PEDIATRIC INFLAMMATORY BOWEL DISEASES: LOOKING TO THE FUTURE

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PEDIATRIC INFLAMMATORY BOWEL DISEASES: LOOKING TO THE FUTURE

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EDITORIAL
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Editorial: Pediatric Inflammatory Bowel Diseases: Looking to the Future

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Editorial on the Research Topic

Pediatric Inflammatory Bowel Diseases: Looking to the Future

The incidence of inflammatory bowel diseases (IBD) is increasing across the globe (1), especially in children (2). This highlights not only the clinical importance of characterizing pediatric IBD (P-IBD) but also provides an opportunity to improve understanding of pathogenesis and patient outcomes. In this editorial, 12 manuscripts are reviewed, all published in the *Research Topic: Pediatric Inflammatory Bowel Diseases: Looking to the Future*. These papers span a diverse array of topics related to P-IBD, from rare complications, through evolving assessment tools and clinical features, to novel pathways, possibly leading to future treatments. These publications focus on features of IBD unique to children, and will serve as a resource for anyone interested in P-IBD.

Clinical expertise incorporates vigilance for recognizing unusual clinical patterns and pursuing appropriate differential diagnoses in such circumstances. Lawrence et al. reported on a 12-year-old girl with Crohn disease who presented with a short history of progressive respiratory distress and hypoxemia, requiring intensive respiratory support. Cardinal factors in this patient's case included profound lymphocytopenia, thiopurine immunosuppression, recent corticosteroid exposure, chest radiographic findings, and a sputum sample for direct microbial testing. Following a diagnosis of *Pneumocystis jirovecii* pneumonia (PJP), targeted therapy was successful. Current pediatric guidelines recommend considering PJP prophylaxis for patients on triple immunosuppression that includes corticosteroid therapy (3).

Collins et al. described a teenager with relapsing Crohn disease whose weight had fallen from the 25th to the 3rd percentile. Weight loss continued in hospital while on full exclusive enteral nutrition (EEN) of 2,400 kcal/day. Weight gain followed a period of 24-h nursing observation. Psychiatric evaluation led to a diagnosis of anorexia nervosa. The sentinel clinical feature was weight loss despite apparently high calorie intake. Anorexia nervosa, rather than Crohn disease, is more often considered as the potential cause of weight loss in young adults. The coexistence of both diagnoses may be associated with poor outcomes (4).

Acute neurological complications in children with IBD are rare, but should prompt consideration of intracranial vascular thrombosis, especially in the setting of active disease and thrombosis risk factors. Martín-Masot et al. describe a 5-year-old patient with refractory ulcerative colitis (UC), who developed a focal headache, upper limb paresis, and seizures within 4 days of

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colectomy and J-pouch creation. Potential risk factors in this case included active preoperative disease resulting in reduced mobility, recent major abdomino-pelvic surgery, and central venous catheter placement. The patient had not been on thromboprophylaxis therapy during the perioperative period. A recent Pediatric clinical guideline recommends thromboprophylaxis in high risk patients, following individual risk assessment (3).

Disease aspects unique to P-IBD are discussed in three additional papers included in this *Research Topic*. Acute severe UC (ASUC) is a medical emergency. Pediatric-onset UC is usually more extensive and follows a more progressive course than in adults (5). Since disease severity has consistently been associated with disease extent, children are especially susceptible to severe refractory flares. With few exceptions, children with ASUC should be admitted to the hospital for intensive medical treatment. Akintimehin et al. published the results of the first population-based study of ASUC in Irish children. In this retrospective study of 55 children >41% required colectomy. Colectomy rates were higher in the pre-infliximab period (88 vs. 36%). The authors did not identify predictive factors of response to infliximab.

Endoscopy is the cornerstone in assessing mucosal healing in IBD. However, this is a relatively invasive and costly procedure. This has led to the search for non-invasive and less expensive markers of intestinal inflammation. In a single center, retrospective cohort study, El-Matary et al. examined the impact of measuring fecal calprotectin (FCAL), a stool marker for intestinal inflammation, on decision-making and clinical care of children with IBD. The majority (86%) of positive FCAL measurements ($\geq$250 μg/g of stools) resulted in treatment adjustments with subsequent clinical improvement. Conversely, 83% of those with normal FCAL remained in clinical remission over the following months without any changes in medical management. This study was limited by its retrospective design, small sample size, and limited data correlating mucosal healing with FCAL. In their commentary article, Day and Adamji highlighted the benefit of non-invasive stool markers in monitoring IBD. They proposed that serial FCAL measurements may be a more effective strategy to inform individual patient care than a one-off level.

Extraintestinal manifestations (EIM) occur in ∼1/3 of P-IBD cases. Pancreatic disease is a less frequently observed EIM, potentially manifesting in multiple ways, as highlighted by Martin-de-Carpi et al. Pancreatic involvement can present as acute pancreatitis, chronic pancreatitis, autoimmune pancreatitis, asymptomatic exocrine pancreatic insufficiency, increased pancreatic enzyme levels, structural abnormalities, and granulomatous inflammation, as well as therapy-associated pancreatitis. Clinicians caring for children with IBD should be familiar with these conditions, diagnostic considerations, and treatment options.

In a separate article, Day and Brown present their experience with using EEN, as an adjunctive therapy, in two cases with Crohn disease complicated by phlegmon to avoid resection, at least in the short term. EEN is recognized as an effective induction agent for active luminal inflammatory disease (6), but in this situation its use in complicated Crohn disease may have prevented the need for bowel resection.

This last section focuses on emerging concepts and potential pathways for future therapies in P-IBD. Multiple factors have been related to the pathogenesis of IBD, including genetic mutations, intestinal immune dysfunction, impaired intestinal microbiota, dietary factors, antibiotics, infections, type of delivery, appendectomy, urban environment, smoking, sleep disorders, and stress. Sun et al. reviewed the role of stress in IBD, explaining in detail the mechanisms by which stress affects IBD in relation to homeostasis and gut motility, intestinal microbiota, and immune and neuroendocrine dysfunction. Stress exerts a deleterious effect at different stages of IBD (onset, disease course, and prognosis). This review highlights that mechanisms related to stress should be considered as a strategy both in prevention and in the treatment of IBD.

Surgical resection is often indicated for complicated fibrostenotic and/or internally penetrating Crohn disease. Recent efforts have focused on more completely controlling inflammation to prevent disease progression and hopefully decrease the need for surgery. As Stenke et al. discuss, while no antifibrotic therapies are currently available for Crohn disease, recent research opens the possibility that targeting the profibrotic transforming growth factor-B (TGF-B) may lead to effective antifibrotic therapies in the future. This may lead to potential therapeutic options to mitigate the effects of fibrosis and reduce the need for surgical intervention.

Another key pathway, well-recognized in the pathogenesis of IBD, involves nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a nuclear transcription factor known to activate pro-inflammatory pathways. Novel approaches to regulate NF-κB and its downstream consequences are reviewed by Zaidi and Wine; these relate to recent discovery of pediatric-specific changes in A20, which is a key regulator of NF-κB, as shown in samples from children with IBD (7). The authors propose that instead of suppressing inflammation, efforts should be made to regulate key pro-inflammatory pathways, thus preventing uncontrolled inflammation to begin with.

