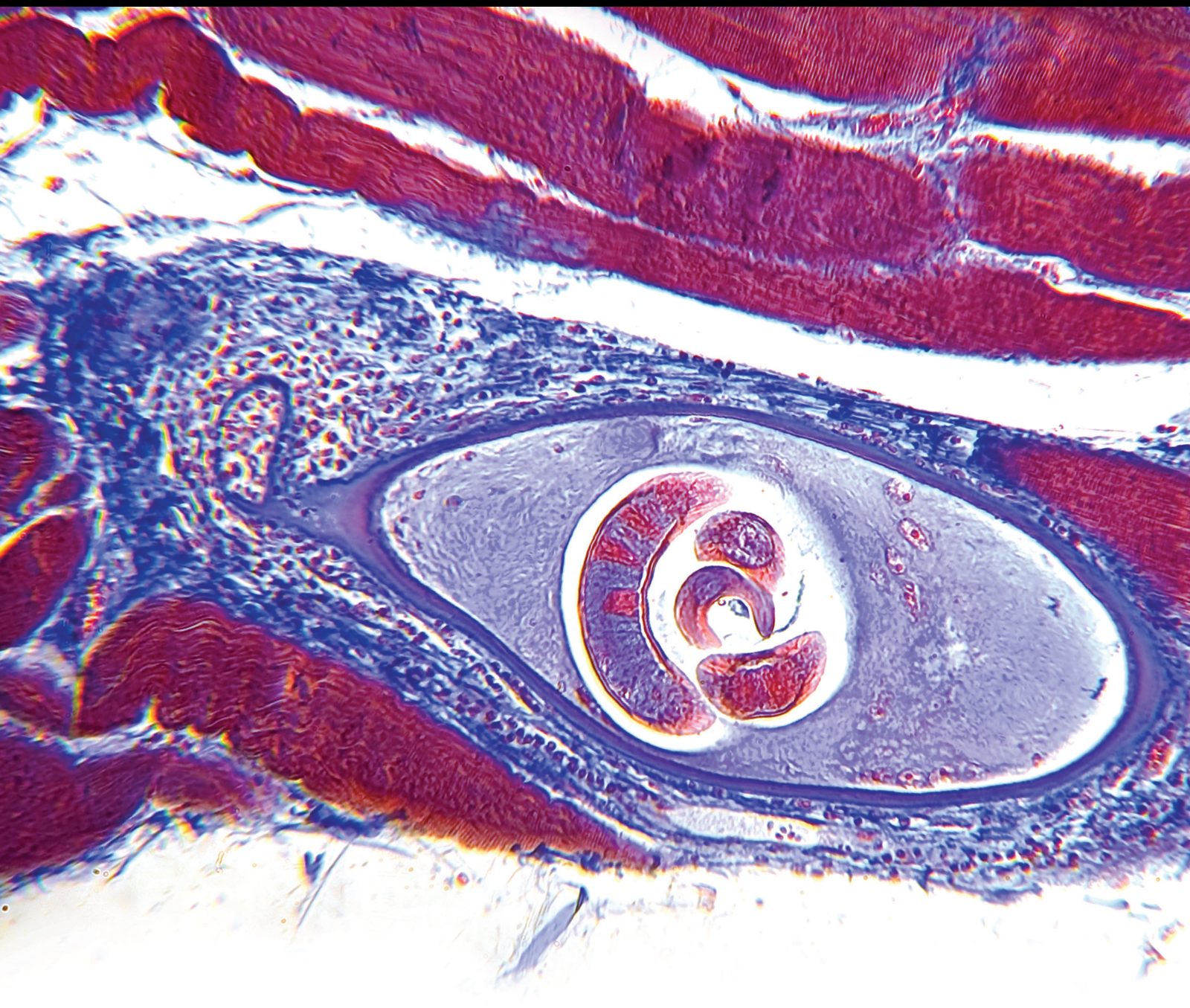
Gastroenterology Research and Practice

Diagnostic and Treatment Options for Inflammatory Bowel Disease

Lead Guest Editor: Elif Sarıtaş Yüksel
Guest Editors: Süleyman Günay, Muhammad Naeem, and Ülkü Dağlı



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Contents

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Research Article

Serum Immune-Inflammation Index Assessment in the Patients with Ulcerative Colitis

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Radiologic and endoscopic diagnostic methods are used to determine disease activity in ulcerative colitis (UC). In order for endoscopic procedures to be invasive and to prevent radiation exposure, especially in young people, studies have been carried out frequently to determine a simple, fast, and reliable activity marker with laboratory methods. Our aim in this study is to determine the usefulness of serum immune-inflammatory index as a noninvasive marker of activation in patients with ulcerative colitis. A total of 82 consecutive patients treated with a diagnosis of ulcerative colitis were included in the study. The disease activation was assessed using the Mayo endoscopic subscore. The site of involvement was grouped into two as left colitis and extensive colitis. Patients were divided into two groups as those who had active disease based on clinical and endoscopic findings and those who were in remission. C-reactive protein (CRP) levels, platelets, neutrophils, and lymphocytes were recorded in all participants. The systemic immune-inflammation index (SII) and CRP values were compared between UC patients with active disease or remission. The correlations between CRP, SII, and Mayo endoscopic subscores were analyzed. In addition, ROC curve analysis for SII was performed to determine the cut-off value, sensitivity, and specificity in determining ulcerative colitis activity. The value of SII was significantly higher in the active group than the remission group (respectively, 1497 ± 1300 and 495 ± 224, $p < 0.001$). In the correlation analysis, a significant correlation was found between SII and Mayo subscore. In ROC curve analysis, SII was found to be significantly effective in determining activity in ulcerative colitis patients. For 0.860 area under the curve, the sensitivity was 68.1% and the specificity was 91.2% at a cut-off value of 781.5. SII is significantly higher in patients with active ulcerative colitis than those in remission. It shows promise for use as a noninvasive marker of active ulcerative colitis.

1. Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease that can involve all colon segments starting from the rectum, causing diffuse and continuous inflammation. It progresses clinically with activation and remissions [1]. The diagnosis of ulcerative colitis is made by clinical, endoscopic findings, and histological evaluation. Determination of disease activity is important in determining the treatment of the patient [2].

Radiologic and endoscopic diagnostic methods are used to determine disease activity. In order for endoscopic methods to be invasive and to prevent radiation exposure, especially in young people, studies have been carried out frequently to determine a simple, fast, and reliable activity marker with laboratory methods [3, 4]. C-reactive protein (CRP) and fecal calprotectin as laboratory methods are currently used for this purpose [5]. Apart from UC, CRP can be elevated in many acute conditions, especially infections. Therefore, its usefulness in ulcerative colitis is limited compared to fecal calprotectin [6]. Fecal

calprotectin levels are affected by bowel movements, and different results can be obtained in the following days [7]. For these reasons, the search for a reliable, fast, and easy noninvasive method to determine the activity in ulcerative colitis still continues.

The systemic immune-inflammation index (SII) is an indicator calculated using neutrophil, platelet, and lymphocyte values. It is obtained by multiplying the neutrophil count and platelet count and dividing by the lymphocyte count. A high index of this index indicates the presence of relatively high neutrophil and platelet counts and low lymphocyte counts. This is indicative of a strong inflammatory response [8]. Its relation with disease prognosis and activity in many cancer types such as pancreatic cancer, colorectal cancer, bladder cancer, and inflammatory diseases has been shown in many studies [9–13].

Our aim in this study is to determine the usefulness of serum immune-inflammatory index as a noninvasive marker of activation in patients with UC and to determine the cut-off value as an activity indicator.

2. Materials and Methods

2.1. Study Population. A total of 82 consecutive patients aged over 18 years treated with a diagnosis of ulcerative colitis in our department between January 2020 and June 2020 were included in the study. Ulcerative colitis diagnosis was based on clinical, endoscopic, and histopathology findings.

2.2. Study Design. The study was planned as a retrospective study. Patients' age, gender, disease duration, treatments, colonoscopy results, and laboratory results were recorded for all patients from hospital software system.

2.3. Colonoscopic Evaluation. The disease activation was assessed using the Mayo endoscopic subscore, 0 points for normal or inactive lesions, 1 point for mild (redness, the reduced texture of blood vessel, and mildly brittle mucosa), 2 points for moderate (significant erythema, disappeared texture of blood vessel, brittle, or eroded mucosa), and 3 points (spontaneous mucosal bleeding or ulceration) [14]. The site of involvement was grouped into two as left colitis and extensive colitis. Patients were divided into two groups as those who had active disease based on clinical and endoscopic findings and those who were in remission.

2.4. Laboratory Evaluation. C-reactive protein levels, platelets, neutrophils, and lymphocytes on the same day as the colonoscopy were recorded in all participants. SII measured for all patients. SII and CRP values were compared between UC patients with active disease or remission. The correlations between CRP, SII, and Mayo endoscopic subscore were analyzed. In addition, ROC curve analysis for SII was performed to determine the cut-off value, sensitivity, and specificity in determining ulcerative colitis activity.

2.5. Exclusion Criteria. Age under 18 years, history of intestinal surgery, presence of active infection, coexistent hepatic and/or renal failure, presence of chronic disorders, pregnancy, lactation, proctitis, patients with hematological disease, malignancy, patients using biological agents, and patients without laboratory values on the same day as colonoscopy were excluded from the study.

2.6. Statistical Analysis. Statistical analysis of the study was done by using SPSS 25.0 (IBM Statistical Package for Social Sciences software version 25). Continuous variables were expressed as a mean ± standard deviation and categorical variables as a percentage. Chi-square test was used to compare categorical values, and the Mann-Whitney U test was used to compare continuous variables between groups. Receiver-operating characteristic (ROC) analysis was performed to calculate the cut-off values. Correlations between SII, CRP, and Mayo endoscopic subscore were determined using Spearman's rho test. A p value of less than 0.05 was considered as statistically significant.

2.7. Ethical Considerations. The study protocol was approved by the local ethics committee (approval no.: 2022-0032).

3. Results

A total of 81 ulcerative colitis patients, 42 (51.2%) female and 39 (48.8%) male, were included in the study. The mean age was 44.0 ± 15.20 years in active disease and 46.9 ± 13.5 years in remission. While 47 (58%) of the patients were in the active period, 34 (42%) were in remission. There was no difference between active and remission patients in terms of gender, age, site of involvement, and the treatment they received. While the Mayo subscore was 0 in all patients in remission, it was 1 in 17, 2 in 18, and 3 in 12 active patients. The comparison between patients with active ulcerative colitis and patients in remission is summarized in Table 1.

3.1. Evaluation of Laboratory Results. The mean CRP levels were 22 mg/dl in ulcerative colitis patients with active disease and 1.1 mg/dl in remission patients ($p < 0.001$).

SII levels were significantly different between active and remission groups (1497 ± 1300 and 495 ± 224, $p < 0.001$, respectively).

In the correlation analysis, a significant correlation was found between SII, CRP, and Mayo subscores. Comparison of laboratory results and correlation analysis between groups is summarized in Table 2.

In ROC curve analysis, SII was found to be significantly effective in determining activity in ulcerative colitis patients. For 0.860 area under curve, the sensitivity was 68.1% and the specificity was 91.2% at a cut-off value of 781.5 (Figure 1).

4. Discussion

The role of neutrophils in the pathogenesis of ulcerative colitis is well known. With the massive infiltration and activation of neutrophils into the inflamed area, proteinase and matrix metalloproteinases are excessively released and oxygen radicals increase. This causes crypt damage. As neutrophils increase in the lamina propria, the crypt epithelium is more commonly damaged and causes ulceration in the mucosa [15, 16]. On the other hand, there is dysregulation in neutrophil apoptosis in ulcerative colitis. Antiapoptotic cytokines such as granulocyte-macrophage colony-stimulating factor

Table 1: Comparison of demographic, colonoscopic, and laboratory findings between the patients with active ulcerative colitis and ulcerative colitis in remission.

	Patients with active UC $n = 47$	Patients with UC in remission $n = 34$	p
Age (year)	44.0 ± 15.20	46.9 ± 13.5	0.379
Sex (F/M)	25/22	17/17	0.477
Site of involvement			0.85
Left-sided	22	22	
Extensive	25	12	
Treatment			
None	3	0	0.26
5-ASA	30	23	0.815
Azatiopurine	10	11	0.309
Steroid	4	0	0.135
Mayo subscore			<0.001
0	0	34	
1	17	0	
2	18	0	
3	12	0	

UC: ulcerative colitis; ASA: acetylsalicylic acid.

Table 2: Correlation analysis and comparison of laboratory findings between the patients with active ulcerative colitis and ulcerative colitis in remission.

	Patients with active UC $n = 47$	Patients with UC in remission $n = 34$	p
SII	1497 ± 1300	495 ± 224	<0.001
CRP (mg/dl)	22 (1.7-161)	1.1 (0-5.1)	<0.001
Correlation analysis			
SII-CRP			<0.001
SII-Mayo subscore			0.004

UC: ulcerative colitis; SII: systemic immune-inflammatory index; CRP: C-reactive protein.

are shown as the cause of this situation. Thus, neutrophils stay in the mucosal inflammation area for a long time and accumulate, causing a delay in the clearance of inflammation [17]. Therefore, the disease activity in ulcerative colitis is parallel to the increase of neutrophils.

It is also known that inflammation in ulcerative colitis is associated with platelets. In active ulcerative colitis, platelet counts increase and there are morphological changes such as increase in size, loss of discoid shape, increase in granular contents, and increase in density. Overproduction and release of many factors such as fibrinogen, P-selectin, von Willebrand factor, and fibrinolitic inhibitors occur from the granular content. There is an increase in the number of receptors associated with cytokines and complement components in the platelet membrane [18]. The result is an increase in the number and activity of platelets in ulcerative colitis. An increase in platelet count is accepted as a biomarker of activation in inflammatory bowel diseases [19, 20].

It has been shown in many studies that SII value is an indicator of poor prognosis in malignancies where the inflammatory reaction is active [21]. Again, it has been demonstrated that SII is a significant activation indicator in active rheumatic diseases in which there is a strong inflammatory response [22]. In this case, as the severity of inflammation increases, the evaluation of SII as an activation marker comes to the fore in ulcerative colitis with increased activity. SII obtained using platelet, neutrophil, and lymphocyte counts can be a strong indicator of inflammatory status in ulcerative colitis patients.

In our study, we compared SII values between patients with active ulcerative colitis and patients in remission, with the hypothesis that the SII value will increase as the inflammation activity increases. We found the mean SII values to be significantly higher in active patients than in patients in remission (1497 ± 1300 and 495 ± 224, $p < 0.001$, respectively). As expected, CRP and Mayo score were significantly higher in active disease ($p < 0.001$). We found a significant positive correlation between SII, CRP, and Mayo scores. In our study, the sensitivity and specificity of SII were 68.1% and 91.2% at the cut-off value of 781.5.

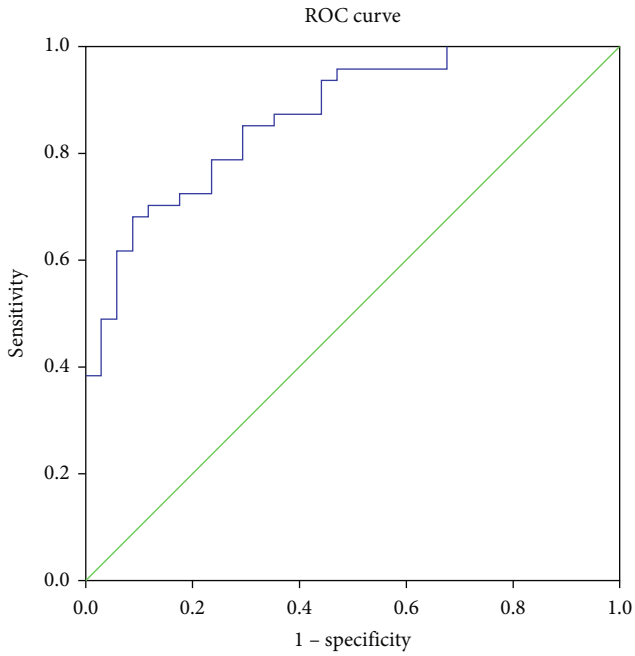

Figure 1: ROC curve analysis of SII values in the patients with active ulcerative colitis.

There are two studies in the literature that evaluated SII in ulcerative colitis patients. In the study published by Xie et al. in 2021, SII was evaluated in ulcerative colitis patients and in the control group, and it was found to be significantly associated with disease activity [23]. Similar to our study, a significant positive correlation was found between SII, Mayo score, and CRP in the correlation analysis. In the study published by Zhang et al. in 2021, SII values in ulcerative colitis patients were found to be significantly higher than the control group and correlated with the Mayo score [24]. On the basis of these findings, we think that SII value is higher in active disease due to the increase in platelet and neutrophil counts with activity in accordance with the pathogenesis of ulcerative colitis. In the light of the results obtained from previous studies and the current study, we think that the use of SII as a noninvasive and radiation-free activity marker in the follow-up of inflammatory bowel disease will be beneficial.

There are some limitations in our study. First of all, the study was done retrospectively. A prospective study with larger patient groups would be more powerful. In addition, histological severity was not evaluated in patients, and therefore, histological severity was not compared with SII values. Because of their small number, patients with proctitis were not included in the study. There was no control group in our study, and the comparison was made between active and remission patients.

In conclusion, SII is an indicator of a strong inflammatory response. Patients with active ulcerative colitis, who have a stronger inflammatory response, have significantly higher SII values than those in remission. It shows promise for use as a noninvasive marker of active ulcerative colitis. These results need to be supported by prospective studies in large patient groups.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

This study was presented as poster presentation in 38, National Gastroenterology Week, November 16-21, 2021, Antalya, Turkey.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Fecal Microbiota Transplants for Inflammatory Bowel Disease Treatment: Synthetic- and Engineered Communities-Based Microbiota Transplants Are the Future

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The human intestine harbors a huge number of diverse microorganisms where a variety of complex interactions take place between the microbes as well as the host and gut microbiota. Significant long-term variations in the gut microbiota (dysbiosis) have been associated with a variety of health conditions including inflammatory bowel disease (IBD). Conventional fecal microbiota transplantations (FMTs) have been utilized to treat IBD and have been proved promising. However, various limitations such as transient results, pathogen transfer, storage, and reproducibility render conventional FMT less safe and less sustainable. Defined synthetic microbial communities (SynCom) have been used to dissect the host-microbiota-associated functions using gnotobiotic animals or *in vitro* cell models. This review focuses on the potential use of SynCom in IBD and its advantages and relative safety over conventional FMT. Additionally, this review reinforces how various technological advances could be combined with SynCom to have a better understanding of the complex microbial interactions in various gut inflammatory diseases including IBD. Some technological advances including the availability of a gut-on-a-chip system, intestinal organoids, ex vivo intestinal cultures, AI-based refining of the microbiome structural and functional data, and multiomic approaches may help in making more practical *in vitro* models of the human host. Additionally, an increase in the cultured diversity from gut microbiota and the availability of their genomic information would further make the design and utilization of SynCom more feasible. Taken together, the combined use of the available knowledge of the gut microbiota in health and disease and recent technological advances and the development of defined SynCom seem to be a promising, safe, and sustainable alternative to conventional FMT in treating IBD.

1. Introduction

The intestinal tract of the majority of animals including human beings is colonized by complex microbial communities since birth, called the microbiome. The composition of the microbiome differs between individuals, shows unique spatiotemporal organizations, and has a significant role in the host health and disease [1]. Thanks to the recent scientific and technological advances, particularly the discovery of high-throughput sequencing techniques, significant progress has been achieved to decipher the structure and function of the gut microbiome. The major players of the gut microbiota include Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria, and Verrucomicrobia [2]. However, we are unable to culture 99% of the microbial majority of the gut microbiota and are thus unable to explore the characteristics of all the individual microbes of the community [1]. Again, the advances in sequencing technologies have enabled us to get insights into important structure-driven functional information of the intestinal microbiome, and analysis of the microbiome of thousands of individuals revealed that each individual has a unique microbiome [2, 3]. Further, long-term dynamic studies (up to 10 years) of the microbiota of healthy human individuals revealed that

the microbiome composition of the gut remains relatively stable (for up to 10 years) as compared to other parts of the body including skin and oral cavity [2, 4]. Moreover, other long-term studies revealed that a variety of biotic and abiotic factors influence the composition of the gut microbiome including dietary intake, the use of antibiotics, intestinal transit, and lifestyles [5, 6].

The recent technological advances and utilization of a variety of accurate approaches have further made it possible to monitor and accurately analyze the intestinal microbial community composition and how the functionality and structure of the microbiome vary in healthy and diseased individuals. This has led to remarkable success in correlating the microbiome in health and disease. Currently, the host intestinal microbiome has been associated with a variety of diseases, including various inflammatory diseases of the intestine, cancer, and obesity which are extensively reviewed recently [7]. Other medical conditions which have been correlated with lower gut microbial diversity include atopic eczema [8], type 1 and type 2 diabetes [9, 10], psoriatic arthritis [11], coeliac disease [12], arterial stiffness [13], and Crohn's disease [14]. The host-microbiome dysbiosis and associated health effects are depicted in Figure 1.

There is a gigantic amount of literature supporting the role of microbiota in health and disease. However, various ethical, medical, and microbiological concerns make it hard to establish causal relations between the microbiota and host. The use of fecal microbiome transplantations (FMTs) is an exception though, in which the intestinal microbiota of the healthy donor is transferred to a diseased individual (recipient). FMT-based interventions in humans recently caught the attention of the scientific community. This is because FMT has shown promising results in treating a variety of diseases, indicating that the intestinal microbiota does play a role in influencing the physiology and health of the host.

Following the success of FMT in treating ulcerative colitis (UC) and recurrent CDI patients over a prolonged period of 56 years, FMT-based treatments are now making their way to target several other diseases. And it all started with an important case to mention, the one reported in 1989 by Dr. Justin D Bennet [15], who suffered himself from a severe UC, and when nothing else worked for the treatment, he was finally cured by FMT from a healthy donor. Since then, FMT has been successfully employed as a potential treatment in a variety of diseases including CDI, UC, IBD, irritable bowel syndrome (IBS), chronic fatigue syndrome [16], and multiple sclerosis. FMT has been employed in more than 500 cases of chronic or recurrent CDI, resulting in an average of 95% recovery of the patients. Thus, currently, FMT is indicated only to treat recurrent CDI [17–19]. However, several clinical trials are in progress to investigate the effectiveness of FMT-based therapy in a variety of other conditions, indicating that this therapy may potentially become a novel cure option for a variety of health conditions. However, various limitations associated with conventional FMT usually end up in transient results or infections. This review focuses on the potential application of FMT in treating IBD as well as various challenges and limitations associated with conventional FMT therapies. This review also reinforces the use of defined synthetic communities (SynCom) to overcome the limitations of conventional FMT and how SynCom approaches could be combined with recent technological advances for more practical use.

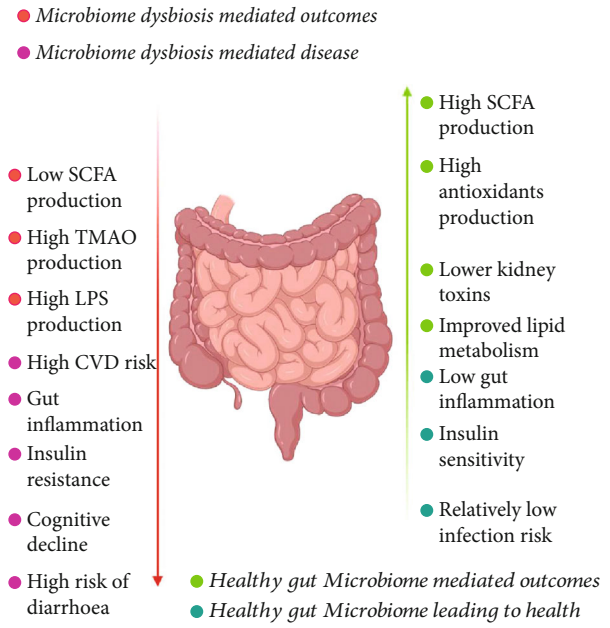

Figure 1: Schematic representation of the gut microbiota-mediated outcomes and their role in health and disease.

2. Inflammatory Bowel Disease (IBD), Pathogenesis, and Drugs-Based Current Treatment Options

Inflammatory bowel disease (IBD) is a worldwide disease in the 21st century [20]. It is a chronic inflammatory disorder of the gastrointestinal (GI) tract with unknown etiology. IBD is divided into two major subtypes: Crohn's disease (CD) and ulcerative colitis (UC). UC classically involves the rectum and may affect the entire colon or part of the colon in a continuous pattern. The inflammation in UC is confined to the mucosal layer. Conversely, CD can affect any part of the GI tract but most commonly involves the ileum and perianal regions in a noncontiguous pattern, causing transmural inflammation [21]. The main symptoms of IBD include diarrhea, rectal bleeding, anorexia, and weight loss that can result in continuous bowel damage with increased risks of hospitalizations, surgeries, and colorectal cancer [22]. Children developing IBD usually have more severe diseases than adults [23]. Collectively, these conditions can result in unbearable physical and psychosocial symptoms for patients and affect society through the loss of schooling, jobs, and health care costs [24].

The exact cause of IBD is unknown. However, an inappropriate mucosal immune response against millions of antigens from food, environment, and microbiome in a genetically and/or immunologically predisposed host is believed to play a role in the pathogenesis of IBD [25].

