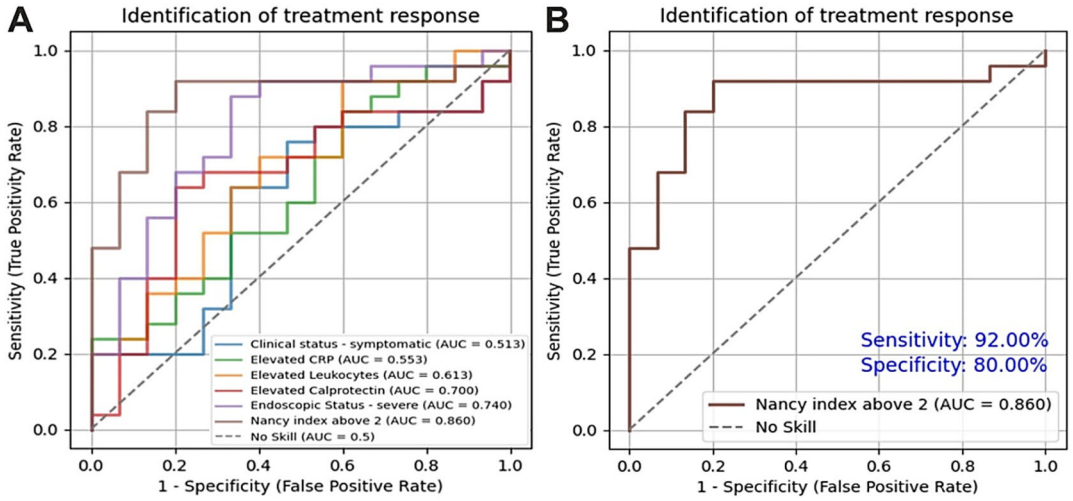

Figure 7 – ***(A and B) Logistic regression analysis, sensitivity and specificity of Nancy Index, and comparison with clinical, biochemical and endoscopic parameters. AUC: Area under the ROC curve; CRP: C-reactive protein; ROC: Receiver operating characteristic.***

Discussions

Over the years, the therapeutic target in IBD shifted from clinical to endoscopic remission. At the same time, histological activity has been recognized as an impactful target. The benefits of microscopic healing are unclouded, but there is scientific debate regarding proper patient management in cases with persistent histological activity despite endoscopic remission and the true impact of each parameter in predicting disease relapse. Our question is, should histological remission be an elective or a must-do target? A single-prospective study from two referral centers might not discern this dilemma but at least ought to draw attention to some practical issues we confront during clinical practice.

Endoscopic healing is the recommended long-term therapeutic target from STRIDE-II consensus [6]. There are a few shortcomings of endoscopic healing that we have to scrutinize and acknowledge.

First, the definitions of endoscopic healing represent a source of ambiguity and confusion. For UC patients, the *International Organization for the Study of IBD* (IOIBD) defines mucosal healing as the absence of ulcers, erosions, and friability [15], while in other clinical trials cMS 1 is accepted for the same target [37]. The lack of consistency in defining endoscopic healing is similarly persistent in CD [38, 39]. The STRIDE-II consensus characterizes endoscopic remission as SES-CD≤2 or CDEIS<3 and absence of ulcerations. The prognosis of a single or multiple ulcers in the same area is not clearly defined. In the CALM and

EXTEND studies (effect of tight control management on CD and role of Adalimumab in ileocolonic CD, respectively), the secondary endpoints of endoscopic remission were defined as CDEIS<4 [40, 41]. In the MUSIC trial, complete remission was defined as CDEIS<3 [42]. The asset of the aforementioned IBD scores is the reasonable reproducibility and level of agreement between experienced endoscopist in referral centers [43, 44]. Nevertheless, it is highly questionable how these scores really serve in routine practice among general practitioners, outside IBD-expert units; if not properly assessed, therapy management and clinical decisions would be biased. Secondly, endoscopic healing is masquerading histological inflammation in a significant number of patients, as proven in previous studies [10, 14, 45–48] and some of these patients will present symptomatic relapse undergoing a suboptimal treatment.

Another issue is the definition of histological remission which is not uniformly defined. There are discrepancies in literature, but most data points towards absence of neutrophils granulocyte infiltration [16]. The complexity of the most well-known scores like Geboes [49] or Robarts [50] might be the barrier that precluded implementation of histological remission as a long-term target. In our opinion, NI could be more easily implemented in daily practice and our study proves its accuracy in assessing treatment response, scoring highest in terms of accuracy, sensitivity and specificity. NI also proved valuable in defining histological remission in a recent meta-analysis [48].