Finally, given the recent attention to food and food additives in the pathogenesis of IBD (8), the unique role of carrageenan and carboxymethylcellulose is reviewed by Martino et al. Both chemicals are commonly-used emulsifiers and food stabilizers. Much of the evidence on the effects of these additives on gut health, and how this might relate to IBD, is based on animal studies, but the relevance of these mechanisms to human IBD is reflected in the utility of new nutritional approaches, such as the Crohn-disease exclusion diet (CDED) (9).

Together, this collection of reports presents recent advances in our understanding of P-IBD and opportunities for improving care through further research and innovation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Impact of Fecal Calprotectin Measurement on Decision-making in Children with Inflammatory Bowel Disease

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Background: The use of fecal calprotectin (FCal) as a marker of intestinal inflammation, in the management of inflammatory bowel disease (IBD) is increasing. The aim of this study was to examine the impact of FCal measurements on decision-making and clinical care of children with IBD.

Materials and methods: In a retrospective cohort study, FCal, clinical activity indices, and blood markers were measured in children with established diagnoses of IBD. Pearson correlation coefficient analysis was performed to examine association between FCal and other markers. Decisions based on FCal measurements were prospectively documented and participants were evaluated 3–6 months later.

Results: A total of 115 fecal samples were collected from 77 children with IBD [median age 14, interquartile range (IQR) 11–15.6 years, 42 females, 37 with Crohn's disease]. FCal positively correlated with clinical activity indices ($r = 0.481$, $P < 0.05$) and erythrocyte sedimentation rate ($r = 0.40$, $P < 0.05$) and negatively correlated with hemoglobin ($r = -0.40$, $P < 0.05$). Sixty four out of 74 (86%) positive FCal measurements (≥250 μg/g of stools) resulted in treatment escalation with subsequent significant clinical improvement while in the FCal negative group, 34 out of 41 (83%) measurements resulted in no change in treatment and were associated with remission on follow-up.

Conclusion: Based on high FCal, the majority of children had treatment escalation that resulted in clinical improvement. FCal measurements were useful and reliable in decision-making and clinical care of children with IBD.

Keywords: calprotectin, children, colitis, Crohn, IBD

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INTRODUCTION

Stool markers such as fecal calprotectin (FCal) have emerged as new diagnostic tools to help in the diagnosis of intestinal inflammation (1). Fecal inflammatory markers include a biologically heterogeneous group of substances that either leak from or are actively released by the inflamed mucosa. Calprotectin is a small calcium binding protein consisting of two heavy and one light

Abbreviations: FCal, fecal calprotectin; IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis.

polypeptide chains (a heterodimer of two S100 proteins) (1). It is found in neutrophilic granulocytes, accounting for 60% of their cytosolic fraction, and in monocytes and macrophages (1–3). It has a homogeneous distribution in feces. In active inflammatory bowel disease (IBD), an increased migration of inflammatory cells including neutrophils to the inflamed intestinal mucosa has been observed. Due to leukocyte shedding in the intestinal lumen, pro-inflammatory proteins such as calprotectin can be detected and measured in the stools. FCal demonstrates good sensitivity and specificity as a marker for mucosal inflammation (1–4).

Inflammatory bowel disease is a chronic disease characterized by remissions and relapses and encompasses two related but distinct disorders of as yet unknown etiology: Crohn's disease (CD) and ulcerative colitis (UC) (5). Both adult and pediatric investigators have recognized the need to optimize and standardize methodology for assessment of disease activity. In children, active disease can have devastating consequences on growth and development. Consequently, it is prudent that disease remission is induced and maintained (6). The goal for IBD therapy is increasingly becoming mucosal healing. Therefore, the need for non-invasive, reliable, and relatively non-expensive biomarkers for IBD disease activity is growing (7).

Fecal calprotectin concentrations may correlate well with both endoscopic and histological IBD activity, which have been traditionally used to evaluate mucosal healing and response to treatment (8–10). However, the impacts of FCal measurement on decision-making and clinical outcomes in children with IBD are currently under-reported. The aim of this study was to examine the impact of FCal measurements on decision-making and clinical care of children with IBD in usual clinical care.

MATERIALS AND METHODS

Study Population

In a single centre cohort study, consecutive children with established diagnoses of IBD assessed between November 2013 and December 2015 in the Pediatric IBD Outpatient Clinic at the Children's Hospital, Winnipeg, MB, Canada, who had developed new symptoms that might had suggested a disease relapse were asked to bring in a stool sample for FCal measurement. Inclusion criteria included all children (<18 years) with confirmed diagnosis of IBD following the North America Society of Pediatric Gastroenterology, Hepatology and Nutrition diagnostic criteria for IBD (11).

Covariate Data

Patients' demographics and disease characteristics and duration were collected from patients' medical records. The most significant symptom or sign as an indication for requesting FCal measurement was prospectively documented for each collected sample.

Fecal Calprotectin

Fecal samples were collected at home and processed at the laboratory of the University of Manitoba IBD Clinical and Research Centre. FCal measurement was performed using the Quantum Blue® Lateral Flow Reader within 24 h of stool collection. FCal ≥ 250 μg/g of stools was considered a positive test, indicative of active IBD. The upper limit of test was >1,800 μg/g of stools.

Clinical Activity Indices

Clinical activity indices [pediatric UC activity index (PUCAI) for UC and pediatric Crohn's activity index (PCDAI) for CD] (12, 13) and the impact of FCal testing on the management of IBD—investigations or changes in therapy—were assessed. Physician global assessment (PGA) for disease activity, whether it is quiescent, mild, moderate, or severe, was also documented (14).

Other Laboratory Markers

Included hemoglobin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and serum albumin performed within 2 weeks of stool collection for FCal.

When patients were asked to bring stool samples, pediatric gastroenterologists were asked to complete a questionnaire. Other investigations such as hemoglobin, serum albumin, and CRP were known to physicians who made no changes in treatment at that time point. All patients with diarrhea had their stools tested for infection screen and only those with negative infection screen had stools for FCal measurements. The clinicians were asked to decide if they would do any additional investigations (mainly colonoscopy) or any changes in therapy, based only on the FCal results. Clinicians were given specific options to choose from for treatment escalation in case FCal comes back as positive. Once the FCal results were known, any investigations and treatment changes were documented and patients were followed up for 3–6 months. Clinical activity indices were measured in the follow-up visits.

If colonoscopy was performed following FCal results, colonoscopy findings and histopathology of collected mucosal biopsy samples performed within 1 month of collecting stool samples were examined. Presence of erythema, loss of vascularity, friability, and/or ulcerations were included in the definition of active disease on endoscopy (active vs. inactive).

Statistical Analysis

Calculations and data analysis were performed using Statistical Package for the Social Sciences (SPSS, IBM Corp., 2013, IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA). Descriptive measures [medians, interquartile range (IQR), means, ranges, and SD] were calculated for continuous variables (such as age and clinical disease activity indices), while frequencies were calculated for categorical variables (such as PGA), along with 95% confidence intervals (CIs) for the means and proportions. Variables were examined for normal distribution. Student's *t*-test was used to compare means. Categorical variable comparisons were performed using Fisher's exact test. Pearson correlation coefficient analysis was performed to examine any possible association between FCal values and clinical disease activity indices, ESR, CRP, serum albumin, hemoglobin (all continuous variables), PGA (as a categorical variable; quiescent, mild, moderate, and severe), and endoscopic activity (as a dichotomous variable; active vs. inactive). A P value < 0.05 was considered significant.