Mucosal immune system cells such as intestinal epithelial cells, innate lymphoid cells, cells of the innate and adaptive immune system, and their secreted mediators are associated with the pathogenesis of IBD. Overall, this dysregulation in the immune system stimulates an inflammatory cascade by producing proinflammatory cytokines leading to chronic intestinal inflammation, also known as mucosal damage [26].

IBD is a public health challenge worldwide for the health professional to treat. Patients with IBD can experience symptoms at a young age [27], necessitating long-term and often costly treatment throughout their lifetimes [28]. To date, there is no cure for IBD. The aim of drug therapy is to achieve and maintain remission from inflammatory episodes. The treatment regimen of IBD consists of anti-inflammatory agents such as 5-aminosalicylates (5-ASAs), corticosteroids, immunosuppressants, and biologic agents such as tumor necrosis factor-alpha (TNF-α) antagonists, anti-interleukins, and anti-integrins. These drugs can induce and maintain remission from inflammatory episodes; however, they can cause serious side effects including increased risk of infections and certain cancers [29]. This may prevent IBD patients from continuing therapy and can lead to failure of therapy and increase patient morbidity and health care costs. Therefore, an utmost need is existing to find an alternative, safe, and effective therapeutic strategy for IBD therapy.

3. Fecal Microbiota Transplantations in Inflammatory Bowel Disease

There is a shred of growing evidence backing the gut microbiome's role in IBD pathogenesis [30]. Diversion of the fecal stream is usually utilized to treat IBD; however, a reexposure to luminal contents and reversal of the fecal stream can lead to a relapse of the disease. Additionally, antibiotics are another choice of induction therapy in IBD; also, remission can be achieved by strict enteral nutrition in CD [31, 32]. Dysbiosis is an established fact in IBD, and it has been known that the gut of IBD patients shows a relatively lower bacterial diversity particularly the loss of anaerobic bacteria [33]. Thus, gut microbiota can be targeted for novel treatment options. Initial reports regarding the use of probiotics did not result in any significant outcomes in IBD treatment [30]. A search on NIH clinical trials.gov (https://clinicaltrials.gov/) was conducted using various search terms such as fecal microbiome transplant, *Clostridium difficile* infection, Ulcerative colitis, IBD, and microbiota transplants to extract information related to trials utilizing FMT in these gut health conditions. A total of 78 clinical trials were enlisted, which were investigating the efficacy of FMT in CD or UC. Among these, 22 trials are completed and only four studies are with results whereas two studies were terminated based on the interim analysis results [34, 35]. These four completed randomized controlled trials (RCTs) revealed promising results in a small subset of UC patients (Table 1) [34–37]. Two of the terminated studies though did not show any significant difference over placebo; still, the FMT treatment outcomes appeared relatively better [34, 35]. In one study, the efficacy of FMT was dependent on donors, and the microbiota profiling of the donors resembled that of the patients who achieved remission after FMT [34]. Moreover, some other single group assignment (SGA) clinical trials also showed that FMT could result in a positive outcome in IBD (Table 1). A recent meta-analysis indicates that FMT-based interventions significantly impact remission than placebo (95% CI 2.196-5.240, $P < 0.001$). However, RCTs are lacking for CD, and various uncontrolled cohort studies with small sample sizes have revealed mixed results. For instance, using meta-analysis, a 52% remission rate was reported among 71 CD patients who received FMT [38]. However, among the studies pooled into the meta-analysis, only a single one was a large cohort study and the remission rate was attributed mainly to it [39]. Furthermore, no endoscopic remission was observed eight weeks post-FMT in CD patients [40]. Because the FMT outcomes in IBD patients are not constant, this treatment option should still be considered an experimental one. More studies are needed regarding suitable donor selection, selection of highly responsive patients, and processing of feces under anaerobic conditions. Moreover, we still do not know what should be the proper timing for FMT interventions in IBD patients. Should FMT be used as the primary treatment or should be applied postinduction therapy? The good thing is that various trials that are currently ongoing may help to address the above questions. Additionally, this will further pave the way for using FMT as a potential treatment option in the future for IBD patients. FMT is also utilized in IBD patients who experience recurrent *Clostridium difficile* infection (rCDI), and a meta-analysis revealed that FMT could result in significant outcomes for treating rCDI in IBD patients (initial cure rate of 81%) compared to non-IBD patients [41]. Additionally, FMT has been known equally significant to treat rCDI both in CD and UC patients. Some of the reported adverse outcomes of FMT include IBD flare; however, it is still debatable whether this flare was associated with FMT intervention or was the result of CDI.

4. Challenges and Limitations Associated with Conventional Fecal Microbiota Transplantation

FMT-based therapies seem to be a promising treatment option for a variety of diseases including IBD; however, it has a variety of limitations resulting in transient and adverse outcomes (Figure 2). One of the major limitations is long-term safety. Although FMT is considered "safe" or "natural" or even "organic" by a majority of the recipients and practitioners, it can potentially be harmful and risky. There is a potential risk that the fecal material from a healthy donor may expose the patient to enteric pathogenic microorganisms and thus spreading and contracting the disease. In a recent study, two patients who recently received FMT were reported being infected with extended-spectrum beta-lactamase (ESBL)-producing pathogenic *Escherichia coli* bacteremia. The source of infection in both patients was tracked back to the same donor stool. One of the two patients expired [42]. FMT-mediated infections have been

Table 1: Completed clinical trials investigating FMT as a potential therapy for IBD.

Feature of study	Completed RCT trials				Completed SGA trials			
	Moayyedi et al.	Rossen et al.	Paramsothy et al.	Costello et al.	NCT02108821	NCT03106844	NCT01560819	NCT02049502
Study design	Double-blind RCT	Double-blind RCT	RCT	RCT	Single group assignment	Single group assignment	Single group assignment	Single group assignment
Number of patients (placebo)	75 (37)	48 (25)	81 (40)	73 (35)	23 (NA)	50 (NA)	9 (NA)	8 (NA)
Treatment regimen	6 FMTs	2 FMTs	40 FMTs	3 FMTs	1 FMT	1 FMT	20 FMT	Single FMT
Comparator (placebo)	Water	Autologous FMT	Water	Autologous FMT	None	None	None	None
Route of administration	Lower GI, enema	Upper GI, duodenal tube	Lower GI, retention	Lower GI, retention	Upper GI, jejunal intubation	Lower GI, colonoscopy	Retention enema	Lower GI, sigmoidoscopy
Stool donor per suspension	Single donor	Single donor	Multiple donors	Multiple donors	NA	NA	Multiple donors	Single donor
Follow-up	6 weeks	12 weeks	8 weeks	8 weeks	26 weeks	8 weeks	4 weeks	13 weeks
Primary endpoint	Endoscopic remission	Endoscopic remission	Endoscopic response	Endoscopic remission	Occurrences of adverse events	Recurrence of CDI in IBD patients	Improvement in PUCAI score	Improvement of pouchitis symptoms based on mPDAI
Primary outcome FMT versus comparator	24% (9/38) versus 5% (2/37) $P = 0.03$	30% (7/23) versus 20% (5/25) $P = 0.51$	27% (11/41) versus 8% (3/40) $P = 0.02$	32% (12/38) versus 9% (3/35) $P < 0.01$	52.17% (12/23)	8.2% (4/49)	Improvement in PUCAI score in all patients; 100% (9/9)	Improvement in mPDAI score in all patients; 100% (9/9)

Abbreviations: FMT: fecal microbiota transplant; GI: gastrointestinal; RCT: randomized controlled trial; UC: ulcerative colitis; mPDAI: modified pouchitis disease activity index; PUCAI: Pediatric Ulcerative Colitis Activity Index; SGA: single group assignment.

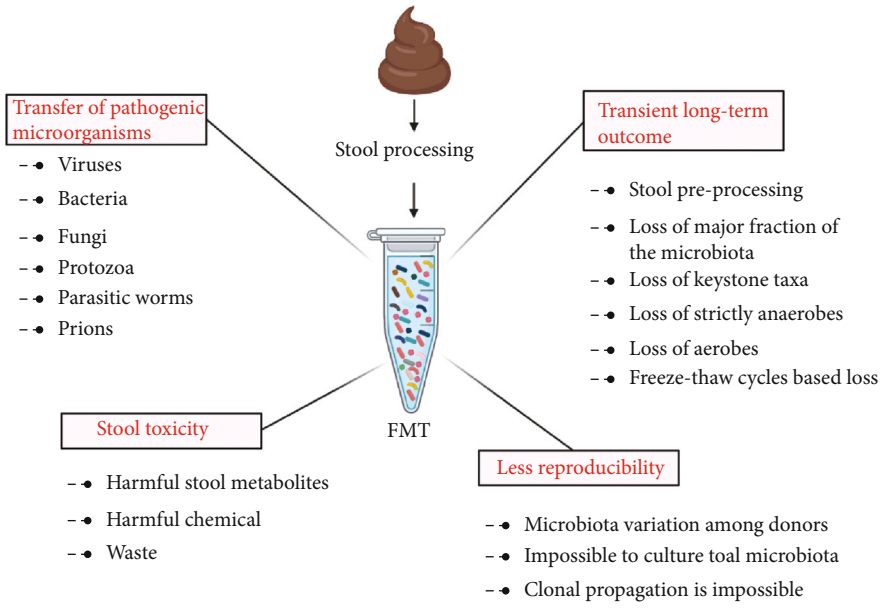

Figure 2: Disadvantages and limitations of conventional FMT. Various disadvantages include pathogen transfer, transient results, stool toxicity, and difficulty in reproduction.

reported in other cases as well, where the source of infection was supposed to have been presented by the fecal microbiome [43–45]. This warrants the need for improved donor screening to minimize the risk prior to FMT-based therapies.

Another important concern is the reproducibility and sustainable long-term use of FMT for a stable outcome. Though FMT shows promising results in the case of CDI, in other diseases such as IBD, it usually ends in the transient outcome. This indicates the complexity of the host-microbiome interactions and the low-key technology and poor practices leading to the loss of the major fraction of the original microbiota. Since each individual carries a unique and stable microbiota, it becomes very important to identify a healthy donor microbiome and ensure the reproducibility of the exact replica of that microbiome for long-term sustainable use and stable clinical outcome. Several factors lead to the loss of a major fraction of the fecal microbiota and thus transient results. For instance, majority of the intestinal microbes are strictly anaerobes, and fecal samples are mostly processed under aerobic conditions, which will instantly kill the anaerobes [46]. On the other hand, if handled anaerobically, the strictly aerobes will vanish. Also, the routinely used storage techniques at low temperatures (-20°C to -80°C) have been known to lead to significant loss of community members of the original microbiota, as a result of the freeze-thaw cycles [47]. This can further result in instability of the clinical outcome in FMT therapy. Moreover, knowledge is lacking for the long-term freeze-based storage (~10 years) of the intestinal fecal materials and their efficacy. Also, stool preprocessing for FMT preparations can lead to significant loss or damage to the major fraction of the microbial community resulting in the loss of approximately 50% of members [48]. Furthermore, cultured-based approaches also seem not suitable, because the gut microbiome is known to be composed of more than 2000 different species majority of which (≥90-99%) cannot be cultured. Also, the stool material itself consists of a variety of harmful chemicals, metabolites, and waste which can pose potential harm to the donor. In summary, there is a large disparity between the currently used technology for FMT-based treatment and the delicate knowledge of the gut microbiota. Therefore, ensuring the long-term safety of the donors in FMT-based treatments should be the primary priority. Also, the production of a reproducible functional microbiome from a single healthy donor may ensure long-term sustainable use and stable outcomes.

5. Synthetic and Engineered Microbial Communities to Understand Microbiota-Assisted Functions

The human gut harbors a diverse array of microorganisms, and it is quite challenging to assess how individual microbes interact with the host and to understand microbiome-mediated functions. Culture-based approaches have been used to decipher host-microbiome interactions; however, various limitations such as only a minute fraction of the total gut microbiota being culturable make these approaches difficult to understand host-microbiome interactions [49–53]. This indicates that conventional screening approaches are not ideal to decipher the host-microbiome complexity and microbiome-assisted functions. A possible solution to this problem is the concept of synthetic bacterial community (SynCom), which is a structurally defined/controlled community. SynCom consists of relatively few known cultured microbial members, and it acts as a representative of the original host-microbiome functions and structure [53]. The SynCom approach has a great advantage in that we can manipulate this community by simply adding, eliminating, or substituting one or a few strains to achieve desired functions including probiotic properties and disease remission (Figure 3). Additionally, such manipulations can even be introduced at the strain genetic levels as well; for instance, individual functions of the SynCom member microbes can be deleted or improved using gene silencing or increased expression, respectively. Because the SynCom microbial members are culturable, this renders the member strains suitable for dissecting the structural complexity and microbiota-associated functions via reductionist approaches. SynCom approaches could be of great use while testing germ-free organisms to decipher the quantitative and qualitative traits of the host driven by the host-associated microbiota. Moreover, the use of SynCom has become an important and practical alternative to the use of conventional FMT as it lacks most of the limitations associated with conventional FMT. These include sustainable use, stable outcome, ease of reproducibility, and long-term safety [53].

The SynCom approach has been widely utilized to determine its safety and functionality in various pathological conditions [54]. Though the source organisms used in these SynCom were derived mostly from humans, none of these were tested back in humans and alternative hosts used were either germ-free mice, rats, or pigs [54]. Next-generation sequencing- (NGS-) based metagenomic studies have been utilized to explore the gut microbial community structures of humans and other animals both in healthy and diseased conditions. Further insights into such NGS data such as relative abundance analysis and network analysis have shed light on microbial dynamics as well as member strains which are crucial for maintaining the structure and function of the microbial community. Moreover, recent advances in culturomics have led to an increased number of cultured organisms from the gut, particularly those which were previously considered unculturable [55]. For instance, various combinations of the long-known popular Altered Schaedler Flora- (ASF-) based SynCom have been utilized in different organisms to see its applicability in improving various pathological conditions (extensively reviewed by [54]). One study used ASF-based SynCom (comprised of 8 different strains) in mice to see how it affects the death rate after *C. botulinum* infection, fecal *C. botulinum* toxin excretion, and colonization pattern [56]. Results revealed that SynCom though did not prevent infection; however, the death rates were significantly lower in mice who received SynCom-based transplantations compared to the nontreated controls. Such functional studies thus indicate that SynCom-based approaches would be a powerful technique to dissect host-

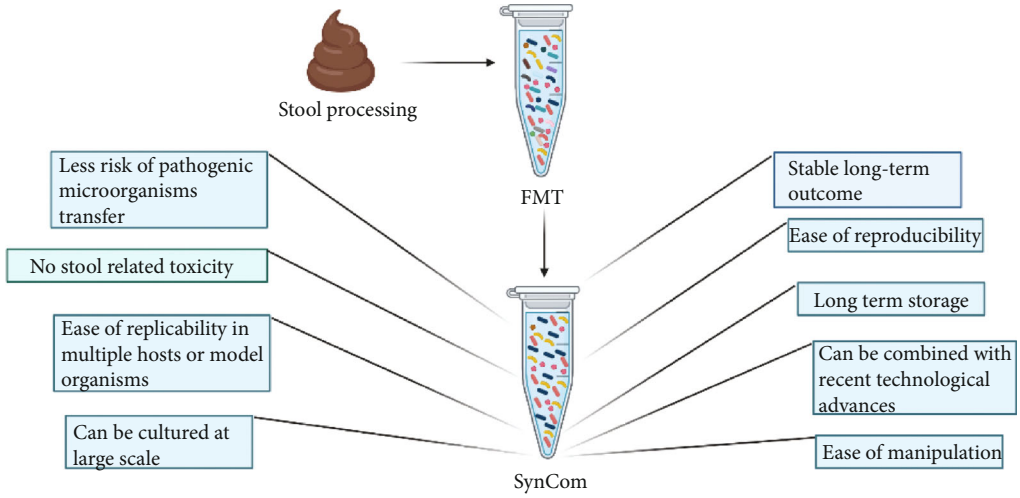

Figure 3: Advantages of SynCom-based transplants over conventional FMT. Various advantages of the SynCom-based FMT include relatively more safety, stable results, relatively less stool toxicity, and ease of reproducibility.

microbiome interactions. The major drawback observed for the ASF communities was that it poorly represents the dominant flora of the gut. Hence, SynCom was further modified to include members representative of the dominant flora as well. Thus, a variety of formulations were tested such as the Oligo-MM (murine microbiota) which consisted of twelve members. The Oligo-MM-based SynCom revealed that this community could provide significant resistance against *Salmonella enterica serovar Typhimurium* colonization and was even relatively better than ASF-based communities [57]. So far, only a single study has been conducted assessing the significance of SynCom in IBD in mice. The role of pathogenic bacteria *Helicobacter hepaticus* in the presence of normal ASF flora was determined in IBD [58]. This flora consisted of eight anaerobic species. Results revealed that even the presence of a single pathogen could lead to IBD conditions in the presence of normal representative flora. This was the pioneering study which revealed that the gut flora has a role in establishing IBD condition.

Though SynCom has a broad range of practical applications in decoding the functional prospects of host-microbiota, still it is unclear whether the SynCom-based outcomes observed in most of the animal models used could be replicated in humans and closely related other hosts as well. The fraction of the organisms cultured so far from the human gut is so small relative to the total microbial diversity of the gut. Therefore, there is a dire need to cultivate more organisms from the gut. Particular emphasis should be on culturing organisms that are abundant in the gut but are still not cultured or with very few cultured members. One such example is the Verrucomicrobia phyla, which are usually present in abundance in the human gut, but so far the cultured members are limited to few representative strains. Once we have enough number of cultured representatives of the gut microbiome, only then will we be able to design better SynCom which could then be of more practical use in human hosts.

6. Perspectives: How SynCom Could Be of Better Use in IBD and Address the Conventional FMT Limitations

The recent technological advances in the field of multiomics including but not limited to structural and functional metagenomics, metatranscriptomics, metaproteomics, metabolomics, and the ease of big data analysis have been largely utilized to elucidate the structure and function of the host-associated microbiota [59]. Few big projects in the fields to mention that have greatly facilitated the understanding and future goals include the Human Microbiome Project, the American Gut, the European microbiome project, and the Asian microbiome project. This has further facilitated the provision of gut bacterial strain banks comprising of diverse isolates as well as has standardized the host-associated microbiota structural and functional profiling protocols [18, 60].

Recently, the concept of the core microbes has been introduced, suggesting that certain bacterial groups are critical for maintaining the structure and function of the gut microbial community [61–63]. However, there is a need for suitable model systems to decipher and test the role of these core microbes and to establish causality in terms of microbiota-assisted functions in animal models. Such model systems will enhance our understanding of the host-microbiota interactions and also interactions among the diverse members within the microbial community. The existence of inherent complex interactions among microbial community members makes it difficult to understand and assign the resultant phenotype to an individual microbe or to a subgroup of that particular microbial community. Such complex interactions should be investigated with the help of suitable model systems. Utilizing culture-based approaches to culture diverse groups of organisms from the gut further decreases the fickleness as a result of the complexity of the microbial community. Further, such approaches make it

feasible to test principles of interactions among the host and its associated microbiota as well as intrinsic interactions among the microbial community members under controlled conditions. There is a dire need for an advanced strategy to dissect the host-microbiome interactions at the gut interface in various gastrointestinal conditions such as IBD. This can further elucidate the individual role of both sides (the gut microbiota and the host immune response) in IBD.

SynCom comprising of several culturable bacterial isolates of the human gut could be one such alternative to address the limitations of conventionally used FMT as well as those posed by the inherent complexity of the gut microbiota. The use of SynCom may shed light on how the dynamics of gut microbial community composition contribute to IBD development in terms of intermicrobial interactions: physical, chemical, and genetic interactions. Moreover, the use of SynCom in model organisms would further explain the underlying functional mechanisms and intermicrobial as well host-microbiota interactions leading to disease or health. Therefore, defined SynCom stand as the only promising validation tool for host-microbiota-associated function dissection *in vivo*. Also, this approach allows one to test and transfer the outcomes in the laboratory, and the output can ultimately be translated and utilized at a broader scale such as the treatment of various gastrointestinal conditions including IBD via SynCom-based transplants.

A variety of clinical trials have assessed the efficacy of conventional FMT in various gastrointestinal disorders including IBD (Table 1). Moreover, various conventionally used combinations of cultured microbes have been utilized in model organisms including mice, rats, and pigs to see if they can lead to a significant outcome [58, 64]. However, most of the studies have neglected postconventional FMT gut microbiota analysis. A significant number of studies are required to investigate the positive and negative outcomes post-FMT transfer and the associated microbial community structure. Comparative analysis of the microbial communities in both cases (positive and negative outcomes) could shed light on which microbial taxa, in particular, are responsible for leading to IBD or curing the IBD. Additionally, the big data coming out from such projects are complex, and such complexity of the microbial community structure and dynamics could potentially be solved by the recent technological advancements in the field of artificial intelligence (AI), including machine learning algorithms that could integrate huge metagenomic and microbiome data [65, 66]. Luckily, a gigantic amount of such comprehensive data has been sufficiently generated in the field of medical sciences. Therefore, AI-based big data comparative analysis of the gut microbiota in normal individuals, in individuals with IBD, and in individuals who have received FMT post-IBD could further enhance our understanding of the intermicrobial interactions, host-microbiome interactions, elucidating the role of the gut microbiota leading to IBD or curing IBD and finally fine-tuning of SynCom for future medical applications.

Various human microbiome-related studies have explored the microbial community structure that lives in association within the human gut [5]. Additionally, the presence of multipartite interactions (host-microbiota interactions and microbe-microbe interactions within the community) have been explored to some extent; however, much is still unknown and various important questions still do exist, and answering those will help in the design and utilization of SynCom for more practical use such as in conditions like IBD. Ultimately, there are still several important questions to be answered. The following are some of those questions: (1) What are the underlying mechanisms that gate and maintain a unique gut microbiota structure and lead to a healthy gut? (2) Is it the human host immune system acting as a gatekeeping system selectively allowing some (but not all) microbes to colonize the gut? (3) Have the gut colonizers evolved specific mechanisms to bypass the host gatekeeping system? (4) Is the differentiation among pathogenic and commensals driven by host genetic factors? (5) What factors (other than genetic) are involved which help hosts in recognizing, nurturing friend microbes, and maintaining a healthy gut? (6) How does the gut microbiota modulate host functions? Is it the dysbiosis of the gut microbiota that lead to disease conditions such as IBD? Or is dysbiosis of the gut microbiota the result of IBD itself? Do the gut microbiota and the host immune system work together in maintaining a healthy gut; if yes, then who does what and to what extent? Much is still waiting answers. There is a dire need to combine the systematic and reductionist approaches to dissect the individual roles of host and associated microbiota in conditions like IBD. For instance, intestinal cell line and stem cells, intestinal organoids (based monocultures, transwell, gut on a chip), ex vivo intestinal cultures, AI-based refining of the microbiome structural and functional data, multiomic approaches, and SynCom approaches could be combined to dissect the microbiota-assisted functions and the role of gut microbiota in various gut diseases such as IBD. Such complementary translational research would not only enhance our understanding of the complex interactions between host and associated gut microbes but also be practically applied to design better SynCom, a safe and sustainable alternative to conventional FMT, and to achieve more controlled and robust treatment of gut inflammatory disorders such as IBD.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Raees Khan and Muhammad Naeem contributed equally to this work.

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Research Article

Evaluation of the Clinical Effects and Frequency of *MEFV* Gene Mutation in Patients with Inflammatory Bowel Disease

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Background. The clinical and pathological features of inflammatory bowel disease (IBD) and Familial Mediterranean Fever (FMF) are similar. *Objective*. Here, the frequency of Mediterranean Fever (MEFV) gene mutation and its effect on the outcome of IBD were evaluated. *Methods*. DNA sequence analysis detected the variants on the MEFV gene in patients with IBD. The relationship between mutations and the need for steroids, immunomodulators, biologics, and surgery was assessed. *Results*. We evaluated 100 patients with IBD (55 with ulcerative colitis (UC) and 45 with Crohn's disease (CD)) and 60 healthy individuals as controls. The frequency of *MEFV* gene mutation was 26.7% ($n = 12$) and 14.5% ($n = 8$) for UC and CD, respectively. No relationship was found between *MEFV* gene mutation and the need for steroids, immunomodulators, and biologics ($p = 0.446$; $p = 0.708$; $p > 0.999$, resp.); however, in UC, the need for surgery in those with mutation ($p = 0.018$) and E148Q mutation alone was significant ($p = 0.037$). *Conclusion*. The rate of *MEFV* gene mutations was high in patients with UC who required surgery. These patients have frequent and severe attacks, indicating that the mutations are related to disease severity. *MEFV* mutation as a modifier factor of IBD should be considered.

1. Introduction

The pathogenesis of inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is affected by environmental factors, leading to an uncontrolled immune response in genetically sensitive people [1]. Several studies have investigated the association of diseases with genes, providing a better understanding of their immunopathogenesis. The *NOD2/CARD15* (nucleotide oligomerization domain 2/caspase recruitment domain 15) gene is responsible for the synthesis of proteins that activate the nuclear factor kappa B (NFκB), which has a role in apoptosis and innate immune response, and the susceptibility gene mutations of *NOD2/CARD15* are the first to be associated with IBD [2]. More than 30 IBD-prone genes have been identified, even if their role is less well known [3]. A meta-analysis examining three major genome-wide association studies (GWAS) related to CD reported that mutations with a deep-rooted relationship with CD constitute only 20% of the genetic variations in CD, meaning there is another genetic infrastructure to be explored [4].