A relatively recent publication brings into discussion a very interesting concept of *histological improvement*, which might be a more realistic target than *histological remission*, with equal or similar benefits in the long-term for IBD patients [17]. Too stringent criteria might lead to unnecessary step-up therapy management, therefore finding the right balance should be more appropriate. There is currently no clear distinction in the management of patients with mucosal healing and persistent histological activity *vs*. patients with complete remission (both mucosal and histological remission). These are several reasons justifying why we have proceeded to assess not only histopathological scores, but also individual acute and chronic inflammatory parameters. Acute inflammation is characterized by the recognition of acute inflammatory cells in the crypt epithelium, *lamina propria* and/or within the surface epithelium [51]. The *ECCO* position paper agreed that only one neutrophil present in the crypt epithelium is sufficient to define *cryptitis*, while identification of a cluster of neutrophils in the crypt lumen refers to a *crypt abscess* [51]. *Mucin depletion* is another important active inflammatory parameter and represents a decrease in the number of goblet cells [52]. Severe mucin depletion is a high sensitivity to differentiate between CD and UC [53]; it is also associated with the risk of relapse in cases with endoscopic remission [53]. The increase in the number of plasma cells (basal plasmacytosis) and lymphocytes (lymphoid aggregates) defines chronic inflammation [54]. *Basal plasmacytosis* is usually regarded as one of the promptest signs of incipient IBD, with a high specificity for diagnosis [55, 56]. This feature is linked to disease relapses in patients with mucosal healing [56]. *Lymphoid aggregates* can be identified basally between crypt bases and the *muscularis mucosae*. Transmural lymphoid aggregates that invade submucosa or *lamina muscularis* may induce deep fissures, ulcers and fibrosis [16]. The presence of Paneth cells distal to the splenic flexure is characteristic for *Paneth cell metaplasia* and represents a marker of longstanding colitis [57, 58]. It has been attributed to the effects of repair and regeneration [59].

There are some knowledge gaps regarding the importance of each individual histological marker in predicting treatment failure or response. For instance, basal plasmacytosis is a feature that is not part of the previous scores that we mentioned (Geboes/Robarts/Nancy) but its presence was related to a higher risk of clinical relapse in patients with clinical remission (OR 4.5) [54]. In our study, basal plasmacytosis was a feature that improved considerably after treatment, particularly in UC patients (present in 55% samples before biological therapy *vs*. 10% during follow-up). Reduced lymphoid aggregates sizes during treatment was also observed in treatment responders, finding in line with another cohort of patients treated with Vedolizumab in a study conducted by Canales-Herrerias *et al.* [60]. Mucin depletion is another feature overleaped by histological scores, despite that mucus abnormalities are described as causative agents in IBD in a recent study [61]. According to the group of Villanacci, complete restoration of mucin expression should be considered a histological marker of mucosal healing [62]. In our cohort, the presence of mucin depletion was significantly reduced in samples obtained after treatment (40% *vs*. 95%).

Due to the relatively small cohort, univariate analysis has not led to statistically significant results, but there are some remarks worth mentioning. The two major factors associated with treatment non-response were a long history of IBD diagnosis (>5 years) and failure to a previous biological therapy. Conversely, factors associated with treatment response were a high burden of mucin depletion, ulcerations and lymphoid aggregates in initial samples (before therapy). Even if it might not seem very intuitive at first glance, we can translate (at least partially) that biological therapy has a beneficial effect in early stages of the disease, when there is an initial high inflammatory burden. This is similar to the molecular mechanisms of biological therapy which may relieve acute obstruction due to inflammatory strictures, whereas there is minimal effect in fibrotic stenosis.

This study has some important strengths. First, it emphasizes the importance of NI which is a simpler tool, yet highly accurate in assessing treatment-response, while high inflammatory histological burden (mucin depletion, lymphoid aggregates and ulcerations) seems to predict treatment-response in biological therapy-treated patients. Secondly, the prospective assessment of individual histological parameters may lead to a more practical approach, easier to implement in general practice. At the same time, we have to recognize the main limitation of this study, which is the small cohort involved, yet we hope it will trigger interest in future studies.

Conclusions

Definitely, intricated histological scores impede consistent and uniform medical management, since not every patient is treated in dedicated expert units. This research addresses an important literature gap, considering that most studies

and clinical trials are centered around complex, intricated histological scores. While histological remission might be a too stringent goal, we do believe that histological improvement of individual parameters should be considered as long-term objectives in future guidelines. Expectantly, there will be vigorous research in the near-future aiming to assess the histological burden in patients with endoscopic remission in order to stratify low- *vs*. high-risk "relapsers" and to avoid under- or over-treatment.

Conflict of interests

The authors declare no conflict of interests.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the local Ethics Committee (Registration No. 45027/October 2022).

Informed Consent Statement:

All patients signed the written informed consent.

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Corresponding author

Mihai Mircea Diculescu, Professor, MD, PhD, 5th Department – Gastroenterology, Carol Davila University of Medicine and Pharmacy, 8 Eroii Sanitari Avenue, Sector 5, 050474 Bucharest, Romania; Phone +40722–300 254, e-mail: mmdiculescu@yahoo.com

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