Ethical Considerations

The study protocol was approved by the University of Manitoba Health Research Ethics Committee. As the study was retrospective, individual consents from participants were not needed.

RESULTS

Over the study period, a total of 115 fecal samples were collected from 77 children with IBD (median age at FCal measurements 14, IQR 11–15.6 years, 42 females, 37 with CD), who were followed up for a median duration of 1.33 (IQR 0.5–3.1) years (mean 2.62 ± 1.84 years). Participants' demographics and disease distribution are summarized in **Table 1**. Base line medications were summarized in **Table 2**.

The most significant symptom or sign such as diarrhea, weight loss, and pallor as an indication for FCal measurement is listed in **Table 3**.

Seventy four (64%) stool samples were positive (≥250 μg/g) and 12 participants had colonoscopy within 4 weeks of FCal measurement as compared with two patients in the group with normal FCal measurements ($P < 0.001$).

Association between FCal and Study Co-Variables

For the whole cohort, 74 (66%) out of 115 fecal samples had high FCal > 250 μg/g of stools. In 33 (44.6%) out 74 positive fecal samples, clinical activity indices were ≥10. On the other hand, 41 (34%) out 115 fecal samples had normal FCal level and only 2 (5%) out of 41 samples had clinical activity indices ≥10. Both patients were scoped and colonoscopy was normal.

Fecal calprotectin measurements had fair correlation with clinical disease activity indices ($r = 0.483$, $P < 0.05$), PGA ($r = 0.40$, $P < 0.05$), ESR ($r = 0.40$, $P < 0.05$), low hemoglobin ($r = -0.40$, $P < 0.05$), with less correlation with low serum albumin ($r = -0.3$, $P < 0.05$) but no correlation with CRP ($r = 0.1$, $P = 0.3$).

When those with CD were compared to the UC/IBD-U group, the correlation of FCal with clinical disease activity indices was stronger for UC compared to CD. High FCal levels correlated with PUCAI ($r = 0.80$, $P < 0.01$) compared to PCDAI ($r = 0.2$, $P = 0.09$).

Correlation was limited ($r = 0.256$, $P = 0.006$) with endoscopic activity as only 14 children had colonoscopy within 4 weeks of FCal measurement.

Impact on Management

Only 14 (12%) out of 115 samples resulted in colonoscopy as guided by physicians' discretion (likely when physicians felt that FCal value was equivocal between 100 and 250); 2 in FCal negative and 12 in the FCal positive group, i.e., for the rest of the cohort [101 (88%) samples], decisions on treatment (escalation or no treatment) were based solely on FCal measurements. Sixty four out of 74 (86%) samples with positive FCal measurements were associated with treatment escalation that resulted in improvements in clinical activity indices (**Table 4**), while in the FCal negative group, 34 out of 41 (83%) measurements were associated with no change in treatment.

Figure 1 summarizes the impact of FCal measurements on the study cohort over time. In the first follow-up visit 3–6 months following changes in treatment in the FCal positive group, of those measurements that resulted in treatment escalation, 80%

TABLE 1 | Demographic characteristics and disease phenotype in participants.

Number	77
Gender	
Male	35
Female	42
Diagnosis	
Ulcerative colitis (UC)	37
Crohn's disease (CD)	38
IBD-U	2
Age	
Median	14
Mean	13.08
Range	3.16–17.25
UC phenotype	
E1	3
E2	5
E3	16
E4	15
CD phenotype	
Location	
Small bowel	7
Colon	8
SB-Colon	23
Upper GI[a]	19
Perianal	12
Behavior	
Inflammatory	29
Stricturing	7
Fistulizing	2

[a]*Upper GI involvement in combination with other phenotypes.*

TABLE 2 | Base line medications of all participants.

Medication	Number[a]
5-ASA	55
Azathioprine	38
Methotrexate	11
Infliximab	31
Adalimumab	9
Enteral nutrition	3
Steroids	11
Antibiotics	1

[a]*Several patients were on more than one medication.*

TABLE 3 | Indications (most significant symptom/sample) for fecal calprotectin measurements in 77 children with inflammatory bowel disease.

Abdominal pain	38
Bleeding per rectum	24
Diarrhea	22
Weight loss	15
Fatigue	8
Others (e.g., constipation, abnormal labs)	8
Total	115

ultimately achieved clinical remission while in the FCal negative group, 32 out 34 (94%) of those who did not have any treatment changes (including 2 patients with clinical activity indices >10), were in clinical remission at the follow-up visit.

DISCUSSION

Fecal calprotectin is a surrogate marker for mucosal inflammation and has been proven as a very beneficial, non-invasive, and relatively an inexpensive test in the work-up of IBD with a considerable high sensitivity (15, 16). Although high FCal can be found in other gastrointestinal disorders, in addition to IBD, such as gastrointestinal infections and polyposis, proper use of this test may reduce the need for frequent colonoscopies (15).

Our study showed a fair positive correlation of high FCal with increased clinical disease activity, a finding that is concordant with other adult and pediatric studies (9, 17–19). It is noteworthy that 55% of persons with increased FCal were in clinical remission by a symptom based activity index. While this may lead some to consider that this suggests that either the FCal is non-specific, or that a substantial proportion of children with IBD in remission have active inflammatory disease. The majority of our subjects with elevated FCal had treatment escalation (even if disease activity index suggested remission). In pediatric IBD practice, our study is unique in that we relied on FCal to direct clinical management and then followed up to determine if our FCal-directed management led to satisfactory clinical outcomes several months later.

Bunn et al. examined FCal in spot fecal samples from 37 children with IBD and 31 control children. FCal was higher in 16 children with UC and in 21 children with CD (18). Similar to our study, FCal concentration correlated negatively with serum albumin concentration (18). In another study from Italy, FCal showed a high correlation with the histologic grade of mucosal inflammation, similar to that observed for endoscopy in 58 children with IBD (19).

In our study, FCal, did not correlate that well with CRP, a finding which was also highlighted in other studies (20, 21). Schoepfer and colleagues concluded that FCal correlated better with endoscopic scoring than that with CRP (20, 21). Correlations with CRP, however, were $r = 0.53$ and 0.55 in 140 ileocolonic CD and 228 UC adult participants, respectively (18, 19). In our study, the assessment of association between high FCal and endoscopic disease activity was limited by the small number of patients who had colonoscopy and the non-validated categorical classification of endoscopic activity into two subcategories (active and inactive disease).

Our study showed a better correlation of FCal with PUCAI compared to PCDAI. FCal has been previously also found to be a stronger marker for UC compared to CD (22).

Monitoring disease activity in patients with IBD is crucial as mucosal healing has become now a new target for treatment. It has been established that calprotectin concentrations correlate well with endoscopic and histological IBD activity (8, 9). However, data on the impact of FCal directing management in an IBD population, especially in children, are sparce. In our study, 86% of positive samples resulted in escalation of treatment with subsequent improvement in the majority of participants and 88% of negative samples resulted in no change of treatment and sustained clinical remission in the majority of participants in subsequent follow-up.