Mediterranean Fever (*MEFV*) gene mutations are associated with Familial Mediterranean Fever (FMF) disease, which is an autosomal recessive disorder characterized by fever, serosal inflammation, and recurrent episodes [5]. The *MEFV* and NOD2/CARD15 genes are localized to the same chromosome 16p13. The product of *MEFV* gene, pyrin protein, and NOD2/CARD15 gene product protein are similar in structure. They belong to the same protein family (death domain superfamily), include a common CARD domain, and play a role in the regulation of apoptosis, cytokine release, and inflammation [6].

Thus, it is important to remember that the inflammation load may be increased in patients with IBD who are carriers

of the *MEFV* mutation. It is noteworthy that mutations may contribute to secondary amyloidosis, and with early diagnosis, colchicine prophylaxis may be beneficial in such cases.

Because *MEFV* gene mutations play a role in controlling inflammation, in this study, we determine the frequency of mutations in IBD and investigate the effects of mutation presence on the course of the disease.

2. Materials and Methods

2.1. Patients' Selection. In the study, we enrolled 100 IBD patients (55 with UC and 45 CD) who were followed up by the IBD Unit, Department of Gastroenterology, İzmir Katip Çelebi University, and 60 healthy controls (HC).

2.2. Exclusion Criteria. We excluded patients with less than one-year follow-up and those with FMF.

2.3. Study Design. We recorded the following data for all the patients: age of onset; disease duration; disease localization; requirements for steroid, immunomodulator, and biologic usage; the need for surgical intervention; and extraintestinal manifestations. Montreal classification and European Crohn's and Colitis Organization (ECCO) guidelines were employed to determine disease localization and disease types, such as UC and CD [7]. In terms of remission, the disease severity, the immunomodulator and biologic usage, and the need for surgery were evaluated according to ECCO guidelines (patients showing disease activity at least two times a year were considered to have frequent recurrent diseases) [8].

2.4. Laboratory Analysis. Blood samples (2 cc from each patient) were collected in EDTA-containing tubes using the QIAamp DNA Blood Mini Kit (Qiagen, Germany). DNA was isolated from peripheral blood leukocytes using a standard procedure. Polymerase chain reaction (PCR) was performed to amplify the targeted gene using Applied Biosystems 9700 Thermal Cycler. Oligonucleotide synthesis was conducted to amplify the second and tenth exons of the *MEFV* gene.

Automated DNA sequencing reaction was performed using the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) according to the manufacturer's instructions. Purified PCR samples were run for 45 min using POP-7 polymer by ABI 3130xl Genetic Analyzers automated capillary electrophoresis device. DNA sequences were analyzed using the SeqScape v2.6 software.

2.5. Statistical Analysis. The Statistical Package for the Social Sciences, version 22.0, was utilized in the analyses. Continuous variables were expressed as means, standard deviations, medians, and min–max values, whereas categorical variables were expressed as frequencies and percentages. Chi-squared and Fisher's exact tests were conducted to compare two categorical variables; however, the differences between two independent samples' means or medians were compared using the Mann–Whitney *U* test or median test. Logistic regression analysis was employed to assess the relationships between two or more variables. $p < 0.05$ was considered statistically significant.

2.6. Ethical Considerations. The İzmir Bozyaka Training and Research Hospital Institutional Ethics Committee approved the study protocol. All patients were informed about the contents of the study, and their written and verbal informed consent was obtained.

3. Results

3.1. Patient Characteristics. In this study, we evaluated 100 patients with IBD (55 with UC and 45 CD) and 60 HC. The mean age of patients with IBD and HC was 44.0 ± 14.0 and 41.2 ± 13.2 years, respectively. The IBD group had 50 females (50%), whereas the control group had 30 females (50%). No statistically significant differences were observed between the IBD and control groups in terms of age and gender ($p > 0.05$). The demographic and clinical characteristics of the patients and control groups are shown in Table 1.

3.2. Mutation Variants and Frequencies. The ratios of FMF-related mutations (M694V, E148Q, V726A, M680I(G-C), M694I, A744S, R761H, and K695R) in the UC, CD, and HC groups were 14.5%, 26.7%, and 15%, respectively. No statistically significant differences were detected in terms of mutation frequency between the UC and CD groups and the control group ($p > 0.05$). For the patients and HC, FMF-related mutation frequency detected in *MEFV* gene exons 2 and 10 and homozygous and heterozygous changes and frequency of mutation types are demonstrated in Tables 2(a) and 2(b), respectively. The most common mutation in CD is M694V (6.6%), while in UC, it is E148Q (3.6%). Upon evaluating all patients for allele frequency, the most common mutations were M694V (3.5%) and E148Q (3%) (Table 3).

3.3. The Effects of Mutations on Clinical Parameters. To examine the effect of *MEFV* gene mutations on phenotypic and clinical variables such as disease behavior and disease activity, patient groups were divided into mutation+ and nonmutation subgroups. Upon assessing the demographic and clinical characteristics of mutation+IBD patients, the number of patients with UC who require surgical intervention was found to be statistically significant compared to those without mutations ($p = 0.018$).

Among the different treatment groups (steroids, biological agents, etc.), none of the medical treatments were associated with the presence of mutations. Moreover, the mutations had no significant effect on the other clinical and phenotypic parameters (Table 4). Using logistic regression analysis, the relationship between mutation types and the need for surgical intervention was evaluated with the following results. The association of E148Q ($p = 0.036$) and M694V ($p = 0.036$) mutations with patients requiring surgery was found to be statistically significant. The presence of the E148Q and M694V mutations increased the need for surgery 6.25 times (OR: 6.25; 95% CI: 1.1–34.6). On the other hand, in patients with UC, the presence of the E148Q mutation alone was significantly associated with the

Table 1: Demographic and clinical characteristics of patients.

		CD ($n = 45$)	UC ($n = 55$)
Demographic characteristics	Age (mean ± std.)	43.6 ± 14.0	45.2 ± 14
	Duration of the disease (year, median)	4 (8)	5 (7)
	Age of onset of the disease (mean ± std.)	37.5 ± 13.9	38.4 ± 14.6
UC localization, *n* (%)	Proctitis	—	2 (3.6)
	Left colitis	—	18 (32.7)
	Extensive colitis	—	35 (63.6)
CD localization, *n* (%)	Ileal	15 (33.4)	—
	Colonic	1 (2.2)	—
	Ileocolic	29 (64.4)	—
CD behavior pattern, *n* (%)	Inflammatory	23 (51.1)	—
	Fibrotic	8 (17.8)	—
	Fistulizing	14 (31.1)	—
	Perianal disease	12 (26.7)	—
Disease activity, *n* (%)	Remission	41 (91.1)	46 (83.6)
	Active	4 (8.9)	9 (16.4)
	Recurrent disease	26 (57.7)	19 (34.5)
	Steroid requirement	34 (75.5)	30 (54.5)
	Immunomodulator use requirement	37 (82.2)	26 (47.2)
	The necessity of using biological agents	20 (44.4)	10 (18.1)
	Surgical requirement	14 (31)	5 (11)
Extraintestinal manifestations, *n* (%)	Arthritis	7 (15.6)	4 (7.2)
	Pyoderma gangrenosum	0	1 (1.8)
	Thromboembolic events	0	1 (1.8)
	Ankylosing spondylitis	2 (4.4)	1 (1.8)
	Absent	36 (80)	48 (87.4)

CD: Crohn's disease; UC: ulcerative colitis; IBD: inflammatory bowel disease.

need for surgery ($p = 0.037$); however, for patients with CD, no significant association was detected. Furthermore, in all groups, there was no relationship between the other demographic and phenotypic characteristics and mutation types.

4. Discussion

Recently, attempts have been made to classify autoinflammatory diseases based on molecular pathophysiology, and the concept of systemic autoinflammatory diseases has been introduced [9]. Many patients with IBD may develop systemic extraintestinal symptoms, not limited to the gastrointestinal tract. Extraintestinal manifestations can affect almost any organ system, potentially harming the quality of life and functional status of the patients.

First, we investigated the effects of *MEFV* gene mutation on the clinical course and severity of IBD. No significant relationship between extraintestinal symptoms and mutations was found. Previous research suggested that the presence of mutation increases the probability of extraintestinal involvement [10]; however, other studies reported that there were indications of this relationship [11, 12]. In terms of the effect of *MEFV* gene mutations on the clinical course, in addition to demographic data, we analyzed the clinical and prognostic parameters such as disease pattern and need for surgical intervention and potent treatments. Upon reviewing the existing literature, it was observed that in CD, the severity of the disease increased with the presence of *MEFV* mutations; moreover, in some case reports, clinical remission could not be achieved with conventional treatments other than biological agents [13]. In some IBD cases, which carry the *MEFV* mutation, the disease activity could not be controlled until the colchicine treatment was initiated [14, 15]. However, in this study, only in the UC group, surgical requirement rates were higher in patients with *MEFV* gene mutations than those without mutation. M694V and E148Q mutations were more frequently found in patients who require surgery. In contrast, in the CD group, there was no indication that mutations increase the need for surgery. This can be explained by the fact that the CD group was predominantly composed of patients with inflammatory behavior that does not require surgery, and most of these patients do not have mutations.

Second, we evaluated the frequency of *MEFV* gene mutations in IBD using a candidate gene approach, identifying genes and variants that increase susceptibility to the disease.

Table 2

(a) FMF-related mutation frequency in the *MEFV* gene

Mutation	Controls ($n = 60$)	CD ($n = 45$)	UC ($n = 55$)
Absent (n, %)	51 (85)	33 (73.3)	47 (85.5)
Present (n, %)	9 (15)	12 (26.7)	8 (14.5)
p values	NA	0.359	>0.999

CD: Crohn's disease; UC: ulcerative colitis; FMF: Familial Mediterranean Fever; *MEFV*: Mediterranean Fever gene.

(b) Frequency of mutation types

Mutation type	UC, n (%)	CD, n (%)	Controls, n (%)
Heterozygous for one	8 (100)	8 (66.8)	9
E148Q/w	4 (50)	2 (17)	3
M694V/w	1 (12.5)	3 (25)	3
R761H/w	0	1 (8.3)	0
V726A/w	1 (12.5)	0	0
M680I/w	0	1 (8.3)	3
K695R/w	1 (12.5)	1 (8.3)	0
R628K/w	1 (12.5)	0	0
Homozygous for one	0	2 (16.6)	0
M694V/M694V	0	1 (8.3)	0
M694I/M694I	0	1 (8.3)	0
Compound heterozygous	0	2 (16.6)	0
M694V/V726A	0	1 (8.3)	0
M680I/V726A	0	1 (8.3)	0

W: wild type; CD: Crohn's disease; UC: ulcerative colitis.

Table 3: Allele frequency of *MEFV* mutations in patient and control groups.

MEFV mutations	CD alleles, $n = 90$ (%)	UC alleles, $n = 110$ (%)	Control alleles, $n = 120$ (%)
E148Q	2 (2.2%)	4 (3.6%)	3 (2.5%)
M694V	6 (6.6%)	1 (0.9%)	3 (2.5%)
R761H	1 (1.1%)	0	0
V726A	2 (2.2%)	1 (0.9%)	0
M680I	2 (2.2%)	0	3 (2.5%)
K695R	1 (1.1%)	1 (0.9%)	0
R628K	0	1 (0.9%)	0
M694I	0	0	0

CD: Crohn's disease; UC: ulcerative colitis; IBD: inflammatory bowel disease; *MEFV*: Mediterranean Fever gene.

In this study, we focus on the frequency of *MEFV* gene mutations in IBD, rather than the cooccurrence or frequency of FMF in IBD. Only two studies discovered that *MEFV* gene mutation was highly frequent in IBD and reported other confusing factors such as kinship marriages or low mutation rates in control groups [16, 17]. However, similar studies demonstrated that the frequency of *MEFV* gene mutations in IBD patients is not significant compared to the healthy population [10–12, 18]. In this study, *MEFV* gene mutation frequency in patient groups (UC: 14.5%; CD:26%) was not statistically different compared to that in the control group (15%).

Most routine examinations investigating mutations in the *MEFV* gene, such as reverse hybridization strip test, allow only studying the common 10–12 mutations. In this study, we scanned 220 variables found in exons 2 and 10, known to be hot spots, using DNA sequence analysis. There are a limited number of studies investigating the relationship between the *MEFV* gene and IBD using DNA sequence analysis [19]. We believe that using DNA sequence analysis strengthens our work. On the other hand, this study had some limitations as follows. We did not evaluate the inflammatory mediators, such as C-reactive protein and fecal calprotectin, in addition to the clinical parameters, when

Table 4: Effect of *MEFV* mutations on clinical variables.

	Variables		Mutation (+)/*n*	Mutation (-)/*n*	*p* value
UC, *n* = 55	Surgical requirement		3/8	2/47	0.018
	Biologic agent requirement		1/8	9/47	>0.999
	Immunomodulator requirement		3/8	23/47	0.708
	Steroid usage		3/8	27/47	o.446
	Recurrent disease		3/8	16/47	>0.999
	UC localization	Extensive colitis	5/8	30/47	>0.999
		Left colitis (+proctitis)	3/8	17/47	
	Extraintestinal manifestations		7/8	41/47	>0.999
	Age of onset of the disease (mean ± std)		36.0 ± 18.0/8	37.8 ± 16.0/47	0.558
CD, *n* = 45	Surgical requirement		5/12	9/33	0.470
	Biologic agent requirement		6/12	14/33	0.651
	Immunomodulator requirement		11/12	26/33	0.419
	Steroid usage		10/12	24/33	0.699
	Recurrent disease		9/12	17/33	0.158
	CD localization	İleal	5/12	10/33	0.496
		Colonic and ileocolonic	7/12	23/33	
	CD behavior	Fibrotic	3/12	5/33	0.661
		Others	9/12	28/33	
		Fistulizing	3/12	11/33	0.725
		Others	9/12	22/33	
	Perianal disease		3/12	9/33	>0.999
	Extraintestinal manifestations		9/12	27/33	0.682
	Age of onset of the disease (mean ± std.)		33.3 ± 11.8/12	39.1 ± 14.5/8	0.253

CD: Crohn's disease; UC: ulcerative colitis; *MEFV*: Mediterranean Fever gene.

investigating the effect of the presence of the *MEFV* gene mutation on the severity of IBD. At the same time, we did not conduct a correlation analysis to explore the relationship between the presence of mutations and the extent and severity of mucosal lesions which could positively affect the strength of the study. Moreover, the candidate gene approach could be supported by evaluating *MEFV* gene expression in tissue biopsies in IBD. Besides, the lack of long-term data regarding the effect of mutations on the course of the disease and not screening for proteinuria or signs for amyloidosis appear to be a shortcoming of the present study.

As a result, in this study, the frequency rate of *MEFV* gene mutations in IBD was not high compared to the healthy population. Moreover, the presence of *MEFV* gene mutation was found to be greatly associated with patients with UC who require surgery. Considering that these patients have frequent and severe attacks, it should be confirmed whether the mutations are related to clinical severity. A serious complication of IBD and FMF is also secondary amyloidosis, and its frequency has been correlated with *MEFV* mutations in certain studies [20, 21].

In light of this information, the presence of *MEFV* mutation as a modifier factor in IBD should be considered and evaluated in terms of its association with clinical severity and disease complications, such as secondary amyloidosis, especially in patients who do not respond to potent immunosuppressive treatments and require surgery.

Abbreviations

CARD: Caspase activation and recruitment domains
CD: Crohn's disease
UC: Ulcerative colitis
IBD: Inflammatory bowel disease
ECCO: European Crohn's and Colitis Organization
FMF: Familial Mediterranean Fever
GWAS: Genome-wide association studies
MEFV: Mediterranean Fever gene
NOD2/CARD1: Nucleotide oligomerization domain 2/caspase recruitment domain 15
NFκB: Nuclear factor kappa B
PCR: Polymerase chain reaction.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

A Systematic Review and Meta-Analysis on the Association between Inflammatory Bowel Disease Family History and Colorectal Cancer

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Background. Colorectal cancer (CRC) and inflammatory bowel disease (IBD) are closely interrelated. However, the effect of having a family history of one disease on the risk of another remains undetermined. *Aim*. The purpose of this meta-analysis was to estimate the prevalence of a family history of CRC among patients with IBD, as well as the prevalence of a family history of IBD among patients with CRC. *Methods*. PubMed, Scopus, Embase, Web of Science, and Google Scholar were searched to identify studies reporting the prevalence of family history of IBD among patients with CRC, in addition to the prevalence of family history of CRC among IBD patients. Criteria for study inclusion consisted of the following: (1) studies that evaluated either IBD or CRC and dysplasia, (2) included all age groups, and (3) evaluated the family history effects for IBD or CRC. The total number of IBD patients and IBD patients with a family history of CRC and the total number of CRC patients and CRC patients with a family history of IBD were reviewed. The pooled prevalence of diseases was also estimated according to degree of relatives and geographical area. Random-effects models were used for estimating pooled prevalence. *Results*. A total of 27 studies were included with 26,576 IBD and 9,181 CRC or dysplasia patients. Eligible studies included 13 case-control, 10 cohort, and 4 cross-sectional types. The pooled prevalence of a family history of CRC among patients with IBD was 6% (95% CI: 4-9%). The pooled prevalence for first- and second-degree relatives (11%, 95% CI: 0-37%) was more than that for the other relative subgroups of relatedness degree. The prevalence in the American regions (8% (95% CI: 5-13%)) was higher than that in the others. The pooled prevalence for a family history of IBD among CRC or dysplasia patients was 11% (95% CI: 6-16%). The pooled prevalence for first-degree relatives (13% (95% CI: 3-28%) was higher than that for the other relative subgroups of relatedness degree; it was also greater in American countries (15%, 95% CI: 8-23%). *Conclusion*. This study emphasizes the relationship between a family history of IBD and CRC development. Additionally, there was notable prevalence for a family history of CRC among IBD patients. American countries and first-degree relatives were identified to have a higher prevalence for both disease processes.

1. Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide [1]. Familial studies have demonstrated that having a family history of CRC may increase an individual's risk of developing CRC and that this risk in individuals with a first-degree relative with CRC is more than 2-folds greater [2, 3]. One of the most important risk factors of CRC is inflammatory bowel disease (IBD). IBD is an immune-mediated gastrointestinal disorder that is identified with subtypes of Crohn's disease (CD) and ulcerative colitis (UC) [4]. Both CD and UC patients are at risk of CRC development [5]. Like CRC, family history is one of the strongest risk factors for development of IBD [6].

It has been shown that CRC is a relatively common and life-threatening consequence of IBD, especially UC. This is likely secondary to proneoplastic effects of chronic intestinal inflammation. Duration, extent and severity of IBD, the existence of inflammatory pseudopolyps, presence of primary sclerosing cholangitis, and a family history of CRC are the main risk factors of IBD-related CRC [7]. A family history of CRC independently increases CRC risk two- to threefolds in patients with UC (OR: 3.7, 95% CI: 1.0–13.2) [8].

There are common factors inducing the development of IBD and CRC, such as the variations in gut microbiota and in the interleukin pathways and tumour necrosis factor, as well as also age, race, genetics, family history, diet composition, obesity, and vitamin and mineral levels [9]. Moreover, it is shown that IBD-related CRC patients are younger and have high prevalence of multiple cancerous lesions [10]. This suggests that in addition to inflammation, other factors may be involved in the pathogenesis of IBD-related CRC.

To determine a quantitative data for the prevalence of a family history of CRC or IBD, there are only a few comprehensive studies. In a previous meta-analysis, the prevalence of CRC in patients with UC has been estimated at 3.7%, across the world [11]. In a study by Shi et al., the prevalence of a family history of IBD among groups of Caucasians, Asians, Blacks, and Hispanics has been estimated at 12%, 0.04%, 0.07%, and 0.13%, respectively [12]. In the other study by Childers et al., it was revealed that the prevalence of a family history of IBD among patients with UC is 12% (range: 0-39%) [13].

Despite CRC and IBD being closely interrelated, the relation between a family history of each disease and the risk of developing the other still has not been quantified. To address this gap, we performed a systematic review and meta-analysis for estimation of the prevalence of a family history of CRC among patients with IBD as well as the prevalence of a family history of IBD among patients with CRC.

2. Methods

2.1. Search Strategy. Our electronic search was limited to the English language, and it was conducted in PubMed, Scopus, Embase, Web of Science, and Google Scholar by using the following keywords: ("inflammatory bowel disease" or "ulcerative colitis" or "crohn's disease") and ("colorectal cancer" or "colon and rectum cancer" or "dysplasia or neoplasia") and ("family history" or "relative" or "familial"). Published studies up to December 2020 were considered, and references of individual studies were searched to find other eligible studies. The Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) guideline was used for reporting this study [14].

2.2. Inclusion and Exclusion Criteria. The authors reviewed titles and abstracts of original full-text articles performed on each of the IBD or CRC patients. The inclusion criteria were as follows: (1) studies that evaluated either IBD or CRC and dysplasia, (2) included all age groups, and (3) evaluated the family history effects for IBD or CRC. The exclusion criteria were as follows: (1) studies with an unknown number of patients with a family history for IBD or CRC and (2) conducted on animals (mice). The authors excluded all reviews or conference abstracts and non-English publications. For quality control of studies, the authors used the Newcastle-Ottawa Scale (NOS) and the high and moderate quality articles considered as eligible [15]. The disagreements among the authors on the choice of the eligible studies were discussed, and finally, any disagreement was evaluated by the senior investigator.

2.3. Data Extraction. For each selected study, the authors extracted the following information: name of the first author, year of publication, country of publication, total sample size, study design, total number of IBD patients and IBD patients with family history of CRC, total number of CRC patients and CRC patients with family history of IBD, and degree of relatives included.

2.4. Outcome of Interest. The main outcomes of this meta-analysis were the prevalence of a family history of IBD in CRC as well as the prevalence of a family history of CRC in IBD.

2.5. Statistical Analysis. All analyses were done using Stata 14 software, and 0.05 was considered as the statistical significance level. For each outcome of interest, the corresponding proportion was calculated via the extracted data from each eligible study. Pooled prevalence with 95% confidence interval (CI) was estimated using the random-effects model wherever the prevalence has been reported. In the process of prevalence merging, the outcomes with zero event were adjusted using the "Freeman-Tukey double arcsine" transformation in the "metaprop" procedure [16]. The heterogeneity was evaluated by using the Cochran's Q test and I^2 statistic and P value. We performed stratified analysis for items that may cause heterogeneity. Publication bias was examined by using Begg's and Egger's tests [17] and also funnel plot [18].

3. Results

3.1. Process of Study Selection. After a comprehensive search of the databases, 131 studies were obtained. We excluded 40 studies after examining the title and abstract. The number of studies selected for primary evaluation was 91. Then, 53 studies were excluded because they did not meet inclusion

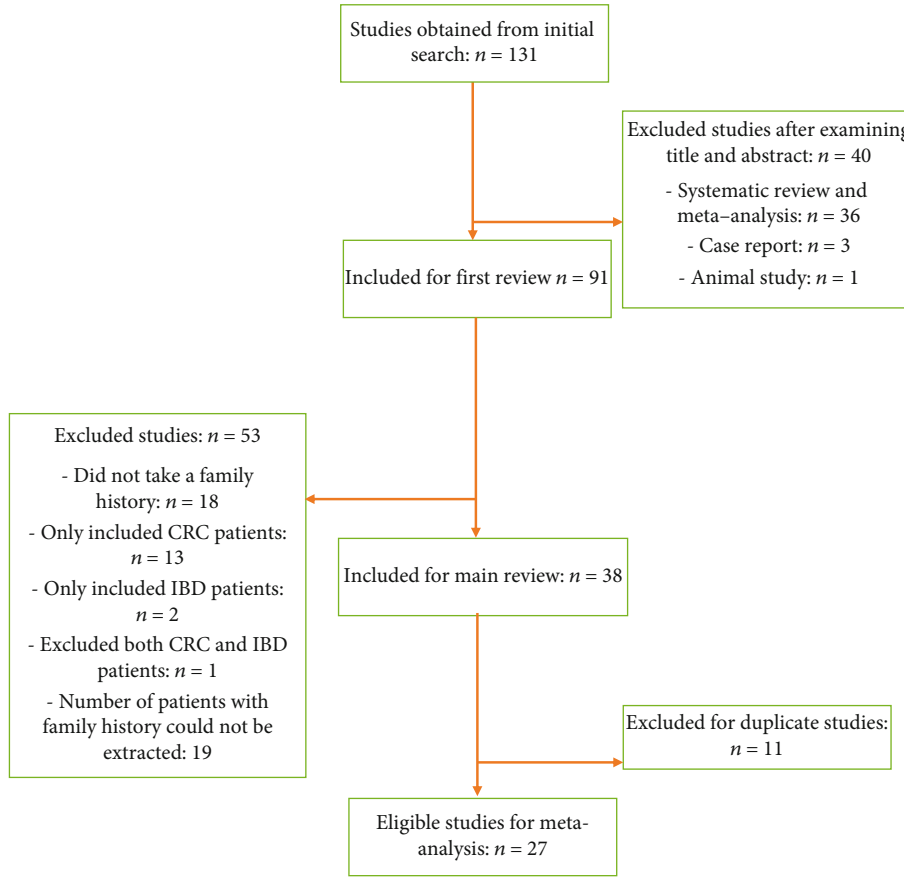


Figure 1: Flow chart for the process of study selection.

criteria or did not report all necessary information. There were 11 republished studies that contained duplicate data samples which were excluded. Finally, 27 studies were considered as eligible and enrolled in the meta-analysis. The details of study selection are presented in Figure 1.

3.2. Characteristics of the Eligible Studies. Eligible studies included 13 case-control, 10 cohort, and 4 cross-sectional types [8, 19–44]. The interest disease which was considered in most studies was IBD-related CRC, and the main target was often evaluation of the factor associated with CRC. Some cohort studies such as Brackmann et al. followed up patients with IBD and evaluated the influence of family history of CRC on survival [19]. Other cohort types including Askling et al.'s study followed up UC and CD patients, and they concluded that having both family histories of IBD and CRC increases the risk of CRC [20]. In some of the case-control studies, the main aim was to review supplementation (aminosalicylate and folic acid) effect on the risk of IBD or CRC [21, 22]. The characteristics of the eligible studies are presented in Table 1. The number of IBD and CRC or dysplasia patients represented by 27 eligible studies was 26,576 and 9,181, respectively. All studies except three reported the age at IBD diagnosis. For IBD patients, the mean age at IBD diagnosis was 34.52 ± 7.79 years (range: 25-29), and for IBD patients with CRC or dysplasia, this mean was 35.35 ± 8.65 years (range: 25-57.4).

3.3. Family History of CRC among IBD Patients. There were 26 studies on the family history of CRC, including 25,819 IBD patients. The pooled overall prevalence of a family history of CRC among patients with IBD was 6% (95% CI: 4-9%, $P < 0.001$) with $I^2 = 96.01\%$, $P < 0.001$. The forest plot of the result is presented in Figure 2(a).

3.3.1. Family History of CRC among IBD Patients by Degree of Relative. The degrees of relatives in the extracted studies were reported as first (including 9 studies with 2,357 IBD patients), first and second (including 3 studies with 594 IBD patients), all degrees (including 5 studies with 22,316 IBD patients), and not reported degree (including 5 studies with 492 IBD patients). More studies were conducted on first-degree relatives, while there were studies that did not report any degree of family connection. The pooled prevalence of a family history of CRC among patients with IBD for first- and second-degree relatives (11%, 95% CI: 0-37) was more than any other degree of relatedness (Figure 2(b)).

3.3.2. Family History of CRC among IBD Patients by Region of Study. The studies were conducted in the regions of the Americas (including the USA with 1,419 IBD patients), Europe (including Sweden, France, Netherlands, England, Portugal, and Norway with 24,279 IBD patients), and Asia (including Japan and India with 89 IBD patients). The pooled prevalence of a family history of CRC among patients

Table 1: Characteristics of studies included in the meta-analysis.

Study	Year	Region	Sample size	Study type	Mean age at diagnosis of IBD (year)	No. of patients with dysplasia and CRC	No. of IBD patients; UC, CD	Family history of CRC	Family history of IBD	Relevant degree	Proportions*
Kisiel et al. [23]	2012	USA	77	Cohort: followed 77 IBD patients	47.6 (14.2–83.2)	31	77 UC patients under polypectomy	12	—	First	$p_1 = \frac{12}{77}$
Bergeron et al. [24]	2010	France	855	Cohort: followed 855 IBD patients	33 (19-47)	14 with AL-LGD[a]	28	2	1	—	$p_1 = \frac{2}{28}; p_2 = \frac{1}{14}$
						28 with CR-LGD[b]	56	1	4	—	$p_1 = \frac{1}{56}; p_2 = \frac{4}{28}$
						33 with advanced neoplasia	66	0	3	—	$p_1 = \frac{0}{66}; p_2 = \frac{3}{33}$
Baars et al. [25]	2011	Netherlands	565	Case- (CRC with IBD) control (IBD)	33 for cases, 31 for control	173 (UC:113; CD: 58; unclassified: 2)	392 (UC: 175; CD: 207; unclassified: 10)	132	—	12 first, 11 second, 109 unknown	$p_1 = \frac{132}{392}$
Rubin et al. [26].	2006	USA	125	Case- (UC with developed dysplasia or CRC) control (UC without neoplasia)	29.5 for cases, 30.5 for control	26	96	9	6	First	$p_1 = \frac{9}{96}; p_2 = \frac{6}{26}$
Lashner et al. [27]	1999	USA	95	Cohort: followed 95 UC patients	26.5 for p53-positive, 29.5 for p53-negative	36	95	1	3	—	$p_1 = \frac{1}{95}$
Askling et al. [8]	2001	Sweden	8810	Cohort: followed 8810 CD patients	—	143	8810	256	—	First, second, or more	$p_1 = \frac{256}{8810}$
Rutter et al. [29]	2004	England	204	Case- (CRC neoplasia) control (UC)	33 for cases, 33 for controls	68	136	18	—	First	$p_1 = \frac{18}{136}$
Wu et al. [28]	2014	USA	44	Cohort: followed 44 UC patients	29.3	22 with LGD	44	—	2	First or second	$p_2 = \frac{2}{22}$
					28.2	43 with adenocarcinoma and pouch dysplasia		—	4		$p_2 = \frac{4}{44}$
Freire et al. [30]	2014	Portugal	76	Cross-sectional in UC	33.3	—	76	7	—	—	$p_1 = \frac{7}{76}$
Connelly et al. [31]	2014	USA	82	Case- (UC with dysplasia/CRC) control (UC without dysplasia/CRC)	34.49	41	41	3	11	—	$p_1 = \frac{3}{41}; p_2 = \frac{11}{41}$

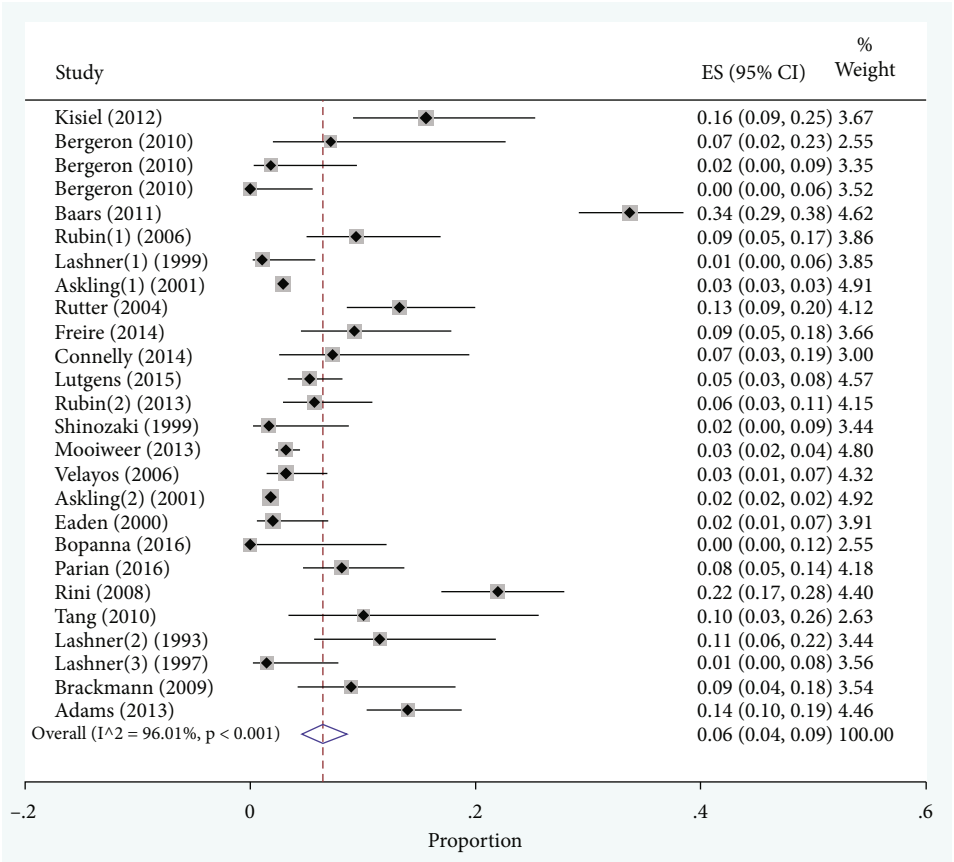
Table 1: Continued.

Study	Year	Region	Sample size	Study type	Mean age at diagnosis of IBD (year)	No. of patients with dysplasia and CRC	No. of IBD patients; UC, CD	Family history of CRC	Family history of IBD	Relevant degree	Proportions*
Lutgens et al. [32]	2015	Netherlands	530	Case- (IBD-CRC) control (IBD)	27.5 for cases, 25.5 for control	188	342	18	—	First	$p_1 = \frac{18}{342}$
Rubin et al. [33]	2013	USA	200	Case- (CRC and UC) control (UC	47.1	59	141	8	—	First or second	$p_1 = \frac{8}{141}$
Shinozaki et al. [34]	1999	Japan	77	Case- (UC with neoplasia) control (UC)	34.3 for cases, 28.4 for control	16	61	1	—	First or second	$p_1 = \frac{1}{61}$
Mooiweer et al. [35]	2013	Netherlands	1018	Cohort: followed 1018 IBD patients	47.7	11	1018	32	—	First	$p_1 = \frac{32}{1018}$
Velayos et al. [36]	2006	USA	376	Case- (UC with CRC) control (UC)	25 for cases, 27 for control	188	188	6	—	First	$p_1 = \frac{6}{188}$
Askling et al. [20]	2001	Sweden	31093	Cohort: followed 31093 IBD patients	37.65	560	13186	234	—	First, second or more	$p_1 = \frac{234}{13186}$
Eaden et al. [37]	2000	Sweden	204	Case- (UC, CRC) control (UC)	57.4 at diagnosis of CRC	102	102	2	5	First	$p_1 = \frac{2}{102}; p_2 = \frac{5}{102}$
Bopanna et al. [38]	2016	India	28	Cross-sectional in UC	28.89	—	28	0	—	—	$p_1 = \frac{0}{28}$
Parian et al. [39]	2016	USA	187	Cross-sectional in UC	36.7 for UC with dysplasia, 29.7 for UC without dysplasia	39	148	12	7	First	$p_1 = \frac{12}{148}; p_2 = \frac{7}{39}$
Rini et al. [40]	2008	USA	223	Cross-sectional in IBD	43.94	—	223: 136 UC, 55 CD, 32 unknown	15 in first degree; 34 in second or more	—	First, second, or more	$p_1 = \frac{49}{223}$
Tang et al. [21]	2010	USA	48	Case- (IBD with CRC) control (IBD)	36.6 for cases, 34.7 for control	18	30	3	3	First, second, or more	$p_1 = \frac{3}{30}; p_2 = \frac{3}{18}$
Lakatos et al. [41]	2006	Hungary	723	Cohort: followed 723 UC	49	13	723	—	0	—	$p_2 = \frac{0}{13}$
Lashner [42]	1993	USA	67	Case- (UC with dysplasia and CRC) control (UC)	—	6	61	7	2	—	$p_1 = \frac{7}{61}; p_2 = \frac{2}{6}$
Derikx et al. [43]	2014	Netherlands	124	Case- (IBD with carcinoma and dysplasia) control (IBD)	25.7 for cases, 25.7 for control	25	99	0	—	—	$p_1 = \frac{0}{99}$

Table 1: Continued.

Study	Year	Region	Sample size	Study type	Mean age at diagnosis of IBD (year)	No. of patients with dysplasia and CRC	No. of IBD patients; UC, CD	Family history of CRC	Family history of IBD	Relevant degree	Proportions*
Lashner et al. [22]	1997	USA	98	Case- (UC with CRC or dysplasia) control (UC without CRC or dysplasia)	25.8 for CRC or dysplasia, 29.5 for no dysplasia	29	69	1	1	—	$p_1 = \frac{1}{69}$; $p_2 = \frac{1}{29}$
Brackmann et al. [19]	2009	Norway	67	Cohort: followed 67 IBD with CRC	25	67	67	6	5	First, second or more	$p_1 = \frac{6}{67}$; $p_2 = \frac{5}{67}$
Adams et al. [44]	2013	USA	7202	Cohort: followed 7202 CRC cases	—	7202	250	35	—	First	$p_1 = \frac{35}{250}$

*p_1 = patient with family history of CRC/patient with IBD; p_2 = patient with family history of IBD/patient with dysplasia or CRC; [a]adenoma-like low-grade dysplasia; [b]colitis-related low-grade dysplasia.



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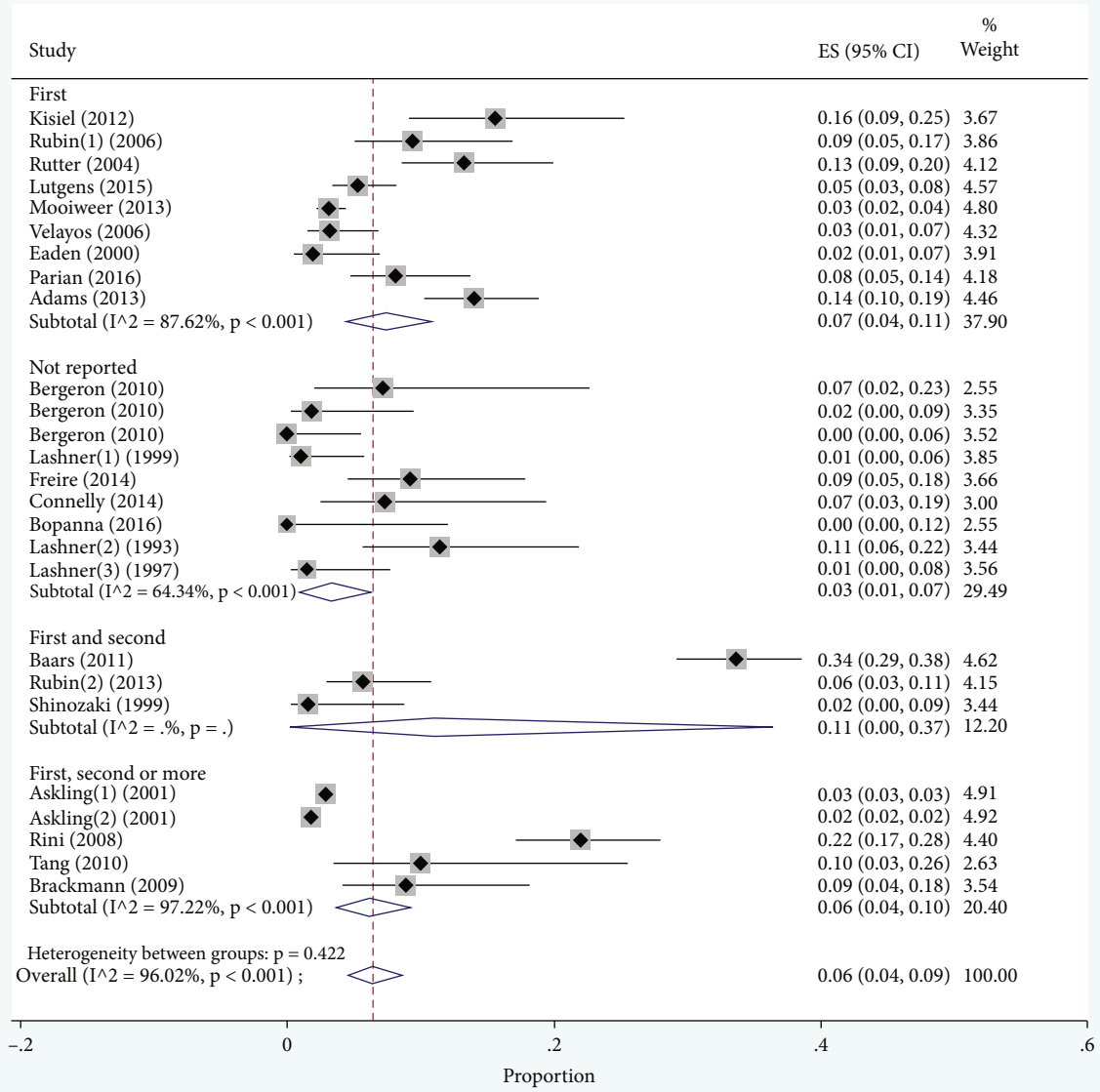

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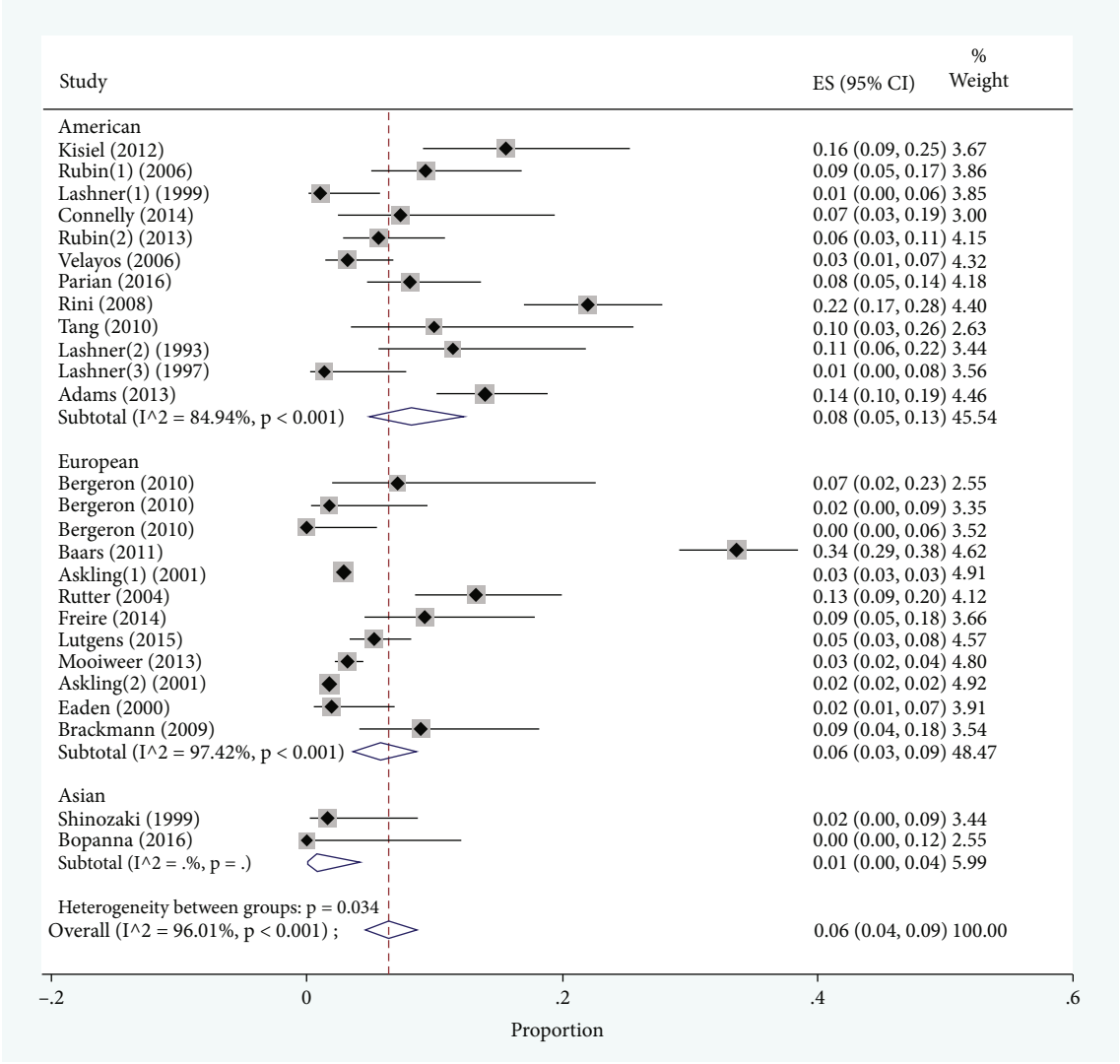


(c)

Figure 2: Forest plot for the prevalence of family history of CRC among IBD patients: (a) overall prevalence; (b) prevalence by degree of relative; (c) prevalence by region of study.

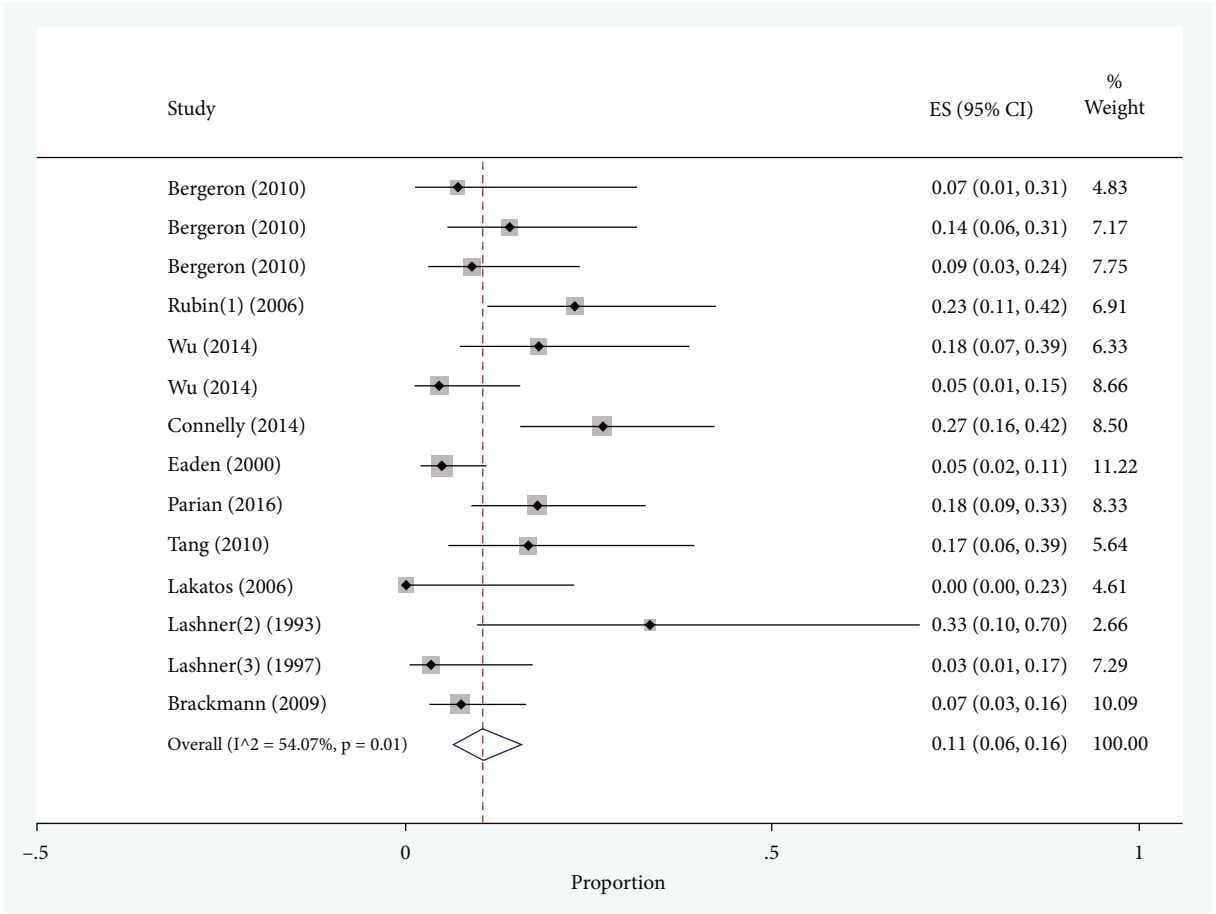
with IBD in the American regions (8% (95% CI: 5-13%)) was higher than that in the others (Figure 2(c)).

3.4. Family History of IBD among Patients with Dysplasia or CRC. The number of studies concerning family history of IBD for CRC patients was 10, including 481 patients with dysplasia or CRC. The pooled prevalence for a family history of IBD among CRC or dysplasia patients was 11% (95% CI: 6-16%, $P < 0.001$) with $I^2 = 54.57\%$, $P = 0.01$. The forest plot is shown in Figure 3(a).

3.4.1. Family History of IBD among Patients with Dysplasia or CRC by Degree of Relative. First degree (including 167 patients with dysplasia or CRC), first and second degrees (including 65 patients with dysplasia or CRC), and first, second, and more degrees (including 85 patients with dysplasia or CRC) were among the reported degree of relatives in the eligible studies. Also, some studies did not report the degree (including 164 patients). The pooled prevalence in the first degree (13% (95% CI: 3-28%) was higher than that in the other groups (Figure 3(b)).

3.4.2. Family History of IBD among Patients with Dysplasia or CRC by Region of Study. The regions of the Americas (including the USA, with 224 dysplasia or CRC patients) and Europe (including France, Norway, Hungary, and Sweden with 257 dysplasia or CRC patients) were the areas represented in the studies reviewed. The pooled prevalence for American countries (15%, 95% CI: 8-23%) was greater than that for the European region (Figure 3(c)).