In a recent study from The Netherlands, clinical activity indices, FCal, and CRP were measured in 62 teenagers with IBD (31 with CD and 31 with UC), who were asked to evaluate their disease control (100% was the best disease control) and their disease control, according to participants' opinion, was above 90% in two successive outpatient clinic visits. Unlike CRP, positive FCal was predictive of a clinical relapse in the subsequent visit in 60% of participants (23). In another prospective multicentre cohort study, stool samples from 101 children who were admitted with severe UC were obtained for measuring four different stool markers for inflammation including FCal. Repeat samples at discharge were obtained from 24 children. Although all markers were initially significantly elevated, FCal did not correlate that well with PUCAI which performed better than the fecal markers in predicting outcome following a course of intravenous corticosteroids. However, perhaps it was too soon to expect a significant drop in FCal following the short course of intravenous corticosteroids (24).

Eccles et al. collected data on the impact that FCal testing from 119 clinic participants including 29 with IBD. FCal testing helped in assessment of disease activity and decision-making in 25 adults with known IBD; 21 had major changes in their treatment while 4 were felt to be in remission on biological therapy (25).

TABLE 4 | Changes in treatment based on 74 fecal samples with abnormally high fecal calprotectin.

Change in medications	Change in medications (%)	Mean disease activity index before the change	Mean disease activity index after the change	*P* value, 95% confidence interval
Starting/switching a biologic	18 (24.3%)	20 ± 15.86	5 ± 2.04	$P < 0.05$, 6.81–14.43
Increase the dose/reduce time interval of a biologic	15 (20.3%)	16.6 ± 9.14	12.03 ± 8.37	N.S, −2.28 to 12.28
Increase dose of the immunomodulator	15 (20.3%)	13.8 ± 7.61	8.63 ± 6.3	N.S, −0.67 to 13.4
Adding an immunomodulator	5 (6.6%)	23 ± 8.3	6.25 ± 4.33	$P < 0.05$, 3.25–26.71
Adding steroids	7 (9.4%)	17.14 ± 9.49	13.12 ± 9.43	N.S, −18.14 to 11.84
Increase/add 5-ASA	7 (9.4%)	15.63 ± 10.48	7.5 ± 6.123	N.S, −6.17 to 22.44
No change of treatment	10 (2 declined change) (13.5%)	10.83 ± 6.05	5.7 ± 4.3	N.S, −5.8 to 18.4
Total	67[a]	12.83 ± 12.68	5.25 ± 7.15	$P < 0.05$, 4.48–11.34

[a]*Sixty-four changes in treatment (three samples resulted in more than one change in medication).*

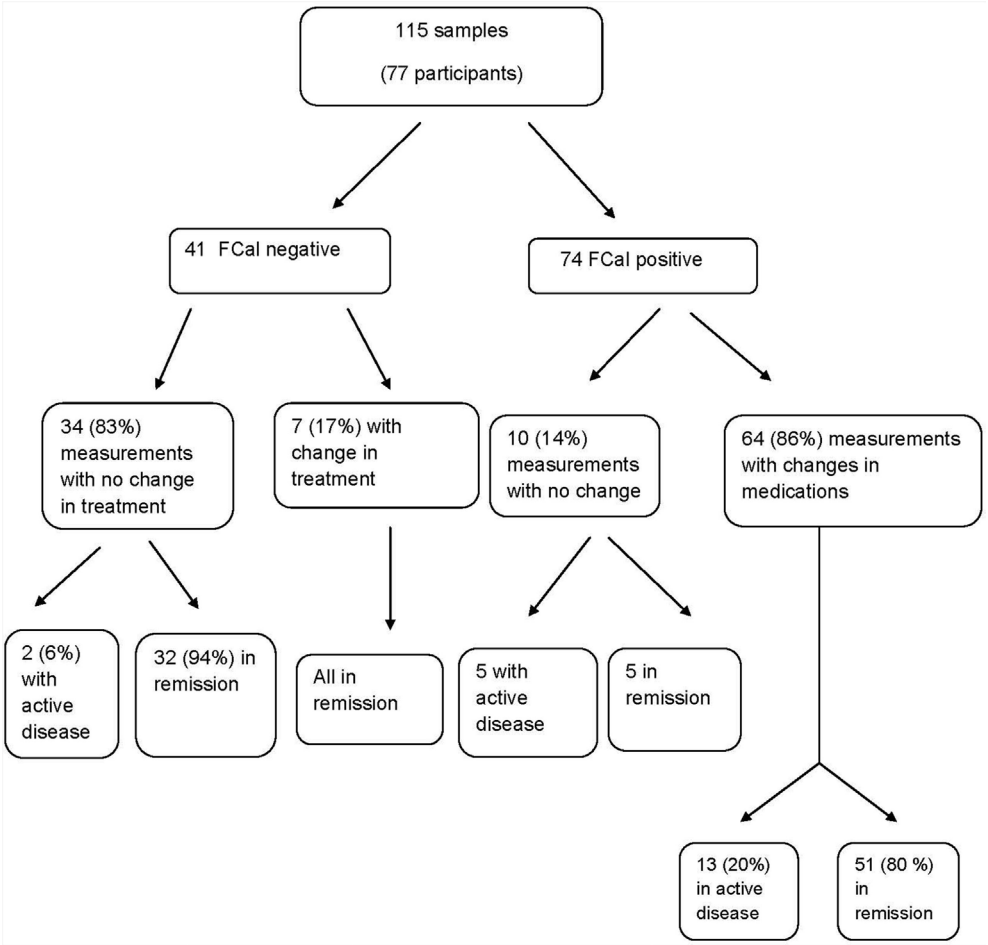

FIGURE 1 | **Flow chart summarizing the impact of fecal calprotectin measurements on the decision-making process among children with inflammatory bowel disease**.

In four pediatric participants with known IBD, one had a normal FCal level and was felt to be in remission but three had elevated levels that resulted in major changes in management. Similar to our study, the investigators concluded that FCal can serve as a marker for disease activity in known IBD and help in the clinical decision-making process (25).

In an open label randomized controlled study, Osterman et al. observed significantly dropping FCal levels in adults with quiescent UC after escalating their mesalamine dose (26). In another randomized study, Lasson et al. used FCal measurements to identify persons with UC who were at risk of relapse and escalated their therapy (27). Both studies, however, were experimental and not reflective of routine real-life clinical practice.

The limitations of our study include the retrospective design and small sample size especially for those who had colonoscopy. However, based on previous research, FCal is known to strongly correlate with endoscopic disease activity (9, 17) and we assumed that it did. Hence, the escalation of treatment in most participants with elevated FCal levels regardless of whether a colonoscopy was performed or not. The study main aim was how FCal measurements, as the major decisive factor, would impact on disease management. A positive FCal most often triggered a clinical response; especially a change in therapy. As we did not do colonoscopy in all subjects we cannot discern how many false negative FCal measurements were represented within our sample. Nonetheless, our results did show that the faith clinicians placed in the FCal result was rewarded with good clinical outcomes. Another limitation was a lack of measurements of FCal in the follow-up visits after implementing changes in therapy but the study outcome was intended to be clinical activity indices. Nonetheless, the study adds significantly to the current literature as little is known about the impact and outcome of FCal measurements in pediatric IBD clinical practice.

CONCLUSION

In children with known IBD, elevated levels of FCal were more likely to be associated with elevated scores of clinical activity indices, high ESR, and low hemoglobin. However, many children with elevated FCal had normal clinical disease activity indices.