3.5. Evaluation of Publication Bias. The results of Egger's ($P = 0.08$) and Begg's ($P = 0.48$) tests revealed that there is no publication bias among the studies from which the prevalence of family history of CRC was. Also, for family history of IBD, the tests of Egger ($P = 0.78$) and Begg ($P = 0.32$)



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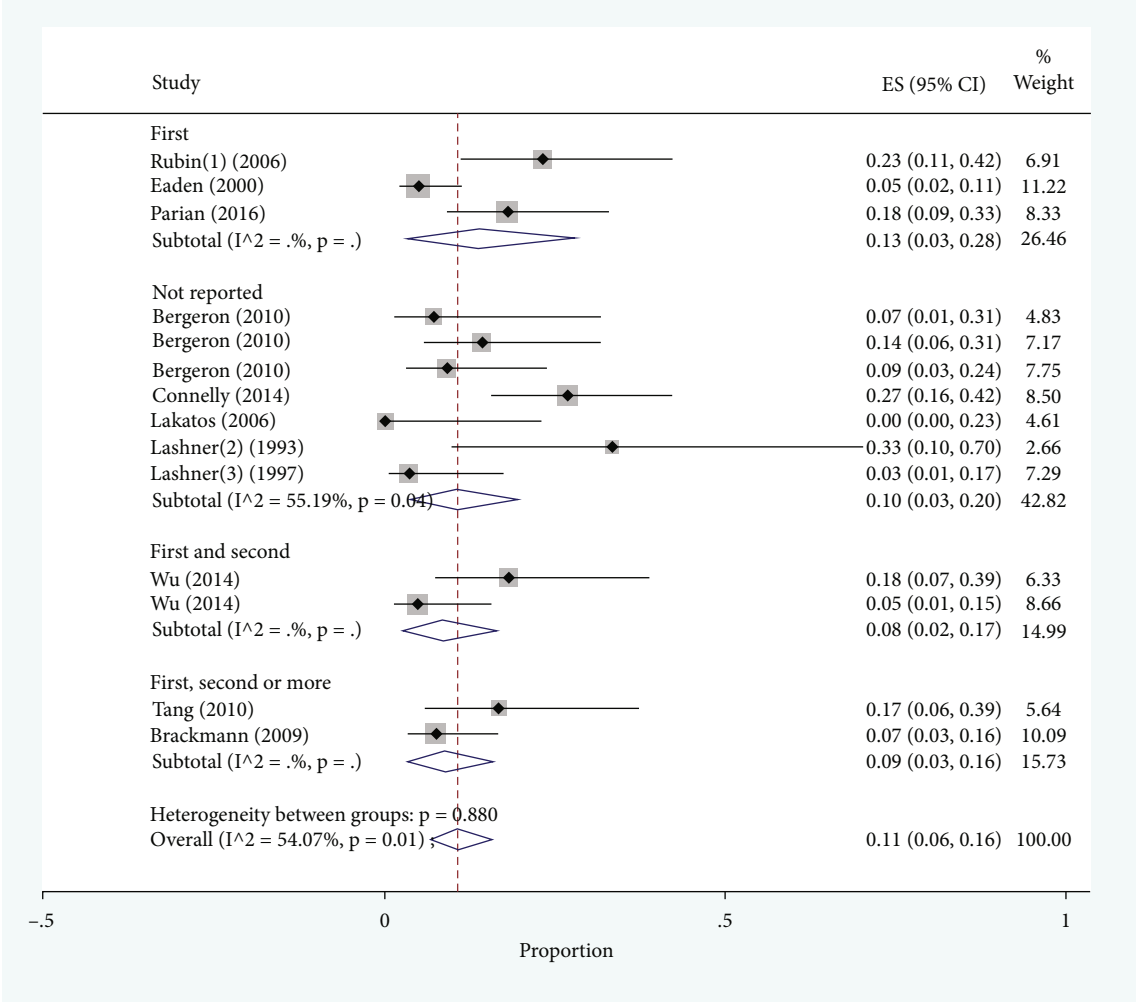

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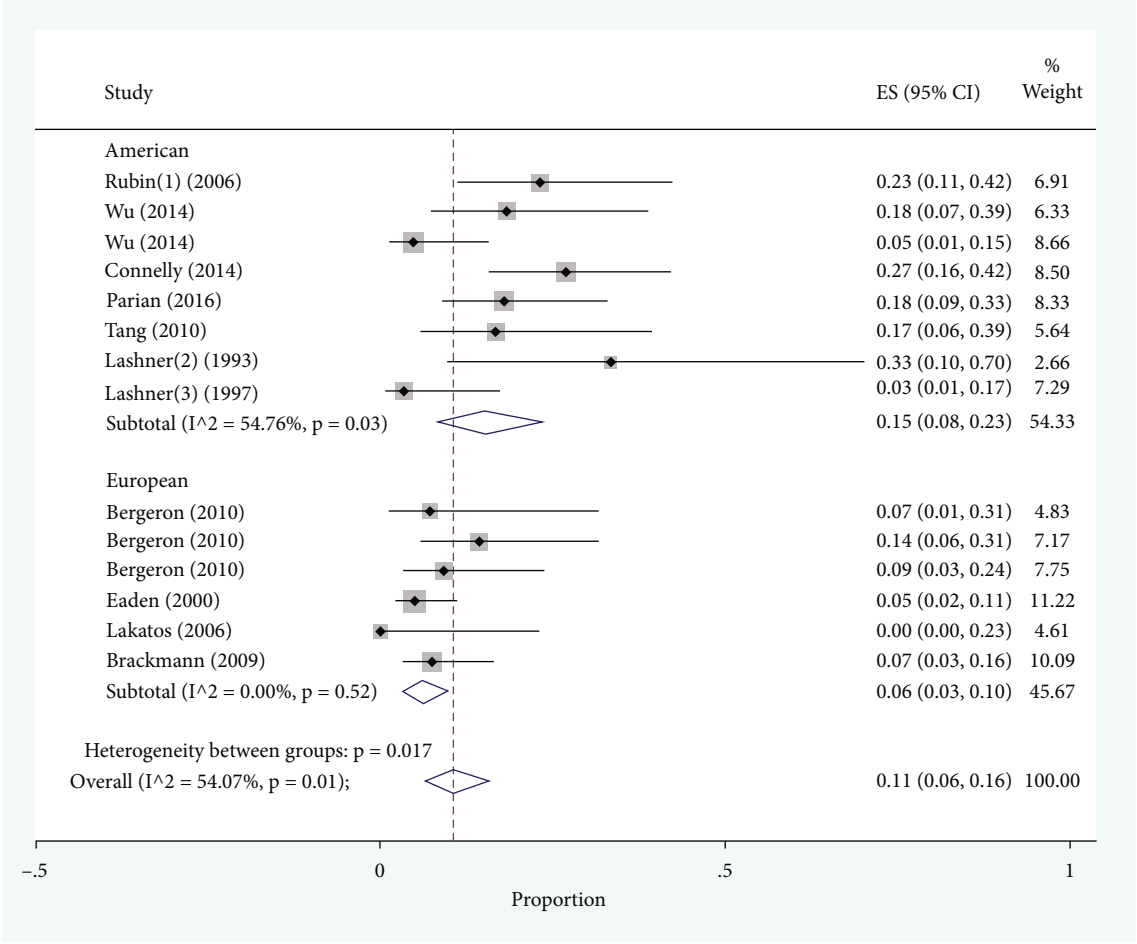

(c)

Figure 3: Forest plot for the prevalence of family history of IBD among dysplasia or CRC patients: (a) overall prevalence; (b) prevalence by degree of relative; (c) prevalence by region of study.

showed similar results. Additionally, the funnel plots showed evidence of an approximate symmetry (Figure 4).

4. Discussion

The present study is the first meta-analysis that estimates the prevalence of a family history of CRC among patients with IBD. Interestingly, we found that among IBD patients, the prevalence of a family history of CRC was 6% (95% CI: 4-9). Additionally, the pooled prevalence of a family history of IBD among patients with CRC or dysplasia was estimated to be 11% (95% CI: 6-16). The reason for the greater latter prevalence may be that previous cohort studies proved that the history of IBD is a factor associated with CRC and the probability of developing CRC for IBD patients in the future is 2-folds higher compared with that for others [45–47]. It is important to note that IBD patients with longer duration and extensive disease and patients with diagnosis at young age are at higher risk of CRC [48]. Among IBD patients, also, the prevalence of a family history of CRC may be notable and the present study confirmed this.

In a recent meta-analysis on the influence of ethnicity in IBD prevalence by Shi et al., the mean age of IBD diagnosis was reported as 30 years [12]. This mean age in other epidemiological studies has varied, with reports of 32.7 [49], 38.46 [50], and 54.1 [51] years of age. These differences may be due to differences in access to health care center for diagnosis and overall awareness about IBD. In our study, we observed that the mean age of IBD diagnosis for IBD patients was close to 34.52 (range: 25-29), and for IBD patients with CRC, this was slightly later (35.35, range: 25-57.4). This may mean that at a later mean age of diagnosis, patients with IBD are at higher risk for CRC than young people [52, 53].

In our study, the authors reported the pooled prevalence of family history of CRC among patients with IBD as well as a pooled prevalence of a family history of IBD among patients with CRC or dysplasia, according to the degree of relatedness. The result demonstrated that the prevalence of family history for first- or first- and second-degree relationships is greater than that for other degrees. Previous studies revealed that the risk of gastrointestinal cancers for individuals with affected family members, especially for first-degree family members, is high and our result is in line with this point [20, 54].

Considering the geographical aspect, the present meta-analysis revealed that the pooled prevalence of family history

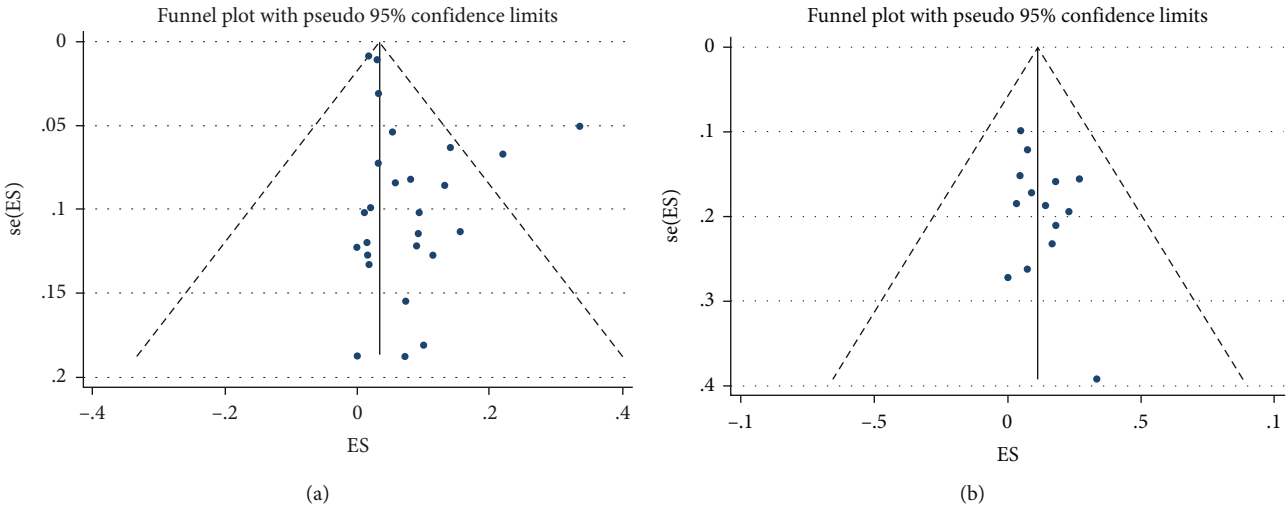

Figure 4: Funnel plot for assessing publication bias in the eligible studies: (a) studies provided data on family history of CRC; (b) studies provided data on family history of IBD.

of either IBD or CRC among Americans was more than that among European and Asian countries. Perhaps the global cancer statistics are helpful for finding the reason: the incidence of CRC is more common in more highly developed countries. The CRC incidence rate in Europe and Northern America is highest in comparison with that in other regions. The reason is that the prevalence of CRC risk factors including obesity and unhealthy diet in these regions is high [55, 56]. Additionally, global comparison for the prevalence of IBD has shown the highest prevalence in European and American areas and the prevalence has remained higher up until 2018 [57].

For IBD, diagnosis and management are complex and utilize clinical presentation, biomarkers, and pathology. Patient manifestation of symptoms may be due to genetic, environmental factors, and possibly molecular mechanisms within the gut microbiota patterns. All of these areas may be targets for personalized IBD treatment. This tailored approach is important for early diagnosis and treatment in the IBD management [58, 59]. Early IBD diagnosis and successful treatment may result in decreased rates or prevention of CRC.

Previous studies observed significant heterogeneity among the results of studies. The heterogeneity is a phenomenon that usually is seen in the meta-analyses of proportion. Instead, we performed a subgroup analysis to create some more homogeneous groups of studies. But the subgroup analysis is performed by dividing studies into stratum, and this may not be useful in all cases. Generally, the more likely cause of heterogeneity, in addition to measurement errors, may be due to the way of constructing the study, including methods as well as differences in the span of the definitions [60].

There are some limitations in the present meta-analysis. First, there is overall a lack of determining the number of patients with affected family member for subtypes of UC or CD in the eligible studies. This limitation caused the authors to not take the two main types of pooled prevalence for each subtype. Second, some eligible studies did not report the degree of relatedness for affected family members. Also, for first-degree relatives, the type of relative (parent or sibling) was not mentioned. With more complete data, the results could be expanded. Further genetic studies are needed to determine the number of subjects with family affected member for both IBD and CRC in details of IBD subtypes, as well as sex in each type.

The present study emphasized the importance of a family history of IBD (or CRC) in the possibility of the CRC onset (or IBD). The advancement of CRC in non-IBD patients, with family history of IBD, leads us to look up further probable factors that may be common among both diseases. Gut microbiota, interleukin, and tumour necrosis factor pathways, race, genetics, family history, and diets are important factors that should be considered in the future studies [9]. The prevalence of a family history of CRC among IBD patients in American and European countries and for first-degree relatives is higher. There is a similar pattern for the prevalence of a family history of IBD among dysplasia or CRC patients. Thus, knowing the prevalence of a family history component for an at-risk population may be helpful in patient's care and managing both CRC and IBD.

Data Availability

There is no raw data associated with this article.

Conflicts of Interest

All authors have no potential conflict of interest relevant to this article.

Authors' Contributions

Hamid Asadzadeh Aghdaei and Hadis Najafimehr are co-first authors.

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Research Article

Prevalence of Sarcopenia and Its Effect on Postoperative Complications in Patients with Crohn's Disease

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Background and Aims. Sarcopenia is a prognostic factor of outcomes for various diseases, but reports on sarcopenia in patients with Crohn's disease (CD) are few. We aim to determine the prevalence of sarcopenia and assess the role of sarcopenia in postoperative complications in patients with CD at a tertiary referral center. *Methods*. Patients who underwent intestinal surgery for CD from January 2013 to October 2019 were retrospectively enrolled. The L3 skeletal muscle mass index (SMI) was used to identify sarcopenia. Demographic data, preoperative laboratory data, surgical details, and hospital outcomes were recorded. The factors associated with postoperative complications were evaluated through univariate and multivariate analyses. *Results*. One hundred and twenty-four patients were enrolled. Thirty-four of them (27.4%), including 11 males, were diagnosed with sarcopenia. Compared with patients without sarcopenia, sarcopenic patients had a significantly lower BMI ($P < 0.001$); lower preoperative serum albumin ($P = 0.006$), prealbumin ($P = 0.030$), and hemoglobin levels ($P < 0.001$); longer hospital stay (34.4 ± 26.8 days vs. 22.8 ± 15.6 days, $P = 0.003$); and more occurrences of complications (41.2% vs. 23.3%, $P = 0.049$). The overall incidence of postoperative complications was 28.2%. Infection (51.4%) and intestinal fistula (22.9%) were the most common among such complications. Through the multivariate analysis, sarcopenia was identified as an independent risk factor for major postoperative complications (odds ratio = 3.974, 95%CI = 1.171–13.489, $P = 0.027$). *Conclusion*. Sarcopenia is common in patients with CD requiring bowel resection, and it significantly increases the risk of major postoperative complications.

1. Introduction

Crohn's disease (CD) is a nonspecific chronic inflammatory disease that affects any segment of the gastrointestinal tract and often causes extraintestinal complications [1–3]. Although the clinical drugs currently available for CD treatment are diverse, up to 80% of individuals with CD undergo at least one operation during their lifetime due to the complex complications of the disease, lack of response to medical treatment, and even malignant transformation in rare instances [4–6]. The incidence of surgical complications in patients with CD ranges from 20% to 40%; these complications include anastomotic leakage, wound rupture and infection, intra-abdominal septic complications, and short bowel syndrome [7]. A number of studies have been conducted to identify the risk factors for patients with CD undergoing surgery. Several risk factors, such as age, hypoalbuminemia, and anemia, are associated with postoperative outcomes [8–11]. However, the role of sarcopenia was not described well in these studies.

Sarcopenia, which is defined as a depletion in lean muscle mass accompanied with a loss of muscle strength, was first described in 1989 by Rosenberg. It is generally developed in aged patients or malnourished individuals with risk factors, such as chronic inflammation, oxidative stress, and hormonal changes [12–15]. Many studies have linked sarcopenia with poor postoperative outcomes in patients with colorectal cancer [16], pancreatic cancer [17], urological cancer [18], and hepatocellular carcinoma [19]. According

to a systematic review, the incidence of sarcopenia is as high as 52% in patients with CD when anatomical criteria are considered without functional strength assessment [13, 20, 21]. However, the correlation between sarcopenia and postoperative complications remains unclear.

This study examined 124 patients with CD who underwent bowel resection. The objective is to assess (1) the prevalence of sarcopenia in patients with CD undergoing bowel resection, (2) evaluate the influence of sarcopenia as a risk factor for postoperative complications on these patients, and (3) compare the BMI, serum albumin level, prealbumin level, and other possible risk factors for postoperative complications of the patients with CD.

2. Methods

2.1. Study Design. The institutional ethics board approved this study. Informed consent was acquired from all patients. Patients who underwent CD-related bowel surgery from January 2013 to October 2019 in our hospital were retrospectively enrolled. CD-related bowel surgery is defined as surgery to cope with major complications, such as obstruction, leakage, and refractory abscess, in CD. The exclusion criteria were as follows: (1) computed tomography (CT) data not available 90 days before surgery or 30 days after surgery; (2) with severe comorbidity, important organ insufficiency, malignancy, or infection with HIV; (3) history of abdominal surgery; and (4) perianal surgery.

2.2. Data Collection. Demographic data included age, gender, marriage status, BMI, smoking history, and alcohol use. Normal BMI, overweight, obesity, and malnutrition were defined as 18.5–25 kg/m^2, 25–30 kg/m^2, >30 kg/m^2, and <18.5 kg/m^2, respectively. Clinical data included disease duration, Montreal classification, and preoperative medication. Disease activity was assessed using the Harvey–Bradshaw index (HBI). Laboratory test results, including serum albumin levels, prealbumin concentration, white blood cell (WBC) count, hemoglobin, and platelet count, were routinely recorded before surgery. Postoperative complications were registered mainly as skin and soft tissue infections, sepsis, venous thrombosis requiring treatment, anastomotic leak, or intra-abdominal abscess.

2.3. Skeletal Muscle Mass Index. The skeletal muscle mass index (SMI) of each patient was rated based on the skeletal muscle mass measured through CT of the abdomen and pelvis. CT scans within three months prior to surgery or in the first month after surgery were selected, and preoperative scans were preferentially used. The total muscle cross-sectional area (cm^2) at L3 vertebra was utilized for the segmentation of skeletal muscle (including the psoas, paraspinal, and abdominal wall muscles). The threshold range for skeletal muscle was from −30 to +150 Hounsfield units (HU) in accordance with reports (Figure 1) [22]. Sarcopenia was identified based on SMI (cm^2/m^2), which is the ratio of the skeletal muscle area (cm^2) to the height squared (m^2). In accordance with a previous work, sarcopenia was identified once a patient fulfilled one of the following criteria: (1) SMI < 41 cm^2/m^2 in women, (2) <43 cm^2/m^2 in men with BMI < 25 kg/m^2, and (3) <53 cm^2/m^2 in men with BMI ≥ 25 kg/m^2 [23].

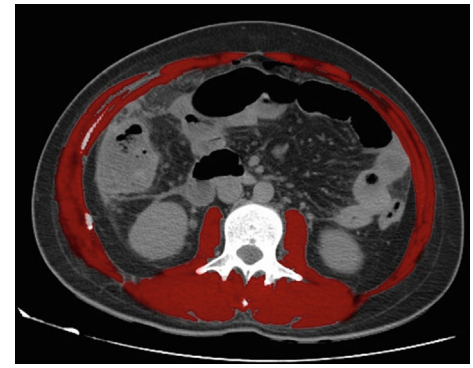

Figure 1: Evaluation of the skeletal muscle mass using a third lumbar computed tomography scan slice. Red: skeletal muscle (including the psoas, paraspinal, and abdominal wall muscles).

2.4. Statistical Analyses. All statistical analyses were performed using SPSS (version 23.0; Inc, Chicago, IL, United States). The mean value and SD were calculated for quantitative and qualitative variables. Data between groups were compared using Student's t-test for normally distributed values, and categorical data were compared using χ^2 or Fisher's exact test, as appropriate. Univariate and multivariate logistic regression analyses were performed to identify the independent predictors of postoperative complications. The Kaplan–Meier curve was applied to estimate the impact of sarcopenia on the length of hospital stay. Linear regression was performed to analyze the linear relationship between SMI and BMI, albumin, and prealbumin level. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Demographic and Clinical Characteristics of CD Patients with or without Sarcopenia. A total of 124 patients who underwent CD-related bowel surgery in our hospital from January 2013 to October 2019 were included in this work. The baseline characteristics of the patients are shown in Table 1. The prevalence of sarcopenia was 27.4% (34/124), in which 32.4% (11/34) was observed in men. This percentage is significantly lower than that for the no-sarcopenia group ($P < 0.001$). Smoking was more prevalent in patients without sarcopenia ($P = 0.035$) than in patients with sarcopenia. Sarcopenic patients had a significantly lower BMI (16.75 ± 2.59 vs. 19.49 ± 3.03, $P < 0.001$), lower serum albumin levels (29.2 ± 6.1 g/L vs. 33.0 ± 8.0 g/L, $P = 0.006$), lower prealbumin levels (141.8 ± 70.2 vs. 175.0 ± 84.9 mg/L, $P = 0.030$), and lower hemoglobin levels (98.3 ± 18.8 vs. 115.1 ± 24.4 g/L, $P < 0.001$) than those without sarcopenia. With regard to disease behavior, the sarcopenic patients had strictures and low B1 and B3 ($P = 0.026$). No significant differences were observed between the two groups in terms of age, disease duration, disease location, perianal disease, preoperative medication, and HBI score (Table 1).

Table 1: Demographic and clinical characteristics in CD patients with or without sarcopenia.

	All patients ($n = 124$)	Sarcopenia ($n = 34$)	Nonsarcopenia ($n = 90$)	P value
Age, years	37.06 ± 13.08	37.03 ± 16.55	37.08 ± 11.61	0.985
Gender				*<0.001*
Male	74 (59.7)	11 (32.4)	63 (70.0)	
Smoking	12 (9.7)	0 (0)	12 (13.3)	*0.035*
Alcohol	7 (5.6)	0 (0)	7 (7.8)	0.188
BMI	18.74 ± 3.15	16.75 ± 2.59	19.49 ± 3.03	*<0.001*
BMI categories				*<0.001*
Underweight	59 (47.6)	29 (85.3)	30 (33.3)	
Normal	57 (46.0)	4 (11.8)	53 (58.9)	
Overweight	8 (6.5)	1 (2.9)	7 (7.8)	
Skeletal muscle index (cm^2/m^2)	53.89 ± 15.13	37.98 ± 3.94	59.90 ± 13.32	*<0.001*
Disease duration (months)	55.81 ± 51.67	54.49 ± 54.45	56.31 ± 50.89	0.867
Age of onset				0.492
≤16 (A1)	9 (7.3)	4 (11.8)	5 (5.6)	
17-40 (A2)	81 (65.3)	21 (61.8)	60 (66.7)	
>40 (A3)	34 (27.4)	9 (26.5)	25 (27.8)	
Disease location				0.063
Ileum (L1)	71 (57.3)	16 (47.1)	55 (61.1)	
Colon (L2)	18 (14.5)	9 (26.5)	9 (10.0)	
Ileocolon (L3)	35 (28.2)	9 (26.5)	26 (28.9)	
Disease behavior				*0.026*
Nonstricturing, nonpenetrating (B1)	22 (17.7)	4 (11.8)	18 (20.0)	
Stricturing (B2)	67 (54.0)	25 (73.5)	42 (46.7)	
Penetrating (B3)	35 (28.2)	5 (14.7)	30 (33.3)	
Perianal disease	34 (27.4)	13 (38.2)	21 (23.3)	0.097
HBI score	6.39 ± 2.81	6.94 ± 2.33	6.18 ± 2.96	0.179
Preoperative therapy				0.712
No therapy	22 (17.7)	8 (23.5)	14 (15.6)	
Steroid	20 (16.1)	6 (17.6)	14 (15.6)	
5ASA	24 (19.4)	8 (23.5)	16 (17.8)	
AZA	36 (29.0)	8 (23.5)	28 (31.1)	
MTX	4 (3.2)	1 (2.9)	3 (3.3)	
IFX	18 (14.5)	3 (8.8)	15 (16.7)	
Hemoglobin (g/L)	110.5 ± 24.1	98.3 ± 18.8	115.1 ± 24.4	*<0.001*
White cell count (cells ×109/L)	6.94 ± 3.97	6.84 ± 4.37	6.98 ± 3.83	0.872
Platelet	265 ± 119	293 ± 130	254 ± 114	0.128
Prealbumin (g/L)	165.9 ± 82.2	141.8 ± 70.2	175.0 ± 84.9	*0.030*
Albumin (g/L)	31.9 ± 7.7	29.2 ± 6.1	33.0 ± 8.0	*0.006*

BMI: body mass index; HBI: Harvey Bradshaw Index; 5-ASA: 5-aminosalicylate; AZA: Azathioprine; MTX: Methotrexate; IFX: Infliximab. P values < 0.05 were highlighted in italic.