Regardless of disease activity index at the time of FCal, based on abnormal measurements of FCal, the majority of children had their treatment escalated which resulted in significant clinical improvement in those with elevated disease activity index or sustained remission in those in whom the index was considered in remission. On the other hand, the majority of those with normal FCal did not have any changes in their investigations or treatment and remained in clinical remission which saved patients and health-care resources from unnecessary interventions.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; drafting the work or revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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MINI REVIEW
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The Role of Carrageenan and Carboxymethylcellulose in the Development of Intestinal Inflammation

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Although the exact pathophysiology remains unknown, the development of inflammatory bowel disease (IBD) is influenced by the interplay between genetics, the immune system, and environmental factors such as diet. The commonly used food additives, carrageenan and carboxymethylcellulose (CMC), are used to develop intestinal inflammation in animal models. These food additives are excluded from current dietary approaches to induce disease remission in Crohn's disease such as exclusive enteral nutrition (EEN) using a polymeric formula. By reviewing the existing scientific literature, this review aims to discuss the role that carrageenan and CMC may play in the development of IBD. Animal studies consistently report that carrageenan and CMC induce histopathological features that are typical of IBD while altering the microbiome, disrupting the intestinal epithelial barrier, inhibiting proteins that provide protection against microorganisms, and stimulating the elaboration of pro-inflammatory cytokines. Similar trials directly assessing the influence of carrageenan and CMC in humans are of course unethical to conduct, but recent studies of human epithelial cells and the human microbiome support the findings from animal studies. Carrageenan and CMC may trigger or magnify an inflammatory response in the human intestine but are unlikely to be identified as the sole environmental factor involved in the development of IBD or in disease recurrence after treatment. However, the widespread use of carrageenan and CMC in foods consumed by the pediatric population in a "Western" diet is on the rise alongside a corresponding increase in IBD incidence, and questions are being raised about the safety of frequent usage of these food additives. Therefore, further research is warranted to elucidate the role of carrageenan and CMC in intestinal inflammation, which may help identify novel nutritional strategies that hinder the development of the disease or prevent disease relapse post-EEN treatment.

Keywords: carboxymethylcellulose, carrageenan, inflammatory bowel disease, microbiota, Crohn's disease, ulcerative colitis

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INTRODUCTION

Crohn's disease (CD) is a chronic relapsing and remitting inflammatory bowel disease (IBD) that causes damage to the mucosal lining of the gastrointestinal tract, resulting in abdominal pain, (bloody) diarrhea, intestinal ulceration, and often progression toward stricturing/penetrating complications requiring surgery, malnutrition, impaired growth, disability, and even mortality (1, 2). Epidemiological

studies have shown an increasing incidence of IBD [composed of CD and ulcerative colitis (UC)] both in areas of the world where the disease has been originally more prevalent (North America, Australia, and Europe) and in countries where IBD was previously infrequent (Asia and South America) (3, 4). It is noteworthy that this increase has occurred in conjunction with the transition toward a "Western" diet characteristically high in processed foods and fats and low in fruits and vegetables (5). Although the exact etiology and pathogenesis of IBD remain obscure, it is believed to result from a combination of genetic, microbial, immunological, and environmental factors including diet (6). In the last couple of years, the field of nutrition in IBD has advanced substantially (7, 8), with several dietary aspects of interest including the food additives used to induce intestinal inflammation and ulceration in animal models of IBD (9–11).

Food additives are substances intentionally added during production, processing, packaging, transportation, or storage of commercial food products. Carrageenan is the name referring to a family of high-molecular-weight sulfated polysaccharides extracted from seaweeds and commonly used as a thickening and emulsifying food additives to improve the texture of commercial food products including infant formulas, dairy products, milk alternatives such as almond milk, processed meats, and soy-based products (9, 12). First patented in the United States in the 1930s, carrageenan's use as a food additive in the Western diet has been substantially increasing over the last 50 years (9, 13). Carboxymethylcellulose (CMC) is a derivative of cellulose, making it affordable and abundant with thickening and emulsifying qualities, and it is found vastly throughout the commercial food industry with a progressive annual increase in its usage as a food additive (14, 15).

THE ROLE OF CARRAGEENAN IN INTESTINAL INFLAMMATION IN ANIMAL MODELS

It has been demonstrated that when guinea pigs are supplied with degraded carrageenan in their drinking water, ulcerations develop in 100% of the animals in their large intestine by the end of a 30-day period (16). The lesions induced by carrageenan in the guinea pigs' large bowel resemble features of human UC (10). Carrageenan has also produced ulcerative lesions in rabbits, mice, and rats that were associated with weight loss, anemia, diarrhea, visible or occult blood, and sometimes mucus in the feces (17). Further, macroscopic features reminiscent of human UC, such as ulcerations predominantly limited to the mucosa along with pseudopolyps and polypoidal formations, were observed, as well as features commonly seen in CD such as strictures in the small intestine leading to obstruction (17). Histological changes associated with exposure to carrageenan in animal models include acute, subacute, and chronic inflammatory changes in the mucosa; occasional crypt abscesses; cystic dilatation or distortion of mucosal glands; mucosal ulceration in various stages of progression and healing; and hyperplastic changes of the glandular epithelium (17). Pricolo et al. noted that animals fed a 2% solution of carrageenan mixed with standard rat chow developed small bowel lesions first (from 2 to 6 weeks) followed by colonic lesions that developed after 8 weeks (11), which may be of particular interest for a subgroup of human CD patients who extend their disease location (18–20).

In recent years, evidence from animal studies has been mounting to suggest that the gut microbiome plays a critical role in the development of inflammation in response to exposure to carrageenan. For example, guinea pigs fed degraded carrageenan develop cecal ulcerations within 21–30 days after carrageenan treatment (21), whereas germ-free guinea pigs fed carrageenan for 6 months or more do not develop any intestinal lesions (22). Concurrent treatment of conventional guinea pigs with carrageenan and metronidazole, an antimicrobial primarily active against anaerobic bacteria, has been shown to prevent the development of intestinal ulcerations (22, 23). Consistent with recent reports in human IBD (24, 25), microorganisms such as *Bacteroides vulgatus* appear to play an important role in the development of the experimental ulcerative lesions in animals (26). For example, animals who were immunized with *B. vulgatus*, prior to carrageenan administration developed experimental disease at a faster rate and more severe lesions than animals that were administered carrageenan alone (23, 27). Mirroring the development of antibodies to components of the intestinal microbiome in human IBD (28, 29), Onderdonk et al. noted that non-immune carrageenan recipients developed antibodies to *B. vulgatus* during carrageenan treatment, implying that an adaptive immune response occurs in the animals following carrageenan-induced intestinal ulceration (26, 27). Factors present on the bacterial outer membrane may mediate these antibody responses, as one study assessed eight strains of *B. vulgatus* and observed the alpha phenon (term used to reflect strain variation of outer membrane proteins) was associated with the enhancement of colitis (30).

The specific mechanism by which carrageenan induces inflammation in experimental animal models is not clearly defined, but carrageenan has been demonstrated to decrease the amount of epithelial glycoproteins in the colon (31) and is capable of inhibiting the interaction between macrophages and lymphocytes (32). The involvement of toll-like receptor-4 (TLR4) and interleukin (IL)-6 in the innate immune response to carrageenan was demonstrated using TLR4- and myeloid differentiation primary response 88-deficient mice (33). Recently, Wei et al. showed in a trinitrobenzenesulfonic acid (TNBS) model of colitis in BALB/c mice that pretreatment with carrageenan aggravated the severity (both clinical and histological) of TNBS colitis, with a concomitant increased expression of IL-6 and tumor necrosis factor alpha (TNF)-α and a reduction in IL-10 (34). Taken together, these animal studies have led to the hypothesis that food emulsifiers such as carrageenan may act as a conditional inflammatory agent that magnifies any existing chronic inflammation of the intestinal tract provoked by pathogens (35). This hypothesis explains why carrageenan has been found to induce intestinal inflammation in most animal studies, but not all. For example, healthy neonatal pigs fed infant formula with carrageenan for 28 days had no effect on blood cytokine evaluations (IL-1β, IL-6, IL-8, and TNF-α) (36), but they would not have had any baseline inflammation and were not exposed to pathogens.

THE ROLE OF CMC IN INTESTINAL INFLAMMATION IN ANIMAL MODELS

In mice given relatively low concentrations of CMC, low-grade inflammation and obesity/metabolic syndrome was induced in wild-type hosts and promoted robust colitis in IL-10$^{-/-}$ and TLR5$^{-/-}$ mice. In the control mice not fed CMC, the closest bacteria were shown to reside about 25 μm from epithelial cells with no bacteria observed within 10 μm, whereas CMC-treated mice exhibited some bacteria in apparent contact with the epithelium, and the average distance was reduced by more than 50% (37). In this study, CMC dramatically altered microbiota composition in both fecal and intestinal-adherent bacteria, and the authors concluded that chronic exposure to CMC resulted in erosion of the protective function of the mucus, increased bacterial adherence, and a more pro-inflammatory microbiota (37). More recently, Chassaing et al. tried to disentangle the effect of inflammation itself on microbiota composition from the effect of CMC on host parameters (that in turn promotes inflammation and subsequently alters the microbiota). They used the Mucosal Simulator of the Gastrointestinal Microbial Ecosystem (M-SHIME) model to examine the effects of emulsifiers on the human microbiota *in vitro* (38). CMC directly acted upon human microbiota to increase its pro-inflammatory potential; the CMC-induced increase in flagellin occurred after 1 day and was driven by altered microbiota gene expression rather than microbiota composition changes (38). Transfer of both emulsifier-treated M-SHIME microbiotas to germ-free recipient mice recapitulated many of the host and microbial alterations observed in mice directly treated with emulsifiers, notably the development of intestinal inflammation (38). These results suggest that the microbiota may be a key direct target of CMC to drive chronic intestinal inflammation.

Swidsinski et al. have demonstrated that exposure to 2% CMC in IBD-susceptible IL-10 gene-deficient mice results in "CD-like effects," reporting that changes in the CMC-treated mice were identical to ileal biopsy findings of CD patients (14). This was supported by the observations that mice treated with 2% CMC demonstrated increased concentrations of bacteria in the ileum, larger spaces between villi, increased amounts of bacteria adherent to the villi, increased white blood cells in the lumen, and, in some of the mice, bacterial infiltration of the epithelium (14).

These findings of the effects of commonly used emulsifiers on the microbiota, mucus layer, and epithelial barrier integrity should be considered in conjunction with the recent work by Desai et al. (39) regarding the role of dietary fiber on mucus barrier maintenance. These authors demonstrated that in gnotobiotic mice colonized with a synthetic human gut microbiota and denied dietary fiber, the microbiota will consume host-secreted mucus glycoproteins as a nutrient source in place of fiber, leading to erosion of the colonic mucus barrier (39). In turn, this erosion of the colonic mucous barrier promoted greater epithelial access and lethal colitis by the murine pathogen *Citrobacter rodentium* (39). Carrageenan and CMC are often added to commercial food products as a fiber (40), instead of other dietary fiber (such as water-insoluble cellulose and resistant starch or water-soluble fiber such as pectin and raffinose) sources. In addition, many CD patients already limit dietary fiber to avoid its bulk-forming and laxative effects. Therefore, a "perfect storm" setting in which CD patients increase their CMC/carrageenan intake but limit their intake of other dietary fibers is created, which could propagate or exacerbate the existing dysbiosis toward more mucin-degradation and which could enhance the susceptibility for mucosa-associated pathogens.

FOOD ADDITIVES AND INTESTINAL INFLAMMATION IN HUMANS

The reports above of commonly used food additives causing intestinal inflammation in animal models have triggered investigations of how they may lead to inflammation in the human gastrointestinal tract, although perfectly corresponding human studies are of course unethical to conduct. It is likely that mechanisms by which carrageenan induces inflammation in the human intestine are similar to animals and multifactorial: the stimulation of pro-inflammatory cytokines, the disruption of the epithelial barrier, and the interference with innate mucosal immune responses to microorganisms (**Figure 1**). Community-level metabolic networks within the microbiome produce bioactive metabolites that have an established role in intestinal immune homeostasis and a healthy gut mucosal barrier (41, 42). This barrier protecting the host from luminal antigens is composed of enterocytes (epithelial cells) and the seal between them provided by the tight junctions (43). With a healthy gut mucosal barrier, an individual maintains a homeostasis where the passage of nutrients, ions, and water across the intestinal epithelium into the mesenteric blood stream is tightly regulated, while translocation of dietary antigens and components of the microbiome is prevented (43), and the active crosstalk between a diverse microbiome and the intestinal immune system leads to immune tolerance. Impaired barrier function can lead to abnormalities in the expression of tight junction protein that may trigger immune activation and the development of inflammatory disease in susceptible individuals (43, 44).

Borthakur et al. provided the first report of the inflammatory response of human intestinal epithelial cells to carrageenan exposure by demonstrating that carrageenan stimulates an inflammatory cascade in colonic epithelial cells in a pathway involving B-cell lymphoma/leukemia 10 gene (Bcl10) activation and the increased production of IL-8 (9), a key pro-inflammatory cytokine. These results were supported when Choi et al., using the human colonic adenocarcinoma cell line HCT-8, demonstrated that carrageenan led to the activation of nuclear factor kappaB, which subsequently increased the gene induction of IL-8 (12). In addition, they reported that carrageenan triggered a disruption of the epithelial barrier, decreasing the density of tight junctional protein zonula occludens (ZO)-1, causing disarray in the distribution of ZO-1 throughout the epithelium, and decreasing the gene expression of this tight junction protein (12). More recently, this group showed that carrageenan exposure triggered the expression of the proapoptotic macrophage inhibitory cytokine 1 (MIC1), which is in turn counteracted by MIC1-induced expression of activating transcription factor 3 (45).