3.2. Surgical Details of CD Patients with or without Sarcopenia. The surgical details of CD patients with or without sarcopenia are listed in Table 2. Twelve percent of the patients had emergency surgery. No significant difference in indication for surgery was found between the groups with and without sarcopenia. The main indications for surgery were strictures (46.0%) and penetrating complications (29.0%). Laparoscopic operations were performed in 26.6% of the cases. Colostomy was performed in 40.3% of the patients, with no significant difference between the two groups.

3.3. Analysis of Postoperative Outcomes and Complications in CD Patients with or without Sarcopenia. The postoperative outcomes and complications are listed in Table 3. The length of hospital admission was 25.98 ± 19.90 days (16.38 ± 17.73

Table 2: Surgical details in CD patients with or without sarcopenia.

	All patients ($n = 124$)	Sarcopenia ($n = 34$)	Nonsarcopenia ($n = 90$)	P value
Emergency surgery	16 (12.9)	2 (5.9)	14 (15.6)	0.152
Indication for surgery				0.365
Bowel obstruction	57 (46.0)	18 (52.9)	39 (43.3)	
Fistula	14 (11.3)	5 (14.7)	9 (10.0)	
Medically refractory disease	17 (13.7)	2 (5.9)	15 (16.7)	
Perforation	36 (29.0)	9 (26.5)	27 (30.0)	
Type of surgery				0.374
Open	91 (73.4)	23 (67.6)	68 (75.6)	
Laparoscopic	33 (26.6)	11 (32.4)	22 (24.4)	
Colostomy	50 (40.3)	16 (47.1)	34 (37.8)	0.347

Table 3: Analysis of postoperative outcomes and complications in CD patients with or without sarcopenia.

	All patients ($n = 124$)	Sarcopenia ($n = 34$)	Nonsarcopenia ($n = 90$)	P value
Length of hospital admission (overall)	25.98 ± 19.90	34.41 ± 26.83	22.79 ± 15.59	*0.003*
Length of hospital admission (postoperation)	16.38 ± 17.73	22.47 ± 24.73	14.08 ± 13.72	*0.018*
Parenteral nutrition	63 (50.8)	20 (58.8)	43 (47.8)	0.272
Complications	35 (28.2)	14 (41.2)	21 (23.3)	*0.049*
Skin or soft tissue infection	18 (14.5)	6 (17.6)	12 (13.3)	
Major intra-abdominal leak	8 (6.5)	4 (11.8)	4 (4.4)	
Postoperative sepsis	4 (3.2)	2 (5.9)	2 (2.2)	
Postoperative thrombosis	2 (1.6)	1 (2.9)	1 (1.1)	
Organ space infection	3 (2.4)	1 (2.9)	2 (2.2)	
Hospital readmission within 30 days	12 (9.7)	4 (11.8)	8 (8.9)	0.629
Reoperation	2 (1.6)	2 (5.9)	0 (0.0)	0.074
ICU admission	3 (2.4)	1 (2.9)	2 (2.2)	0.621
Death	1 (0.8)	1 (2.9)	0 (0.0)	0.274

P values < 0.05 were highlighted in italic.

days of postoperative stay). A significant difference was observed in the length of hospital stay and postoperative stay between patients with sarcopenia and those without (34.41 ± 26.83 vs. 22.79 ± 15.59 days, $P = 0.003$; 22.47 ± 24.73 vs. 14.08 ± 13.72, $P = 0.018$) (Figure 2). Sixty-three patients required PN therapy postoperatively. In total, 35 complications were reported (28.2%) (Table 3). The sarcopenic CD patients had much more complications (41.2% vs. 23.3%, $P = 0.049$) than the nonsarcopenic CD patients. Of the 124 patients, 12 (9.7%) required hospital readmission within 30 days of surgery, among which 3 patients required ICU admission and 2 patients needed reoperations. No significant difference was found in terms of hospital readmission, reoperation, ICU admission, and death between sarcopenic and nonsarcopenic groups.

3.4. Univariate and Multivariate Analysis for Postoperative Complications. Statistical analyses were performed to identify the potential risk factors for the occurrence of postoperative complications. In the univariate analysis, low BMI ($P = 0.049$), low HBI ($P = 0.027$), low preoperative levels of serum albumin ($P = 0.001$), low prealbumin levels ($P = 0.009$), emergency surgery ($P = 0.002$), and colostomy and sarcopenia ($P = 0.026$) were identified as factors related to postoperative complications (Table 4). In the multivariate regression analysis, sarcopenia (OR: 3.974; 95% CI: 1.171–13.489; $P = 0.027$) and male gender (OR: 4.080; 95% CI: 1.205–13.814; $P = 0.024$) were identified as independent risk factors associated with postoperative complications (Table 4).

3.5. Factors That Affect SMI in Patients with CD. Multiple linear regression analyses were performed to investigate possible associations between SMI and disease duration, BMI, body weight, serum albumin levels, and prealbumin levels. The values for body weight and serum albumin levels were significantly correlated with SMI ($R^2 = 0.52$, Table 5). The higher the albumin level was, the higher SMI was and the lower the prevalence of sarcopenia was. Body weight exerted more influence on SMI than albumin level. The linear regression equation is shown in Table 5.

4. Discussion

Many studies indicate that CD may cause skeletal muscle loss or sarcopenia [22, 24, 25]. However, the prevalence of

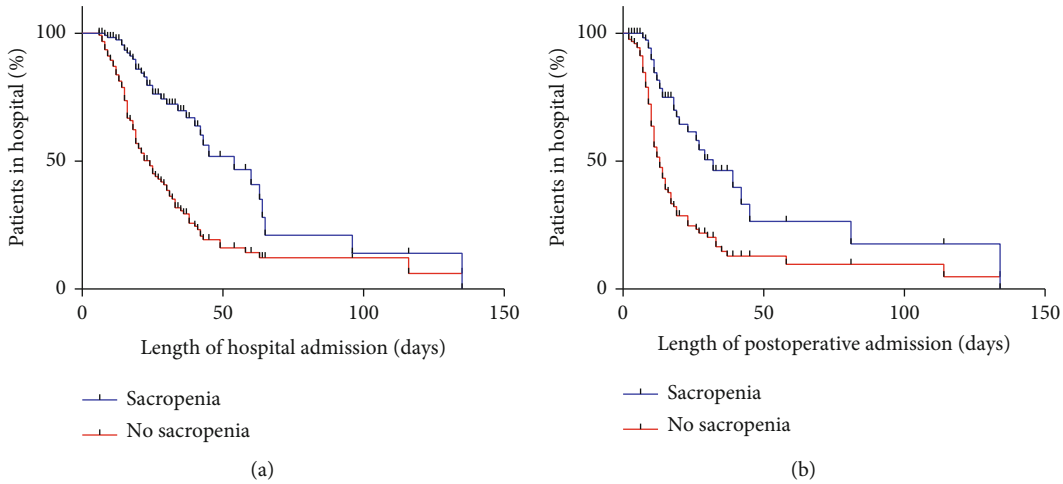

Figure 2: Length of overall hospital stay and postoperative hospital stay dependent on with or without sarcopenia. (a) Length of overall hospital stay (34.41 ± 26.83 vs. 22.79 ± 15.59 days, $P = 0.003$). (b) Length of postoperative hospital stay (22.47 ± 24.73 vs. 14.08 ± 13.72 days, $P = 0.018$).

sarcopenia among patients with CD varies significantly in relevant studies. Schneider et al. discovered that the prevalence of sarcopenia is high at 60% among remission phase CD patients through measurements obtained via dual-energy X-ray absorptiometry (DEXA) [21]. Zhang et al. obtained a prevalence rate of 61.4% in 114 CD patients in China through the measurement of the skeletal muscle area via abdominal CT with SMI of less than $55\,cm^2/m^2$ in male and SMI of less than $39\,cm^2/m^2$ in female patients [20]. A US study in Iowa calculated the total psoas index via CT among IBD patients and derived a 24.7% prevalence rate of sarcopenia [25].

In this study, we found a sarcopenia prevalence of 27.4% based on the threshold set by Martin et al., who reported an SMI of less than $41\,cm^2/m^2$ in females and an SMI of less than $43\,cm^2/m^2$ ($BMI < 25\,kg/m^2$) or SMI of less than $53\,cm^2/m^2$ ($BMI \geq 25\,kg/m^2$) in males. The prevalence in this study is within the range in other literature but relatively low [20–22, 26, 27]. On one hand, this is because the measurement methods in various studies differed either due to DEXA being affected by fat tissue and body water or the different SMI thresholds used. On the other hand, the majority of the patients in this study underwent elective surgery and have thus been receiving total or partial enteral nutrition support, which might lead to the reduced prevalence of sarcopenia. We also discovered that 14.7% of sarcopenia patients in the study were of normal weight or overweight. Therefore, they are not identified as malnourished under the traditional body mass index (BMI) method. The diagnosis of sarcopenia is important to patients' nutrition evaluation and treatment, and even normal-weight and overweight patients deserve special attention.

A few studies have indicated that the incidence of sarcopenia can cause multiple adverse events, such as osteoporosis with pathologic fracture, repeated hospitalization, mobility difficulty, and reduction in the quality of life [11–13, 25]. Chen et al. diagnosed 11.8% of 313 gastric cancer patients with postlaparoscopic gastrectomy as sarcopenic [28]. Sarcopenia significantly increased the postoperative complications, hospital stay days, and total financial costs of the patients with sarcopenia compared with the patients without it [26, 29].

Reports on postoperative complications among CD patients are scarce. Therefore, the 124 postoperative patients were studied using logistic single-factor regression and multiple-factor regression, which revealed that only sarcopenia (OR = 3.974, $P = 0.027$) and male gender (OR = 4.080, $P = 0.024$) can be considered independent risk factors for postoperative complications. SMI can reflect patients' nutrition status and predict the incidence of complications more accurately compared with conventional nutrition indicators, such as BMI, prealbumin level, and albumin level [9, 11, 30]. Therefore, improving patients' perioperative nutrition status is important in reducing the occurrence of sarcopenia and postoperative complications.

Research has shown that elevation of serum cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin 6, reduces insulin-like growth factor-1 (IGF-1) in the serum and muscle of CD patients. This reduction, in turn, results in growth hormone resistance in the liver and skeletal muscle, leading to a downward moderation of the mTOR pathway into activation of the ubiquitin ligand and expression of proteolytic enzyme, which cause skeletal muscle mass reduction and impairment of muscle contraction [13, 14]. Current interventions for sarcopenia include exercise therapy, nutrition therapy, and medication. Most experts recommend that CD patients undergo exercise therapy for 6 weeks to 3 months to improve the patients' general condition, increase the oxygenation index, and correct malnutrition [3, 10]. The guidelines of the European Society for Clinical Nutrition and Metabolism recommend that active (adult) IBD patients increase their protein intake to 1.2–1.5 g/kg/d (with 50% high-quality protein) higher than that recommended for the general population to increase the skeletal muscle cell volume, inhibit proteolysis, and reverse muscle mass reduction and functionality decline [31]. Medication for sarcopenia is still in the exploration phase, and no specific drug has been invented yet. However, Subramaniam

Table 4: Univariate and multivariate regression analysis for risk factors of postoperative complication.

Variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Male	2.041	0.878-4.746	0.098	4.080	1.205-13.814	*0.024*
Smoking	2.089	0.434-10.055	0.358			
Alcohol	2.458	0.285-21.191	0.413			
BMI			*0.049*			0.147
Underweight	4.308	0.499-37.207	0.184	6.353	0.444-90.812	0.173
Normal	1.628	0.179-14.771	0.625	2.501	0.165-37.947	0.509
Overweight	1			1		
Sarcopenia	2.587	1.124-5.959	*0.026*	3.974	1.171-13.489	*0.027*
Duration (months)	0.996	0.988-1.004	0.313			
Age of onset			0.493			
<16 years (A1)	1					
16-40 years (A2)	1.677	0.496-5.665	0.405			
>40 years (A3)	1.037	0.265-4.052	0.958			
Disease location			0.493			
Ileum (L1)	1					
Colon (L2)	0.964	0.247-3.768	0.958			
Ileocolon (L3)	1.617	0.636-4.109	0.312			
Disease behavior			0.371			
Nonstricturing, nonpenetrating (B1)	1					
Stricturing (B2)	0.635	0.199-2.029	0.443			
Penetrating (B3)	0.531	0.219-1.288	0.161			
Perianal disease	1.742	0.678-4.475	0.249			
HBI score			*0.027*	1.167	0.911-1.495	0.381
Remission (<5)	1					
Mild active (5-8)	3.103	1.240-7.761	*0.016*	2.485	0.674-9.160	0.172
Sever active (>8)	4.583	1.124-18.693	*0.034*	2.591	0.376-17.843	0.334
Hemoglobin	0.988	0.972-1.004	0.141	1.000	0.974-1.028	0.986
White cell count	1.098	0.998-1.208	0.056	0.947	0.826-1.085	0.431
Platelet	1.001	0.998-1.004	0.552			
Prealbumin (g/L)	0.992	0.986-0.998	*0.009*	0.994	0.982-1.007	0.362
Albumin (g/L)	0.911	0.860-0.965	*0.001*	1.014	0.993-1.152	0.830
Emergency surgery	5.533	1.830-16.731	*0.002*	4.225	0.730-24.459	0.108
Indication for surgery			*0.049*			0.687
Stricture	1			1		
Fistula	2.323	0.649-8.321	0.195	1.383	0.251-7.625	0.710
Medically refractory disease	0.896	0.219-3.670	0.879	3.221	0.457-22.695	0.240
Perforation	3.345	1.320-8.479	*0.011*	1.258	0.382-4.140	0.706
Type of surgery	2.754	0.967-7.848	0.058	1.393	1.345-5.633	0.642
Colostomy	4.401	1.916-10.108	*<0.001*	1.965	0.665-5.805	0.222

BMI: body mass index; HBI: Harvey Bradshaw Index; 5-ASA: 5-aminosalicylate; AZA: Azathioprine; MTX: Methotrexate; IFX: Infliximab. *P* values < 0.05 were highlighted in italic.

et al. proved that a TNF-α inhibitor, infliximab, can inhibit the activation of NF-κB, reduce proteolysis and sarcolysis, and accelerate muscle formation to reverse sarcopenia [32]. Thus, gastroenterologists, surgeons, radiologists, and nutritionists should work closely together to determine an early intervention plan and a proper operation timing to reduce the incidence of postoperative complications.

However, the current study has several potential limitations. First, selection bias may exist in this retrospective work. Second, the lack of consensus regarding the adoption of an SMI threshold remains unaddressed. This study used the widely applied CT measurement method proposed by Martin et al., so it cannot be compared directly with other studies. Third, this retrospective study's diagnosis of

Table 5: Factors affecting skeletal muscle mass index in patients with CD.

Variable	β	95% CI	P value
Body weight	0.939	0.758-1.119	*<0.001*
Serum albumin level	0.355	0.103-1.606	*0.006*

Linear regression equation $Y = -7.310 + 0.939 * \text{body weight} + 0.355 * \text{albumin} R^2 = 0.520$.

sarcopenia consisted of skeletal muscle mass reduction and muscle strength decline, which requires the measurement of grip strength and walking pace. These measurements could be performed in any further prospective study to achieve an accurate diagnosis of sarcopenia. Despite these limitations, this study revealed that sarcopenia can be used as an independent risk factor to predict the incidence of complications in patients with CD.

5. Conclusion

Sarcopenia is common in patients with CD and is unlikely to be recognized by a routine clinical assessment using BMI alone. Detection of sarcopenia is important; it can serve as a prognostic factor for the prediction of postoperative complications in patients with CD undergoing bowel resections. Therefore, the use of the SMI index together with a routine assessment of nutritional status should be a cornerstone in clinical practice.

Data Availability

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

The study received approval from the institutional review board of Ruijin Hospital, Shanghai Jiaotong University School of Medicine.

Consent

Written informed consent for publication was obtained from all participants.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Zhengting Wang and Jie Zhong contributed to the conception of the study. Chen Zhang and Dingye Yu performed the data analyses and wrote the manuscript. Liwen Hong and Tianyu Zhang helped perform the analysis with constructive discussions. All authors provided critical feedback and helped shape the research, analysis, and manuscript. Chen Zhang and Dingye Yu contributed equally to this work.

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Research Article

Age and Gender: Affecting the Positive Rates of Serum PAB and ANCA in Patients with Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) is a group of immune-mediated conditions. Immune activity is varied by age and gender. The present study is aimed at investigating the effect of age and gender on the positive rates of anti-Saccharomyces cerevisiae antibodies (ASCA), anti-neutrophil cytoplasmic antibodies (ANCA), anti-intestinal goblet cell antibodies (GAB), and antibodies to exocrine pancreas (PAB) in IBD patients. A total of 1871 hospitalized patients with confirmed IBD were included in this study. Sera were obtained from each subject for antibody measurement by indirect immunofluorescence assay. The positive rates of ANCA IgG and IgA were higher in female patients than those in male patients ($P < 0.001$) while the positive rate of PAB IgG was just reversed ($P < 0.001$). Moreover, the median ages of patients with positive ANCA IgG and IgA were higher than patients with negative antibodies ($P = 0.0019$ and $P = 0.0110$, respectively), while the median ages of patients with positive PAB IgG and IgA were significantly lower than patients with negative PAB ($P < 0.0001$). The serum levels of ANCA IgG and IgA were potentiated in old female patients, while serum PAB IgG was easy to be detected in the young male patients with IBD.

1. Introduction

Inflammatory bowel disease (IBD) mainly comprises Crohn's disease (CD) and ulcerative colitis (UC). It is a group of progressive immune-mediated conditions characterized by chronic inflammation of bowel and usually with a long-term treatment and unpredictable course [1, 2]. The incidence and prevalence of IBD are increasing globally, and the prevalence was more than 0.3% of the total population in many developed countries [3, 4]. The prolonged chronic inflammation also increases chance of developing colorectal cancers [5]. The diagnosis of IBD mainly depends on clinical manifestations, endoscopy, imaging, histology, and biochemistry detection. Increasing evidences show that anti-*Saccharomyces cerevisiae* antibodies (ASCA), anti-neutrophil cytoplasmic antibodies (ANCA), anti-intestinal goblet cell antibodies (GAB), and antibodies to exocrine pancreas (PAB) play a role in assisting diagnosis of IBD, especially in differential diagnosis of UC and CD [6, 7].

ASCA is directed against the oligomannosidic epitopes of *Saccharomyces cerevisiae* wall [8] and was elevated in the serum of CD patients [9]. ANCA is detected in 40%–80% of UC patients and in 6%–20% of CD patients [10]. Meanwhile, ASCA and ANCA were widely combined to serve as valuable serological tools for differential diagnosis of UC and CD [11, 12]. Similarly, PAB was directed against pancreatic antigens and was found in 30% to 40% of patients with CD. PAB have also been nominated as serological diagnostic markers for CD, though it was not correlated with clinical features of CD [13–15]. GAB was directed against goblet cells in the intestine and was found in up to 30% of patients with UC [15, 16].

Most studies to date have focused solely on the positive rates of these antibodies for the differentiation of CD from UC. Desplat-Jégo et al. [13] reported that CD patients in children who are under 15 years old displayed a higher positive rate of ASCA than in adults (mean age: 31 years), while in adults, patients under 20 years also exhibited a higher

frequency of serum ASCA than patients over 20 years old in France. Similar results had also been found in the study which reported that the positive rate of PAB was higher in adult patients less than 20 years old compared to patients over 20 years old [13]. Authors also stated that CD patients more than 35 years of age were significantly less likely to express PAB, though sex was not a significant factor in PAB or GAB expression [6]. It seems that the positive rates of serum ASCA and PAB are high when the age of onset is young, or the positive rates of serum ASCA and PAB are related to the age of onset of CD, not the sex of the patients.

However, the effect of age and gender on the positive rates of ASCA, ANCA, GAB, and PAB in the IBD patients remains obscure. Herein, we investigated the relationship between age, gender, and positive rates of ASCA, ANCA, GAB, and PAB in IBD patients with large simple size and determined the effect of age and gender on the positive rates of these antibodies in IBD patients.

2. Materials and Methods

2.1. Study Subjects. This study was conducted at the 1st Affiliated Hospital of Fujian Medical University, from January 2015 to December 2019. The protocols for the study and informed consents were approved by the Fujian Medical University ethics committee (Approval number: 201536). A total of 1871 hospitalized patients with confirmed IBD were included in this study.

The inclusion criteria of the participants are as follows: (1) participants were admitted to hospital, (2) participants were diagnosed with IBD according to the Consensus on diagnosis and treatment of inflammatory bowel disease (Guangzhou, 2012) [17], and (3) written informed consents were filled correctly.

The exclusion criteria were as follows: (1) patients with gastrointestinal tumor; (2) patients with other gastrointestinal diseases, such as irritable bowel syndrome, ischemic bowel disease, intestinal polyps, intestinal vascular malformations, and eosinophilic gastroenteritis; (3) patients with bacterial or viral infection; and (4) patients with other autoimmune diseases.

2.2. Antibody Measurement. Venous blood 2 mL was obtained from each subject. Sera were separated immediately and kept at -80°C before analysis. IgA and IgG of ASCA, ANCA, GAB, and PAB were detected by indirect immunofluorescence (IIF) using commercially available detection kits (EUROIMMUN Medical Diagnostics Co., Ltd.) according to the manufacturer's instructions. In brief, sera were diluted 1 : 10 in phosphate buffer. 25 mL of the diluted sera was incubated for 30 min on slides with smears of Saccharomyces cerevisiae, ethanol-fixed human neutrophil, monkey small intestine, and monkey pancreas for ASCA, ANCA, GAB, and PAB, respectively. After a washing step, fluorescent-conjugated goat anti-human IgG or IgA was added to detect IgG or IgA of these antibodies, respectively.

2.3. Statistical Analysis. All data were analyzed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Graphs were plotted with the GraphPad Prism 5 software. Continuous variables were expressed as median (interquartile range) and calculated by Mann–Whitney test. Categorical variables were expressed as percentage and compared by χ^2 test for unpaired data and McNemar's test for paired data between groups. $P < 0.05$ was considered statistically significant.

Table 1: Positive rate of ASCA, GAB, PAB, and ANCA in patients with IBD.

Antibodies	Total ($N = 1871$)	No. (%) Female ($n = 757$)	Male ($n = 1114$)	P value
ASCA IgG	149 (7.96)	71 (9.38)	78 (7.00)	0.062
ASCA IgA	174 (9.30)	65 (8.59)	109 (9.78)	0.381
GAB IgG	642(34.31)	249 (32.89)	393 (35.28)	0.286
GAB IgA	48 (2.57)	21 (2.77)	27 (2.42)	0.638
PAB IgG	244 (13.04)	74 (9.78)	170 (15.26)	<0.001
PAB IgA	97 (5.18)	39 (5.15)	58 (5.21)	0.958
ANCA IgG	155 (8.28)	82 (10.83)	73 (6.55)	<0.001
ANCA IgA	96 (5.13)	55 (7.27)	41 (3.68)	<0.001

Data were presented as No. (percentage) and calculated by using χ^2 test. ASCA: anti-*Saccharomyces cerevisiae* antibodies; GAB: anti-intestinal goblet cell antibodies; PAB: antibodies to exocrine pancreas; ANCA: anti-neutrophil cytoplasmic antibodies; IBD: inflammatory bowel disease. P values indicate differences between female and male patients with IBD. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Presenting Characteristics. The study cohort included 1871 hospitalized patients with confirmed IBD. The median age was 39 years (IQR, 26-55 years), and 757 (40.5%) were women.

3.2. Positive Rates of ASCA, GAB, PAB, and ANCA in Patients with IBD. To assess the positive rates of antibodies known to be associated with IBD, the antibodies including ASCA IgG, ASCA IgA, GAB IgG, GAB IgA, PAB IgG, PAB IgA, ANCA IgG, and ANCA IgA were measured (Table 1 and Figure 1). Our results showed that the positive rate of GAB IgG was 34.31%. It was the highest among these antibodies (all $P < 0.001$). Next to GAB IgG, the positive rate of PAB IgG was 13.04%, which was the second highest among these antibodies. And the positive rate of other antibodies ranged from 2.57% to 9.30%. Moreover, the positive rates of ANCA IgG and ANCA IgA were higher in female patients than those in male patients (10.83% vs. 6.55%, $P < 0.001$ and 7.27% vs. 3.68%, $P < 0.001$, respectively), while the positive rate of PAB IgG was significantly lower in female patients than that in male patients (9.78% vs. 15.26%, $P < 0.001$). No distinguishing positive rates of other antibodies were observed in female patients and as compared with male patients with IBD.