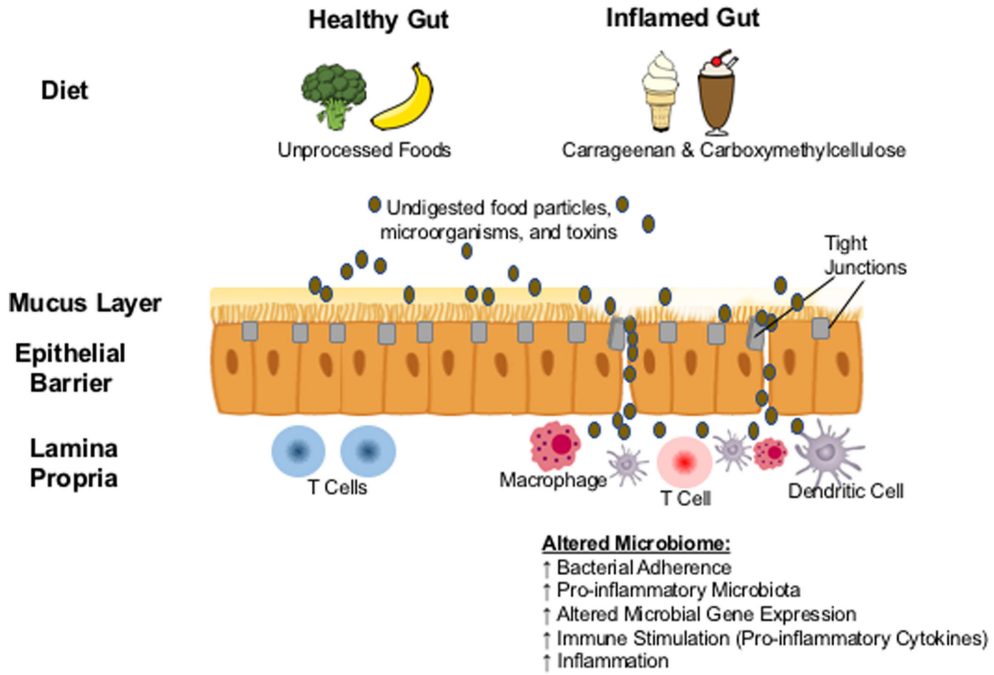

FIGURE 1 | Proposed biological mechanism: carrageenan and carboxymethylcellulose in processed foods result in the erosion of the protective mucus layer and in the abnormal expression of tight junction proteins. Undigested food particles, toxins, and microorganisms are then able to pass though the intestinal epithelium resulting in translocation of microbes and the overcolonization by pathobionts that can alter the composition of the gut microbiome, triggering the activation of the immune system and the development of inflammation.

Carrageenan has been reported to interact with the glycoprotein deleted in malignant brain tumors 1 (DMBT1). DMBT1 functions as a pattern recognition molecule with a peptide domain capable of binding and aggregating a wide spectrum of bacteria (46) and has been demonstrated to prevent the invasion of bacteria into intestinal epithelial cells *in vitro* (47). Carrageenan inhibits the bacterial aggregating function of DMBT1 *via* binding to the specific peptide that recognizes bacteria, and it has been suggested that carrageenan disrupts the mucosal protection provided by DMBT1 (48). This raises the possibility that carrageenan is capable of disrupting an innate mucosal immune function provided by DMBT1, which can then trigger the initiation or perpetuation of an inflammatory response to intestinal bacteria or bacterial antigens (48).

In human cells, carrageenan has also been demonstrated to trigger innate immune responses through pathways that involve Bc110, TLR4, NF-κB, and AP-1 (9, 35, 49), leading to the upregulation of TNF-α secretion (50). For example, Jiang et al. (51) demonstrated that carrageenan-induced TNF-α secretion is the main contributor to cellular damage in Caco-2 monolayers exposed to carrageenan. However, researchers in the field note that in these studies, the degree of inflammation caused by carrageenan alone is low, while the inflammation generated through these pathways when a pathogen is additionally present is high, supporting the hypothesis that an interaction effect is present in which carrageenan serves as a pro-inflammatory agent to amplify existing intestinal inflammation (35).

In view of the recent benchmark reports by Chassaing et al. (38) and Desai et al. (39), more studies of the intestinal response to the widely used emulsifiers, carrageenan and CMC, together with other features of a Western diet such as reduced dietary fiber intake are eagerly awaited (52). It is noteworthy that the Food and Drug Administration regulations do not contain a definition of dietary fiber but rather have relied on analytical methods for measuring levels of dietary fiber present in food. Therefore, an isolated or synthetic non-digestible carbohydrate such as carrageenan and CMC can be added to foods and quantified as a dietary fiber even if it does not provide the beneficial physiological effect to human health that dietary fiber should provide. For example, CMC is listed among the 26 most common fibers being added to food and declared on the Nutrition Facts label as dietary fiber (40).

WIDESPREAD USE OF FOOD ADDITIVES

Patented in the United States in the 1930s, carrageenan was granted GRAS (generally regarded as safe) status in 1959 and currently remains included as a food additive that holds GRAS status in the Code of Federal Regulations in the United States (13). Carrageenan acts to thicken, stabilize, and emulsify a wide variety of foods typically consumed in the Western diet including dairy products such as chocolate milk, ice cream, cottage cheese, sour cream, and yogurt; processed meats; soymilk; almond milk; mayonnaise; and infant formula (53). Estimates regarding the average daily intake of carrageenan vary from 20 to 200 mg/day

(13, 54) and are currently difficult to put into context without a standard for comparison within human studies or with animal studies.

Carboxymethylcellulose is used broadly throughout the food industry in products typically consumed by children including candy, chewing gum, "snack foods," ketchup, and various baked goods, and currently, there are no quantitative restrictions on its use nor does its addition to food require to be declared (14, 15). CMC is listed in the Food and Drug Administration's database of GRAS substances (55), and CMC is also included in the Safety and Toxicity of Excipients for Paediatrics (STEP) database (56, 57), a resource developed by the European Paediatric Formulation Initiative and the United States Paediatric Formulation Initiative in collaboration for storage and rapid/effortless access to the safety and toxicological data of commonly used excipients.

ROLE OF DIET IN IBD: EPIDEMIOLOGY AND TREATMENT

The increasing incidence rate of IBD worldwide, with the highest incidence and prevalence being in Westernized countries (3), has been associated with increased consumption of a Western diet (5). A case–control study conducted in Japan assessed environmental risk factors for UC in 101 patients and observed that consumption of foods typical of a Western diet including bread, butter, and sausage was significantly related to an increase in UC risk (58). Moreover, D'Souza et al. demonstrated that the consumption of fried and fast foods, meats, snacks, and desserts, which they labeled as a traditional Western dietary pattern, increased the likelihood of acquiring CD in adolescent females (59). Conversely, they found that both males and females exposed to a prudent dietary pattern, consisting of mostly vegetables, fish, grains, and nuts, had a decreased likelihood of developing CD (59).

Exclusive enteral nutrition (EEN) is recommended as a first-line therapy in pediatric CD and involves the use of a liquid formula diet as the only source of nutrition over several weeks (8, 60).The mechanism of action of EEN has not been clearly established; however, it may provide efficacy *via* eliminating certain components of the Western diet and through modulation of the microbiome (61–67). More recently, novel dietary therapy regimens have been described, which involve avoidance of processed foods such as the CD exclusion diet (CDED) (68) and the Specific Carbohydrate Diet (69). Sigall-Boneh et al. demonstrated that the CDED, which avoids exposure to many components of a Western diet such as dairy products and processed foods containing food additives, led to remission in 70% of patients (8). Through large network studies such as the Canadian Institutes for Health Research supported Inflammation, Microbiome, Alimentation, GastroIntestinal and Neuropsychiatric effects (IMAGINe), considerable efforts are being made to investigate the potential contributions of dietary factors to IBD pathophysiology as a dysregulated interplay of genetic, environmental, microbial, and immunological factors (6, 59), and so more details on the role of diet, including food additives such as carrageenan and CMC, in IBD may be discovered in the near future and inform dietary recommendations and medical treatment of IBD.