3.3. Ages of ASCA, GAB, PAB, and ANCA in Patients with IBD. To further evaluate the characteristics of age distribution in patients with IBD, the ages of patients with and without the antibodies, together with the ages of female and male

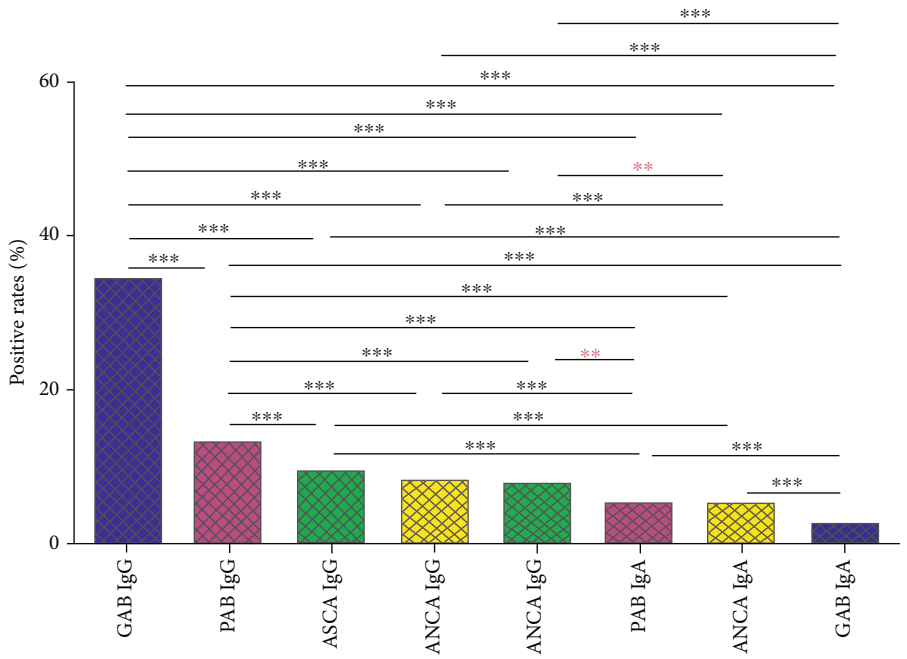

Figure 1: Positive rate of ASCA, GAB, PAB, and ANCA in patients with IBD. Positive rate was presented as percentage and calculated by using McNemar's test. ASCA: anti-*Saccharomyces cerevisiae* antibodies; GAB: anti-intestinal goblet cell antibodies; PAB: antibodies to exocrine pancreas; ANCA: anti-neutrophil cytoplasmic antibodies; IBD: inflammatory bowel disease; $^{***}P < 0.0001$; $^{**}P < 0.001$.

Table 2: Ages of ASCA, GAB, PAB, and ANCA in patients with IBD.

Antibodies	Median (IQR), years		*P* value
	Positive	Negative	
ASCA IgG	40.0 (25.0-63.0)	39.0 (26.0-54.0)	0.0615
ASCA IgA	43.0 (23.8-64.3)	39.0 (26.0-53.0)	0.0203
GAB IgG	37.0 (26.8-53.0)	41.0 (25.0-56.0)	0.3446
GAB IgA	31.5 (23.3-49.8)	39.0 (26.0-55.0)	0.0887
PAB IgG	27.5 (22.0-34.0)	42.0 (27.0-56.0)	<0.0001
PAB IgA	26.0 (20.0-33.0)	40.0 (26.0-55.3)	<0.0001
ANCA IgG	45.0 (31.0-57.0)	39.0 (25.0-54.0)	0.0019
ANCA IgA	44.5 (31.3-55.8)	39.0 (25.0-55.0)	0.0110

Data were presented as median (IQR) and calculated by Mann–Whitney test. IQR: interquartile range; ASCA: anti-*Saccharomyces cerevisiae* antibodies; GAB: anti-intestinal goblet cell antibodies; PAB: antibodies to exocrine pancreas; ANCA: anti-neutrophil cytoplasmic antibodies; IBD: inflammatory bowel disease. *P* values indicate differences between antibody positive and negative patients. $P < 0.05$ was considered statistically significant.

patients with the positive antibodies, were analyzed. For ASCA IgA, ANCA IgG, and ANCA IgA, the median ages of patients with these antibodies were higher than those of patients without these antibodies (43.0 vs. 39.0, $P = 0.0203$; 45.0 vs. 39.0, $P = 0.0019$; and 44.5 vs. 39.0, $P = 0.0110$, respectively), while the median ages of patients with PAB IgG and PAB IgA positive were significantly lower than those of patients without PAB IgG and PAB IgA (27.5 vs. 42.0, $P < 0.0001$ and 26.0 vs. 40.0, $P < 0.0001$, respectively). There was no significant difference in the median ages between antibody-positive patients and antibody-negative patients for ASCA IgG, GAB IgG, and GAB IgA (Table 2).

Interestingly, there was a higher median age in the female patients compared to male patients with positive ASCA IgA (52.0 vs. 40.0, $P = 0.0354$), while there was no significant difference in the median ages between female and male patients with the other positive antibodies (Table 3).

4. Discussion

In this study, we investigated the relationship between age, gender, and the positive rates of ASCA, ANCA, GAB, and PAB in IBD patients. Our study found that GAB IgG had a positive rate of 34.31%, which was the highest positive rate among these antibodies in IBD patients. Interestingly, the positive rates of ANCA IgG and IgA were higher in female patients than those in male patients while the positive rate of PAB IgG was significantly lower in female patients than that in male patients. Moreover, the median ages of patients with positive ASCA IgA, ANCA IgG, and ANCA IgA were higher than patients with negative antibodies, while the median ages of patients with positive PAB IgG and IgA were significantly lower than patients with negative PAB.

The intestinal infection, disorder of immune regulation in the intestinal mucosal, and gene susceptibility are main factors related to IBD [18]; therefore, serological antibodies, especially ASCA, ANCA, GAB, and PAB, were often measured to aid diagnosis of IBD or distinguish CD from UC. Unfortunately, the expressions of these antibodies are not high. For patients with CD, ASCA was positive in approximately 40% to 54%, while PAB was detected in 30% to 46% of patients [6, 7, 13]. And for patients with UC, ANCA was positive in approximately 50% to 75%, while GAB was detectable in 2%-46.4% of patients [6, 7, 13]. In line with

Table 3: Ages of patients with positive ASCA, GAB, PAB, and ANCA.

Antibodies	Median (IQR), y		*P* value
	Female	Male	
ASCA IgG	47.0 (27.0-65.0)	35.0 (23.0-62.0)	0.1099
ASCA IgA	52.0 (27.5-66.5)	40.0 (21.0-61.0)	0.0354
GAB IgG	38.0 (27.0-53.5)	36.0 (26.0-52.5)	0.2218
GAB IgA	32.0 (25.0-47.5)	31.0 (22.0-50.0)	0.7949
PAB IgG	27.5 (22.0-37.3)	27.5 (21.0-34.0)	0.3483
PAB IgA	25.0 (19.0-36.0)	28.0 (20.0-32.0)	0.9736
ANCA IgG	48.0 (33.3-58.8)	40.0 (29.0-52.5)	0.0728
ANCA IgA	47.0 (33.5-61.0)	40.0 (29.0-52.5)	0.1035

Data were expressed as median (IQR) and calculated by Mann–Whitney test. IQR: interquartile range; ASCA: anti-*Saccharomyces cerevisiae* antibodies; GAB: anti-intestinal goblet cell antibodies; PAB: antibodies to exocrine pancreas; ANCA: anti-neutrophil cytoplasmic antibodies; IBD: inflammatory bowel disease. *P* values indicate differences between female and male patients. $P < 0.05$ was considered statistically significant.

these studies, our results showed that the positive rate of GAB IgG was 34.31%. And the expressions of PAB, ASCA, and ANCA were between 2.57% and 13.04% in our study, which was not as high as previous reported [6, 13]. One possible reason for this difference was that the positive rates of these antibodies were from all the patients including CD and UC patients. The expression rates of these antibodies for CD patients or UC patients only were not calculated. In addition, the expression rates of these antibodies may be related to different ethnic group. A similar finding also showed that the positive rates of ANCA, ASCA, and PAB were significantly different between Chinese and Caucasian patients with IBD [6].

In our study, pancreatic antibody PAB was also detected in the patients with IBD, and the positive rate was 13.04%. PAB was also measured in the patients with IBD by Desplat-Jégo et al. [13] and Lawrance et al. [6];Vimal Bodiwala, Timothy Marshall, Kiron M Das, Steven R Brant, Darren N Seril they reported that PAB expression was highly specific for CD, though it was without any correlation with clinical characteristics of the disease including whether the patients have pancreatitis or not. So, there is no evidence for a direct pathogenic role for PAB in CD. Patients with IBD often have pancreatic diseases such as acute pancreatitis, chronic pancreatitis, or pancreatic exocrine dysfunction [19], which may lead to pancreatic antigen release from pancreas and stimulate the production of pancreatic antibodies. IBD is a group of immune-mediated conditions, and autoantibodies including pancreatic antibodies may be exhibited in the abnormal activation of immune system. These mechanisms may partially explain the presence of PAB in IBD patients with pancreatic diseases. But why did PAB exhibit in the IBD patients without pancreatic disease? Maybe the generation of PAB is the result of a cross-reactivity with enteric microbial antigens [20], which has recently been demonstrated for ANCA in UC [21]. Maybe the pancreas like antigens was expressed in the regional intestine under the chronic inflammation of the intestine. Anyway, up until now, a mechanism association between PAB and CD had not been demonstrated. Therefore, the mechanisms of PAB appeared in the IBD patients need to be further studied in the future.

Growing evidences indicate that autoimmunity is influenced by gender. The immunoreactivity including antigen presenting activity and mitogenic responses of lymphocytes and monocytes in females is more enhanced in females than in males. The immunoglobulin levels in females are also higher than males, due to the influence of sex hormones and sex related genes [22]. And IBD should be regarded as an autoimmune disease because of the reactivity of lymphocytes to their own antigens [23] and the autoimmune extraintestinal manifestations [24]. Consistent with these theories, our study found that the positive rates of ANCA IgG and ANCA IgA were higher in female patients than in male patients. Our results are also agreed with Hornig et al. [25] who reported that the level of serum antinuclear was increased in women than in men. Contradictory to those studies [22, 25], the positive rate of PAB IgG was significantly lower in female patients compared to male patients. And we failed to see a significant difference between female and male patients for the positive rates of ASCA and GAB. Therefore, our study reflected that gender can affect the expressions of autoantibodies, and some of which were highly expressed in women while some of which are highly expressed in men.

Age may also be related to the positive rates of autoantibodies. And elderly patients have a relative immunodeficiency compared to younger patients [26]. In our study, we found that the median ages of patients with ASCA IgA, ANCA IgG, and ANCA IgA were higher than patients without these antibodies. Our results were similar with the study conducted in USA which reported that cystic fibrosis patients who were ASCA seropositive were older than the seronegative patients [27]. But our results were partly contrasted with Desplat-Jégo et al. [13] who documented that young CD patients (under 15 years old in children or under 20 years old in adults) had a higher ASCA seropositivity than old patients in France. These discrepancies probably relate to distinct racial and ethnic groups [28] and dietary habits or environmental exposures, because bread is a great source of *Saccharomyces cerevisiae* and is also a major source of gluten which was usually eaten in France [13]. Consistent with previous studies [6, 13], our results also found that the median ages of patients with PAB IgG and PAB IgA positive were significantly lower than patients without PAB IgG and PAB IgA. Additionally, a higher median age in the female patients with positive ASCA IgA was also found in our study. Therefore, our study suggests that age may also have influence on autoantibodies.

This study had some limitations. First, we did not access the influences of age and gender on the expression of autoantibodies in CD and UC subgroups. Second, how age and gender affect the expression of these autoantibodies was not included in the study.

5. Conclusion

Age and gender can affect the expressions of antoantibodies in patients with IBD. The levels of ANCA IgG and IgA were

potentiated in old female patients, while the level of PAB IgG was easy to be detected in the young male patients. Our study inflects that the influence of age and gender on the results of autoantibodies should be considered in the clinical application.

Data Availability

The data used to support the findings of this study are currently under embargo while the research findings are commercialized. Requests for data, 12 months after publication of this article, will be considered by the corresponding author.

Conflicts of Interest

The authors have no competing interests to declare.

Acknowledgments

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Research Article

Usefulness of Measuring Thiopurine Metabolites in Children with Inflammatory Bowel Disease and Autoimmunological Hepatitis, Treated with Azathioprine

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Introduction. Thiopurines, such as azathioprine (AZA) and 6-mercaptopurine (6-MP), are immunomodulatory agents, used for the maintenance of remission in children with inflammatory bowel disease (IBD)—Crohn's disease (CD) and ulcerative colitis (UC), as well as with autoimmunological hepatitis (AIH). Measurements of thiopurine metabolites may allow identifying patients at risk for toxicity and nonadherence. It can also provide an explanation for the ineffectiveness of the treatment, observed in some patients. *Patients and Methods*. A retrospective analysis was carried out of sixty-eight patients (thirty-six patients with CD, eighteen with UC, and fourteen with AIH), treated with AZA. Thiopurine metabolites, 6-thioguanine nucleotide (6-TGN) and 6-methylmercaptopurine (6-MMP), were assayed by high-performance liquid chromatography (HPLC), and the AZA dose was adjusted when 6-TGN concentration was known. *Result*. Only twenty-five (41%) children had therapeutic 6-TGN concentrations, ten (16%) subjects had suboptimal 6-TGN concentrations, and twenty-six subjects (43%) had 6-TGN concentrations above the recommended therapeutic range. 6-MMP was not above the therapeutic range in any case. Seven subjects revealed undetectable 6-TGN and 6-MMP levels, indicating nonadherence. The mean AZA dose after the 6-TGN concentration-related adjustment did not differ, in comparison to the initial dose, either in IBD or AIH groups. The mean AZA dose was lower in AIH than in IBD. The subjects with an optimal 6-TGN level presented with a higher ratio of remission (88%) than the under- or overdosed patients (60% and 69%), respectively (Chi – square test = 3.87, $p < 0.05$). *Conclusion*. Timely measurements of thiopurine metabolites can be a useful tool to identify nonadherent patients before a decision is taken to switch to another drug. We may also spot the patients who receive either too low or too high doses, compensating dose deviations in an appropriate way. The patients with optimal 6-TGN levels presented a higher percentage of remission than the under- or overdosed patients. In most patients, both initial and adjusted AZA doses, lower than suggested in guidelines, appeared to be sufficient to maintain remission.

1. Introduction

Immunosuppressants are crucial drugs for the treatment of autoimmune disorders, including autoimmune hepatitis (AIH) and inflammatory bowel diseases (IBD). Azathioprine (AZA) has been proven to be a suitable medication with regard to its efficacy and side-effect profile.

AZA was synthesized in 1957 as a derivative of 6-mercaptopurine (6-MP) but earlier, in 1951, George Herbert Hitchings and Gertrude Elion discovered 6-MP and

thioguanine (TG) as a result of searching for antimetabolites of nucleic acid bases that could arrest cell proliferation [1]. Thiopurines are prodrugs, metabolised by, at least, four different pathways until the final molecules, called thioguanine nucleotides (TGN), are obtained [1].

The metabolism of 6-MP involves three competing pathways: the first one being a degradation to thiouric acid (TUA), which is then excreted, the second one leads through methylation by thiopurine S-methyltransferase (TPMT) into 6-methylmercaptopurine (6-MMP), and the third one involves the breakdown of 6-MP into thioinosine monophosphate (TIMP), catalysed by hypoxanthine phosphoribosyltransferase (HPRT). TIMP is then further metabolised via inosine monophosphate dehydrogenase (IMPDH) into thioguanine triphosphate (TGMP). Kinases convert this into the TGNs [2]. TGNs are the active metabolites which exert immunomodulatory effects, whereas 6-MMP and 6-MMPR are the inactive and potentially toxic metabolites. These processes are presented in Figure 1.

AZA and 6-MP are immunosuppressants with short half-lives (3 and 1.5 hours, respectively) and, therefore, measuring their metabolites is a more appropriate method, both for adherence assessment and therapeutic drug monitoring. An intracellular accumulation of AZA/6-MP metabolites occurs over a period of 2–3 weeks [3]. Various studies have examined the relationship between 6-TGN levels in red blood cells and a clinical response to thiopurine therapy. There is an evidence that 6-TGN levels above 230 pmol/8×10^8 erythrocytes correspond to a good clinical effect [4, 5], however, they do not guarantee remission. On the other hand, a 6-TGN level above 450 pmol/8×10^8 erythrocytes may lead to an increased risk of myelotoxicity [6]. The concentrations of 6-MMP above 5400 pmol/8×10^8 erythrocytes have been related to the development of hepatotoxicity [7]. Thiopurine metabolite measurements become more and more available, although their routine use is still limited by costs and technical requirements at laboratories. The primary aim of this study was to assess the usefulness of monitoring thiopurine metabolites in paediatric patients with IBD and AIH to assess their adherence to therapy and treatment safety. According to our knowledge, evaluating the use of metabolite measurement in children receiving thiopurine treatment was not performed in Polish children population. It is probably that the population from which the sample comes from could influence the results of the study. The secondary aim of the study was to compare AZA doses in both diseases and among the subgroups of patients, stratified according to the disease activity.

2. Material and Methods

2.1. Subjects. Sixty-eight children (thirty-one girls) with IBD and AIH, receiving azathioprine therapy in a consistent dose to maintain remission for at least 3 months, treated in one regional paediatric gastroenterology centre between April 2017 and May 2020, were identified by means of a retrospective review of their medical records. Within that group, there were thirty-six patients with CD, eighteen with UC, and fourteen with AIH. All the IBD children were treated according

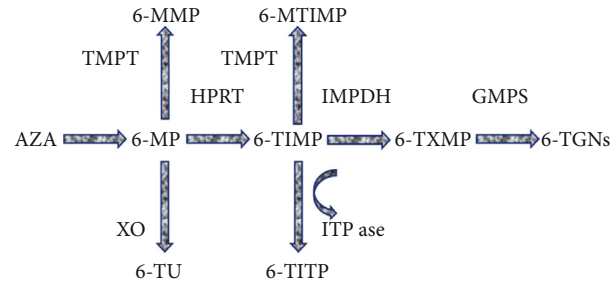

Figure 1: Azathioprine metabolism. AZA: azathioprine; HPRT: hypoxanthine phosphoribosyltransferase; IMPDH: inosine monophosphate dehydrogenase; GMPS: guanosine monophosphate synthase; ITPase: inosine triphosphatase; XO: xanthine oxidase; TPMT: thiopurine S-methyltransferase; 6-MP: 6-mercaptopurine; 6-MMP: 6-methylmercaptopurine; 6-MTIMP: 6-methylthioinosine monophosphate; 6-TXMP: 6-thioxanthylic acid; 6-TGNs: 6 thioguanine nucleotides; 6-TIMP: 6-thioinosine monophosphate; 6-TITP: 6-thioinosine triphosphate; 6-TU: 6-thiouric acid.

to the ECCO (European Crohn's and Colitis Organization) guidelines [8, 9], and the AIH children were treated according to ESPGHAN Hepatology Committee [10]. Seven patients with undetectable 6-TGN and 6-MMP levels were excluded from a detailed analysis concerning the assessment of the mean values of the AZA dose, initial and after correction, 6-TGN, 6-MMP, and a statistical analysis of those variables.

Among the remaining subjects, the following data were collected: demographics, body mass, type of disease, and laboratory data including white blood cell count, haemoglobin, aspartate aminotransferase (AST), alanine transaminase (ALT), and amylase and thiopurine metabolites (6-TGN, 6-MMP). IBD activity was determined, using the respective scales: PUCAI (the Paediatric Ulcerative Colitis Activity Index) and PCDAI (the Paediatric Crohn's Disease Activity Index). Biochemical remission in AIH was defined as a normalisation of transaminase activity and IgG concentration [10]. The mean duration of AZA therapy, before azathioprine metabolites were assayed, was 397 days (the range: 127-1294). The characteristics of the study group are presented in Table 1.

2.2. Methods. Azathioprine metabolite (6-TGN and 6-MMP) levels were determined at an external analytical laboratory. In summary, cells, isolated from venous EDTA blood samples, were first three times washed with an isotonic buffer and then lysed, using the thermal disruption method. Subsequently, the lysates were deproteinised by incubation in acidic conditions and centrifuged for at least 15 min. at >10 000 rcf to remove cellular debris. The cleared lysates were analysed by high-performance liquid chromatography (HPLC) against a reversed-phase (RP) and by detection at 300-350 nm (using a UV-VIS detector). The obtained concentrations were quantified, using the AUC (area under curve) method, comparing the values against a standard curve, obtained with synthetic calibrators of known concentrations. Such raw reads were normalised, based on the RBC (red blood cell count) of each sample. The final results were calculated as pmol/8×10^8

Table 1: Background information about the group receiving azathioprine.

Characteristics	Values
Total number of patients	61
Females (percent)	27 (44%)
Age (years ± SD)	14.97 ± 2.6
Crohn's disease (CD)	31 (50%)
Ulcerative colitis (UC)	16 (26%)
Autoimmune hepatitis (AIH)	14 (23%)
Weight SDS (mean ± SD)	0.13 ± 1.11
CD	−0.23 ± 1.0
UC	−0.39 ± 0.7
AIH	0.53 ± 0.8
Height SDS (mean ± SD)	−0.43 ± 0.8
CD	−0.54 ± 0.9
UC	−0.60 ± 0.7
AIH	0.11 ± 0.7
BMI SDS (mean ± SD)	0.06 ± 0.9
CD	−0.04 ± 0.9
UC	−0.12 ± 0.8
AIH	−0.03 ± 2.7
Premonitoring azathioprine dose mg/kg (mean ± SD)	1.15 ± 0.3
CD	1.22 ± 0.3
UC	1.23 ± 0.3
AIH	0.94 ± 0.3
Postmonitoring azathioprine dose mg/kg (mean ± SD)	1.08 ± 0.4
CD	1.11 ± 0.4
UC	1.26.±0.3
AIH	0.84 ± 0.3
6-TGN (mean ± SD)	494.7 ± 345.3
CD	534.9 ± 371.8
UC	371.0 ± 174.5
AIH	535.6 ± 398.4
6-MMP (mean ± SD)	1288 ± 886.0
CD	1236 ± 816
UC	1505 ± 990
AIH	1175 ± 952
Disease activity:	
Remission/mild form of IBD	32 (68%)
Moderate form of IBD	10 (21%)
Severe form of IBD	5 (11%)
Remission of AIH	14 (100%)

SD: standard deviation; 6-MMP: 6-methylmercaptopurine; 6-TGN: 6-thioguanine; IBD: inflammatory bowel disease; AIH: autoimmunological hepatitis; CD: Crohn's disease; UC: ulcerative colitis.

erythrocytes. Figure 2 presents a typical spectrum, obtained for azathioprine metabolite measurement using HPLC resolved using water to methanol biphasic system.

2.3. Interpretation of the Results. The thresholds of 6-TGN and 6-MMP measurements and the interpretation of obtained results are presented in Table 2. Thus, it was aimed at keeping 6-TGN levels in the range of 230-450 pmol/8×10^8 erythrocytes and 6-MMP below 5700 pmol/8×10^8 erythrocytes.

3. Statistical Analysis

A statistical analysis was performed, using the Statistica software (StatSoft, Tulsa, OK, USA). Descriptive statistics for continuous variables were presented as mean values and standard deviations. The Shapiro-Wilk test was applied to verify the normality of data distribution. For a comparative analysis (comparisons between study groups), the applied statistical tools included the Student's t-test for independent samples or the Mann–Whitney U-test (for data with normal or abnormal distribution). The analysis of variance (ANOVA) with a post hoc least significance difference (LSD) test was used when more than 2 subgroups were compared. The longitudinal comparisons between the measurements, obtained at baseline and during follow-up, were assessed by the t-test for dependent samples or the Wilcoxon signed-rank test, whichever was appropriate, according to the data distribution. A correlation analysis was done by Pearson's or Spearman's correlation tests, whichever was appropriate, according to data distribution. Qualitative features were presented, juxtaposing the number of subjects with the percentage values in the defined subgroups. Comparisons of qualitative feature prevalence rates were performed by the Chi-square test. Significance for results in all the statistical analyses was assumed at $p < 0.05$.

The caregivers of the patients have consented to the use of their medical data in anonymised forms for statistical, educational, and scientific purposes, which is a standard procedure at the hospital. The current data analysis has been approved by the hospital authorities.

4. Results

4.1. Measurements of 6-TG and 6-MMP: An Interpretation of Metabolite Levels in the Group Receiving AZA. The mean 6-TGN values in the whole group, as well as in CD, UC, and AIH patients, were 494.7 ± 343.5, 534.9 − ±371, 8 371.0 ± 174.5, and 535.6 ± 398.4 pmol/8×10^8 erythrocytes, respectively. The mean 6-MMP values in the whole group, as well as in CD, UC, and AIH patients, were 1288 ± 886, 1236 ± 816, 1505 ± 990, and 1175 ± 952 pmol/8×10^8 erythrocytes, respectively (see Figure 3).

Twenty-five (41%) children had therapeutic 6-TGN concentrations with a normal 6-MMP range. Ten (16%) had suboptimal 6-TGN concentrations with a normal 6-MMP range, which indicates that the AZA dose was below the therapeutic level. Twenty-six subjects (38%) had 6-TGN concentrations above the required therapeutic range with a normal 6-MMP concentration, which indicated hypomethylation

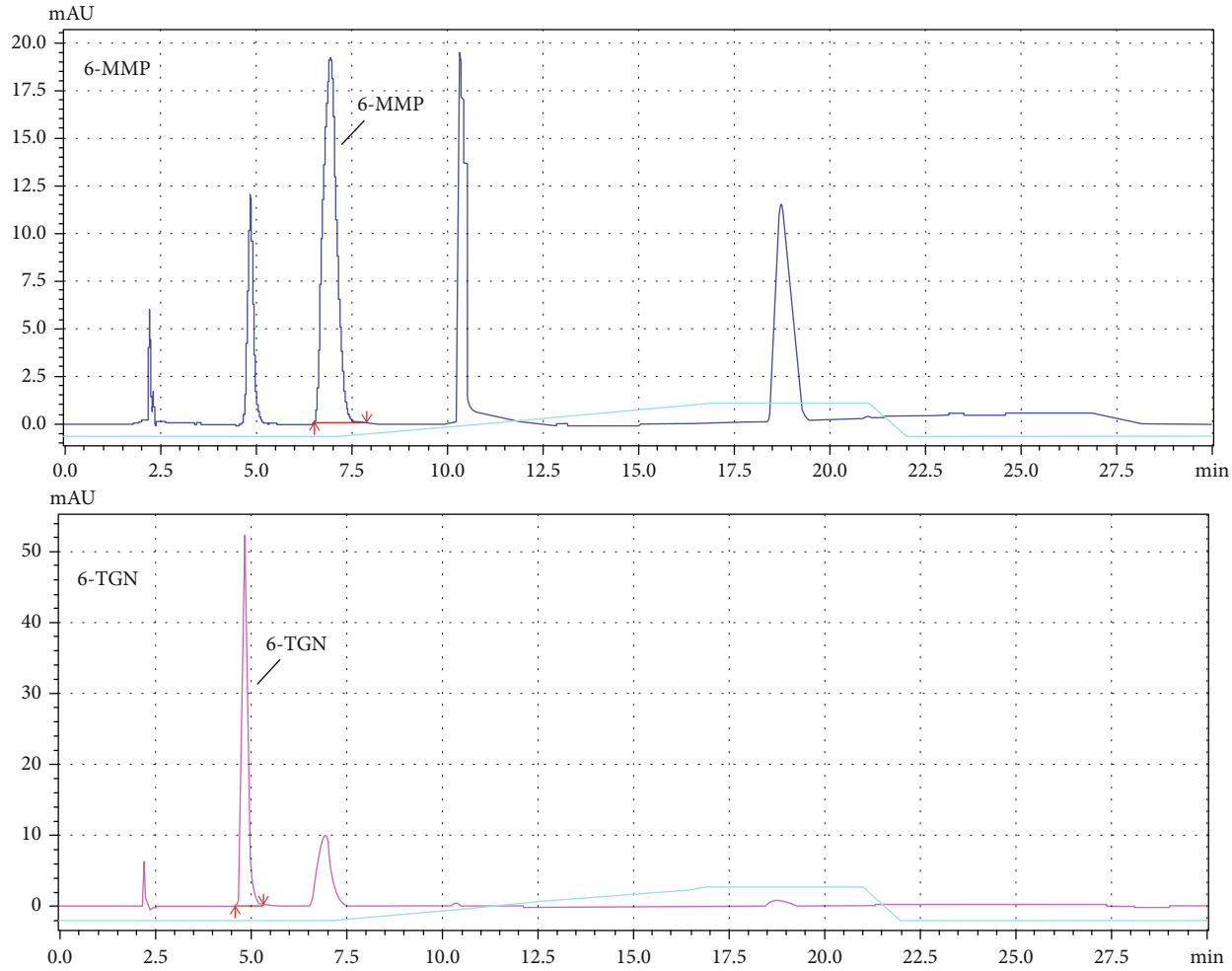

Figure 2: Representative examples of full HPLC spectra acquired for the measurements of thiopurine metabolites, 6-MMP (top) and 6-TGN (bottom). The marked peaks are specific for 6-MMP and 6-TGN, respectively. The remaining peaks do not have an influence on result interpretation.

Table 2: Interpretation of metabolite levels (measured in pmol/8×10^8 erythrocytes) and recommended approaches. TPMT: thiopurine methyltransferase; 6-MMP: 6-methylmercaptopurine; 6-TGN: 6-thioguanine.

6-TGN	6-MMP	Interpretation	Recommendation
Very low	Very low	Nonadherence	Improve adherence
Low (<230)	Normal (<5700)	Insufficient dose	Consider dose increase
Normal (230-450)	Normal (<5700)	Therapeutic optimum	Further monitoring of treatment
High (>450)	High (>5700)	Overdosing	Dose reduction
Low (<230)	High (>5700)	Hypermethylation, risk of hepatotoxicity	Consider changing treatment or adding allopurinol with low doses of AZA
High (>450)	Normal (<5700)	Potential TPMT deficiency, risk of myelotoxicity	Monitoring blood test, consider dose reduction
Normal (230-450)	High (>5700)	Hypomethylation, risk of hepatotoxicity	Monitoring liver enzymes, split or reduce the dose
>1000	Undetectable	Potential TMPT absence, lack of methylation, risk of acute toxicity	Discontinuation of treatment

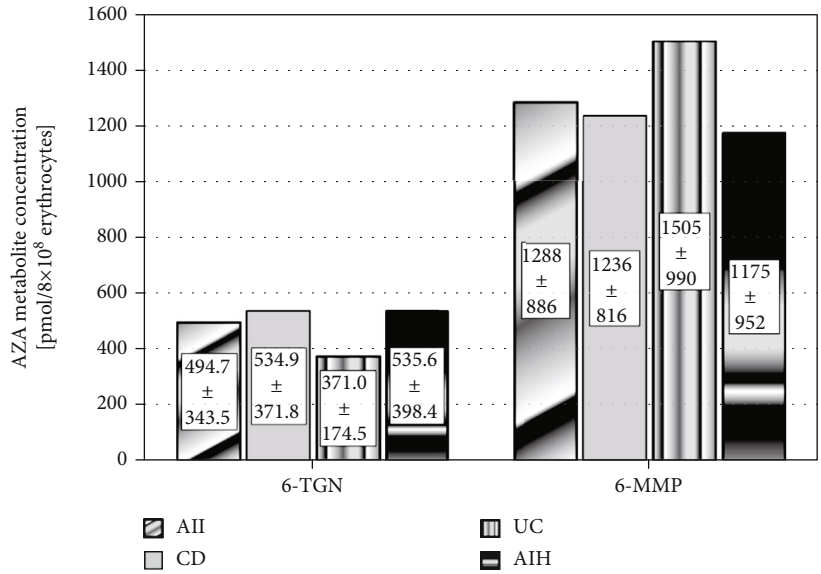

Figure 3: Interpretation of metabolite levels in the group receiving AZA. AZA: azathioprine; CD: Crohn's disease; UC: ulcerative colitis; AIH: autoimmunological hepatitis; 6-MMP: methylmercaptopurine; 6-TGN: 6-thioguanine.

and a potential toxicity for the bone marrow. Seven subjects had undetectable 6-TGN and 6-MMP levels, which indicated nonadherence to the therapy. 6-MMP concentrations above the range, which would indicate potential hepatotoxicity, were not identified in any case (see Table 3).

4.2. The Mean AZA Dose, Initial and after Adjustment

4.2.1. The Difference between Pre- and Postadjustment of AZA Dose, Both in IBD (CD, CU) and AIH Subjects. The presented mean initial AZA dose in the whole study group, as well as in CD, UC, and AIH subgroups, was 1.15 ± 0.35, 1.22 ± 0.37, 1.23 ± 0.32, and 0.94 ± 0.30 (mg/kg/day), respectively. The dose after adjustment, based on 6-TGN concentrations, did not differ significantly from the initial AZA dose, either in the whole study group or in CD, UC, or AIH, and was 1.08 ± 0.44, 1.11 ± 0.4, 1.26 ± 0.32, and 0.84 ± 0.31 (mg/kg/day), respectively (see Figure 4). Neither was there any difference between the girls and the boys nor between the subgroups, defined according to the various methods of treatment or the disease activity, neither at baseline nor at the second assay.

4.2.2. The Difference between IBD (CD, UC) and AIH Subjects, Both for Pre- and Postadjustment AZA Doses. A significant difference was revealed between IBD (CD, UC) and AIH subjects for the pre- and postadjustment AZA doses ($p < 0.05$), namely, the mean AZA dose was lower in AIH than in IBD patients. There was no correlation between the initial AZA dose and 6-TGN levels; after dose adjustment, based on 6-TGN concentrations that correlation could be clearly observed ($R = -0.43$, $p < 0.005$).

4.3. The Effect of Metabolite Measurements on Dose Modification in the Group Enrolled to the Study ($n = 68$). In 7 cases, the level was undetectable and AZA was reintroduced. In 46% (28/61) cases, the dose was not changed. In the other cases (55%), the AZA dose was corrected. In 16% (10/61) cases, the AZA levels were below the range but the dose was increased only in 15% (9/61) subjects because of slightly decreased levels of leucocytes in the remaining patients of the study group. In 42% cases (26/61), 6-TGN-levels were above the range but dose modification was introduced only in 39% (24/61) subjects. In two patients, 6-TGN concentration was slightly above the range; the dose was maintained for the lack of remission (see Figure 5).

The subject with optimal 6-TGN levels presented a higher ratio of remission (88%) than those who were either under- or overdosed (60% and 69%), respectively (Chi − square test = 3.87, $p < 0.05$).

4.4. Adverse Outcomes. One patient (1.6%) developed leucopoenia (<3.5 WBCx $\times 10^9$), while none of the studied subjects developed any elevation of the liver enzymes. No other side effects, such as pancreatitis, glomerulonephritis, or lymphoma, were found in any of the patients throughout the study period.

4.5. A Correlation between 6-TGN Levels and Faecal Calprotectin. No correlation was found between faecal calprotectin and 6-TGN levels.

5. Discussion

Monitoring of thiopurine metabolites is a part of the safe treatment strategy and, together with other actions, such as the pretreatment screening for virus infections, a routine monitoring of leucocytes and aminotransferase, dose splitting strategies, allopurinol supplementation, and testing for TPMT deficiency, it helps reduce the risk of side-effects [11].

5.1. Nonadherence Rate. 6-TGN levels are useful to identify nonadherence to thiopurine therapy, and it has been suggested that routine observations of the metabolites can help

Table 3: Metabolite levels. Mean standard deviations and the range of values of 6-thioguanine (6-TGN), 6-methylmercaptopurine (6-MMPN), measured in pmol/8×10^8 erythrocytes.

6-TGN levels (all subjects)	6-MMPN levels (all subjects)	Interpretation
Mean level: 494 ± 343	1288 ± 886	
Within range 41% (25/61)	Within range	Therapeutic optimum
Below range 16% (10/61)	Within range	Insufficient dose
Above range 43%(26/61)	Within range	Potential TPMT deficiency (potential bone marrow toxicity)
Undetectable 7 subjects	Undetectable	Nonadherence

6-MMP: methylmercaptopurine; 6-TGN: 6-thioguanine; TPMT: thiopurine S-methyltransferase.

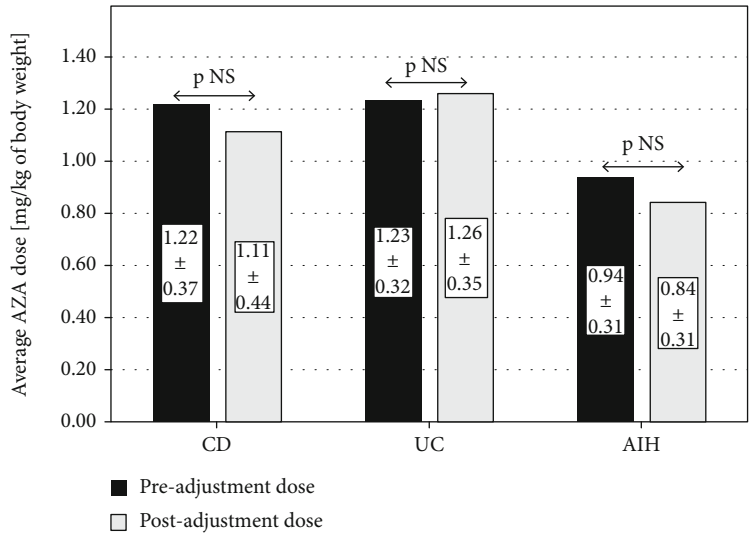

Figure 4: The difference between pre- and postadjustment AZA doses, both in IBD (CD, UC) and AIH subjects. AZA: azathioprine; CD: Crohn's disease; UC: ulcerative colitis; AIH: autoimmunological hepatitis.

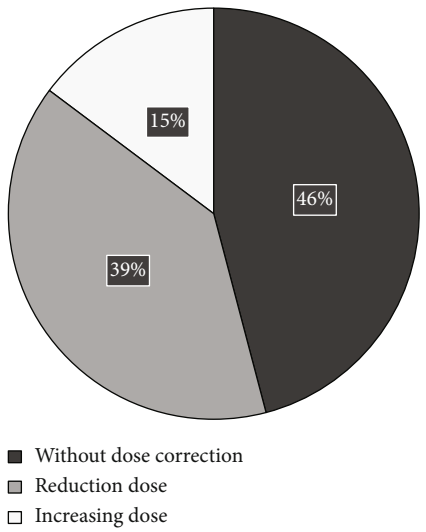

Figure 5: Therapeutic decision based on 6-TG concentrations. 6-TGN: 6-thioguanine.

improve the adherence rates [12]. Several factors, such as sociodemographic, individual, family, disease regimen, and health care system, influence nonadherence. Indeed, in our study, only in 46% of cases, 6-TGN levels were within the therapeutic range and the dose was not changed. In that group of patients, the subjects with optimal 6-TGN levels presented higher a higher remission percent (88%) than those who were either under- or overdosed. That observation applied to all the AIH and IBD subjects. In a large study, where metabolite levels were reviewed in 9187 patients, the therapeutic goal was achieved only in 2444 patients (27%) [13].

In our study, seven subjects had undetectable 6-TGN and 6-MMP levels. A detailed medical history, regarding medicine intake regularity, revealed that those patients had not been taking the prescribed medicines for fear of side effects. In that group, there were five CD and two UC patients. We did not asses the adherence to treatment, using any specific questionnaires, although a multimethod assessment is more widely used [3]. In the future, we plan to assess adherence not only by objective methods but also with a specific questionnaire for both: parents and children. The lack of remission was an indication to consider the reintroduction of

AZA therapy. In a Spanish study, the authors did not find any high rates of nonadherence (6.45%) but they strongly emphasised that the measurements of thiopurine metabolite concentrations could be useful to identify nonresponders before replacing or combining thiopurines with other alternative treatments (generally biological agents), with a consequent increase in both, a potential toxicity and costs [2]. Bokemeyer et al. [14] revealed in their study that, in a group of 65 adult CD patients, six (9.2%) had metabolite profiles that were indicative of nonadherence. The rate of nonadherence is comparable to the values in previously published studies [13, 15]. Hommel et al., evaluating adherence in 42 IBD adolescents, found that the majority of the sample (93%) had demonstrated quantifiable 6-TGN levels but only 14% were within the therapeutic range what indicated that nonadherence assessment was especially important in the group of adolescents, faced with learning to manage a chronic condition and negotiate normal developmental issues [16]. Alsous et al. [3], using a binary logistic regression analysis, identified the age to be independently predictive of adherence, with adolescents more likely to be classified as nonadherent. The mean age of our nonadherent subjects was 15 years. The patients, who are nonadherent, are more likely to have a more severe course of disease, potentially necessitating the need for a more aggressive medical treatment, such as an increased corticosteroid use or surgery, present a higher risk of disease recurrence, in addition to these medical consequences, and, eventually, suffer of poor psychosocial functioning and low quality of life [17].

5.2. Underdosing. The regular measurements of metabolites can also identify patients who receive too low or too high drug dose, with available information about thiopurine methyltransferase (TPMT). In the Caucasian population, 0.3% subjects have TMPT deficiency, 6-11% have moderately reduced levels of TPMT activity, and 89-94% have normal TMPT activity. Tests for TPMT deficiency, prior to the onset of thiopurine therapy, should be the first step in personalising thiopurine therapy; however, cytopenia may still occur, despite normal TPMT activity, which does not identify patients at risk of other toxic or allergic adverse events, either. The latter information may help differentiate patients between those who have received a suboptimal AZA dose and those who have had higher TPMT activity, shifting AZA metabolism towards 6-MMP production. However, the cost and availability significantly reduce the use of the tests in routine practice. For this reason, we did not perform this test before the beginning of treatment at our hospital.

In our study, 10 cases (15%) had 6-TGN levels below the therapeutic range with 6-MMP within the range that indicated underdosing. It could also indicate irregular medication intake, so a detailed medical history is essential. 6-TG below the therapeutic range and 6-MMP above the range could indicate preferential metabolism via the TPMT pathway but it was not observed in our study. Another study showed even a higher percentage of underdosing [46].

It is recommended to keep 6-TGN levels between 250 and 450 pmol to maintain remission in inflammatory bowel disease [8, 9, 18, 19]. In one of the recent studies, it was revealed that serial thiopurine metabolite level assessments and dose adjustment aiming to maintain higher 6-TGN levels could be helpful to improve long-term outcomes in patients with IBD. The median 6-TGN levels were significantly higher in the patients who did not relapse, as compared with the levels in those patients who did relapse (233 vs. 167 pmol per 8×10^8 erythrocytes, $p = 0.025$) [5]. Dubinsky et al. demonstrated that, in paediatric patients, 6 – TGN level $\geq$ 235 pmol per 8×10^8 erythrocytes was associated with a therapeutic response to 6-MP [20]. Wright et al. also revealed that those patients, who developed active disease, accumulated significantly lower 6-TGN concentrations than those who remained in remission (175 vs. 236 pmol per 8×10^8 erythrocytes, respectively). This study shows additionally that, due to intrapatient variability in 6-TGN production and the high incidence of compliance problems, a single 6-TGN reading may not be reflective of drug metabolism and serial measurements could be more useful [15].

All our children, whose 6-TGN level was below the therapeutic range, were IBD patients. They reported a regular intake of AZA, so AZA dose was increased. An ideal therapeutic 6-TGN-level for AIH was not determined [21]. The abovementioned Sheiko study revealed that 87% of 66 children maintained sustained biochemical remission in association with low 6-TGN levels, ranging from 50 to 250 pmol [21]. In a French study [22], the subjects in remission had similar-6-TGN levels (mean 6-TGN 436 pmol) as those with active disease (mean 6-TGN 406 pmol), which demonstrated the lack of correlation between 6-TGN levels and remission induction. After dose modification, follow-up measurements were carried out after three months.

5.3. Overdosing. In 39%, 6-TGN levels were above the range, with 6-MMP levels within the range, which indicated a potential TPMT deficiency and potential bone marrow toxicity. We did not observe 6 – TGN > 1000 pmol per 8×10^8 erythrocytes with undetectable 6-MMP, which could suggest TPMT absence. A high concentration of 6-TGN is associated with an increased occurrence of adverse events. In a study by Lee et al. [23], the occurrence of leucocytopaenia and lymphopenia was associated with high concentrations of 6-TGN. Also, Pavlovska et al. showed similar results [24]. However, we did not observe this correlation in our study; the possibility of serious side effects should be considered in case of high 6-TGN levels. An interpretation of the range as high (>450 pmol per 8×10^8 erythrocytes) depends on clinical features. In cases of active diseases, high 6-TGN levels suggest a thiopurine refractory case, prompting for an alternative treatment [25]. In case of remission or mild disease, dose reduction should be considered. In our study, dose modification was decided in 24 patients (39%), and in 2 patients, 6-TGN concentration was only slightly above the range, so the dose was maintained.

As in some other studies, we did not find any correlation between thiopurine dose and 6-TGN levels; therefore, increasing the drug dose may not be sufficient to reach the desired 6-TGN target [24, 26]. This may be explained by an increased methylation of intermediate 6-MP metabolites by inherited high levels of TPMT activity [5]. Other

explanations refer to changes in azathioprine absorption, depending on disease activity, AZA formulation, or interactions with other drugs, such as mesalazine or and sulphasalazine [27]. On the contrary, Lee et al. found a positive correlation between the dose of AZA and the concentrations of 6-TGN ($p < 0.0001$) [23]. Despite the trend, favouring individualised dosing, other studies show no statistically significant differences in treatment efficacy between individualised dosing, based on baseline TPMT activity, and dosing, subsequently adjusted, according to the 6-TGN concentrations and weight-based AZA dosing [28].

5.4. Mean AZA Dose. In our study, the mean initial AZA dose and the dose after adjustment, based on 6-TGN concentrations, were lower than those, proposed in ECCO and ESPGHAN guidelines, both in IBD and AIH patients.

In AIH patients, the initial AZA dose was 0.5 mg/kg/day and then it was increased up to a maximum of 2.0-2.5 mg/kg/day. Our observation was similar to that in another study. Sheiko et al. [21] revealed that AZA dose of approximately 1.2–1.6 mg/kg/day was sufficient to maintain biochemical remission in the majority of patients. There was no correlation between AZA dose and 6-TGN levels, which was a similar conclusion to that in our study. The AZA dose in AIH was significantly lower, not only than proposed by ESPGHAN but also than that in IBD subjects. Those observations are coherent with Sheiko observation [22].

In inflammatory bowel diseases (CD and UC), the recommended dose is 2.0–2.5 mg/kg, and for its prodrug, 6-mercaptopurine, 1.0–1.5 mg/kg once daily. Our study revealed that the mean initial AZA dose in Crohn's disease and after modification was also lower than recommended. Neither was there any difference between the initial AZA dose and the dose after adjustment, based on 6-TGN concentrations, most likely for low AZA dose at baseline. The decision about the starting dose was made individually by a gastroenterologist. Various practical approaches among practitioners included thiopurine dosage, decisions about continuing thiopurines, and timing of metabolite assays. The other reason for lower AZA doses was the fact that, according to the previous studies, a lower dose of azathioprine is effective to induce and maintain remission in active Crohn's disease. Qian et al., in a prospective observational study, revealed that azathioprine,1.5 mg/kg/d, combined with steroids was as effective as AZA 2.0 mg/kg/d to induce remission of active CD in the first 6 months and to maintain remission of inactive CD in the first 2 years, without higher recurrence rate of active CD [29]. Another Chinese study confirmed that observation [30].

The mean AZA dose in CU patients, both at the beginning and after modification, was also lower than recommended (0.97, 0.87, and 2.0–2.5 mg/kg/day, respectively). The mean 6-TGN concentration was within the range (349, 47, 11 pmol/8 × 108 erythrocytes). Hibi et al. revealed the same observation in adults [31]. Walker et al. [26] also showed that, in the paediatric IBD children, the AZA dose, sufficient to maintain remission, was 1.3 ± 0.4 mg/kg; after excluding children on biologics, the effective azathioprine dose was 1.4 ± 0.5 mg/kg. Apart from receiving low AZA doses, 75% of IBD patients in our study were in remission. Those results suggest that therapeutic thiopurine metabolites can be achieved with a dose lower than recommended, although, since TMPT activity was not determined at the beginning of treatment, we could not know then that lower AZA doses could be sufficient to maintain remission.

5.5. Side Effects. Myelotoxicity is one of the most serious thiopurine-induced side effects and may occur at any time during the treatment. It is strongly linked to low TPMT enzyme activity and high 6-TGN blood levels. Myelotoxicity may also occur with normal TPMT activity, necessitating regular full blood count monitoring in clinical practice. In a review of 66 studies, including more than 8,000 thiopurine-treated patients, the incidence rate of drug-induced myelotoxicity was 3% per patient year of treatment [32]. In our study, only one patient (1.6%) developed leukopenia (<3.5 WBCx $\times 10^9$), but it was not related to high 6-TG levels. None of the children developed azathioprine toxicity, as defined by abnormal liver function tests. That observation was similar to that in another study [26]. On the contrary, Pavlovska et al. reported a higher percentage of adverse effects [24]. The most common treatment-related complication was leucocytopaenia (42.9%), followed by elevated transaminase levels (28.6%), aphthous ulcers (14.3%), and elevated amylase in serum (14.3%).

We did not find any correlation between faecal calprotectin (FC) and 6 T-GN levels. On the contrary, another study showed that, in patients with CD on AZA monotherapy, 6-TGN concentrations within a defined range (250–450 pmol/8 $\times 10^8$ erythrocytes) were associated with significantly lower FC [33].

We acknowledge the limitations of this study, including the lack of TPMT activity tests, justified by cost and availability issues, the sample size, and the retrospective design.

Measurements of 6-TGN and 6-MMP levels in IBD and AIH patients on AZA/6-MP may help identify patients at risk for toxicity and provide an explanation for the ineffectiveness of the treatment, observed in some patients. Thiopurine metabolite measurements become more and more available, although their routine use is still limited by costs and laboratory gear.

In conclusion, timely measurements of thiopurine metabolites can be a useful tool for the identification of non-adherent patients before adding or switching to another drug. This method can also identify patients receiving too low or too high doses, enabling subsequent corrections of drug doses, as the patients with optimal 6-TGN levels presented a higher percentage of remission than those who were under- or overdosed.

Data Availability

Data are available on request through the authors themselves. Contact: bak-drabik@wp.pl

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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