CONCLUSION

Carrageenan and CMC administered in animal models consistently result in intestinal ulcerations with histopathological features similar to human IBD. Although the set of precise mechanisms through which these emulsifiers induce lesions and inflammation remains unknown, disruption of the epithelial barrier and dysregulation of the immune response to the gut microbiome have been repeatedly implicated. These findings raise concern because carrageenan and CMC are used extensively in processed food products that are consumed by the pediatric population, and the incidence rate of childhood IBD is increasing concurrently with a rise in the adoption of a Western diet. The only successful dietary interventions to have induced CD remission exclude processed foods containing carrageenan and CMC, further supporting the possibility that carrageenan and CMC are potential triggering or magnifying substances of inflammation in IBD. Further research is warranted to clarify the role of carrageenan and CMC in the microbiome alteration of intestinal inflammation together with an improved appreciation of the complex interplay with the consumption of dietary fibers, and such studies could lead to novel nutritional strategies that help prevent the development of IBD or help induce and sustain remission.

AUTHOR CONTRIBUTIONS

All authors listed have made substantial, direct, and intellectual contribution to the work and approved it for publication.

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GENERAL COMMENTARY
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Commentary: Impact of Fecal Calprotectin Measurement on Decision-Making in Children with Inflammatory Bowel Disease

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In their recent publication, El-Matary et al. (1) describe the utility of measurement of fecal calprotectin (FC) in the ongoing management of children with known inflammatory bowel disease (IBD). In this cohort of 77 children, FC was measured upon presentation with key changes in symptoms (most commonly abdominal pain and hematochezia). The child's management was then adjusted according to the level of the FC, with a cutoff of 250 μg/g stool. Almost 90% of those with elevated FC had a change in their management, which resulted in a reduction in clinical activity indices over the subsequent 3–6 months. Repeated FC measurements were not available. Conversely, the majority of those with low FC (below the cutoff) had no change in management in the following months. Reassuringly, 94% of these children were judged to be in remission at their follow-up visit.

Non-invasive markers, especially those measured in stool, have become increasingly important and relevant in recent years in the management of IBD. Numerous markers have now been described. Although calprotectin has been the most utilized to date, other promising markers include S100A12, osteoprotegerin, lactoferrin, and M2 pyruvate kinase (2–4).

Generally, markers such as calprotectin provide greater specificity and sensitivity for the presence of gut inflammation than standard serum-based markers. In one assessment of routine serum markers at the time of diagnosis, erythrocyte sedimentation rate, C-reactive protein, albumin, and platelet counts were each normal in 19 (13%) and all abnormal in just 52 (36%) of 146 children with CD (5). Overall, the platelet count was most often abnormal in this group. Both FC and fecal S100A12 provided much greater utility than any of the same four serum markers in an earlier cohort of 31 children with IBD (6).

Although fecal markers have particular roles in the investigation of an individual with undifferentiated symptoms (to assess the need for further investigations and to reach a diagnosis), they also clearly have important roles in individuals diagnosed with IBD. Such roles include the monitoring of disease control, assessment of response to an intervention, assessment of mucosal healing (MH), and to provide an assessment of the risk of relapse in the coming months.

The current report focused upon children with a change in symptoms, such as the development of rectal bleeding. The key issues in this context are to ascertain whether the change in symptoms is related to an exacerbation of disease, or due to other factors such as an intercurrent infection or due to functional overlap. Both enteric infections and irritable bowel syndrome can result in an elevated FC.

Prompt access to FC measurement clearly provides guidance as to the clinical management required. In practice, however, one would otherwise consider the pattern of symptoms, the routine blood tests, and examination findings and anthropometry as well. It would seem reasonable and appropriate to consider FC measurement in addition to these assessments, rather than instead.

There are some data suggesting that FC levels may vary according to disease location, with it being less reliable in small bowel inflammation (7). Assessment at baseline, at the time of initial diagnostic endoscopy, along with serial measurements over time may be of assistance for the individual patient. Accordingly, a change in FC from a prior measurement may be more helpful than a one-off level.

The other potential roles of fecal markers in monitoring disease progress are also important, especially in children. Several reports indicate that the serial measurement of S100A12 or FC in individuals in clinical remission may predict the risk of a future relapse (8, 9). In addition, fecal markers may enable the early prediction of recurrence after ileo-colonic resection (10). The role of FC in predicting the achievement of MH is less clear: the data are not yet conclusive as to the correlation between FC and MH (11). In addition, FC may have a role in guiding the appropriate indication for repeat endoscopic assessment in children with established disease: optimizing the timing of endoscopy in children will clearly be of benefit to health administrations (given the cost of endoscopy) and also for children and their parents (given the inconvenience of endoscopy for children).

Clearly, the potential roles for FC and other non-invasive markers are likely to expand. Further understanding of the differential information provided by the various markers may lead to the use of more than one marker, or indeed to the use of a panel of markers.

In conclusion, the work of El-Matary and colleagues (1) provides further important information of the utility of FC in children with IBD. Additional prospective assessments are required, ideally with comparisons between available markers and with serial estimations over time.

AUTHOR CONTRIBUTIONS

AD formulated the plan for this commentary along with initial draft manuscript. MA reviewed the draft manuscript and assisted in revisions of the drafts of the manuscript.

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MINI REVIEW
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Crohn's Strictures—Moving Away from the Knife

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Crohn's disease (CD) is a lifelong inflammatory bowel disease with a rapidly rising incidence in the pediatric population. A common complication of CD is the development of fibrotic strictures, which may be present at initial diagnosis or develop many years later. Clinical presentation depends on stricture location and degree of obstruction, and strictures frequently contain a mixture of inflammatory and fibrotic tissue. Histological examination of Crohn's strictures shows thickening of the muscular layers and the submucosa, where increased collagen deposition by activated myofibroblasts is concentrated around islands of smooth muscle cells and at the superficial margin of the muscularis propria. No antifibrotic therapies for Crohn's strictures exist. Profibrotic transforming growth factor-β (TGFβ)/bone morphogenetic protein signaling stimulates myofibroblast differentiation and extracellular matrix deposition. Understanding and targeting TGFβ1 downstream signaling is the main focus of current research, raising the possibility of specific antifibrotic therapy in CD becoming available in the future.

Keywords: Crohn's disease, inflammatory bowel disease, fibrosis, intestine, reactive oxygen species, NADPH oxidase

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INTRODUCTION

The incidence of Crohn's disease (CD) is rapidly rising in the pediatric population (1). Crohn's inflammation is transmural and can occur throughout the gastrointestinal tract, unlike ulcerative colitis where mucosal inflammation is confined to the colon. CD is phenotyped according to disease behavior (inflammatory/penetrating/stricturing) and location using the Paris classification for pediatric patients (2), a modification of the Montreal classification (3). Fibrotic narrowing of the intestinal tract (stricturing disease) is present in approximately 10–17% of children at diagnosis (4), affecting up to 40% by 10 years after diagnosis (5–7). Strictures occur predominantly in the ileum, reflecting the common distribution of inflammation, but can arise throughout the digestive tract (8). Presenting features vary from overt intestinal obstruction to subacute and non-specific symptoms. The conventional conceptual framework of the pathobiology of fibrosis in CD is one of chronic inflammation leading to ongoing frustrated attempts at healing with formation of disorganized tissue. If this occurs circumferentially in a relatively narrow diameter organ such as the small intestine, the result is a fibrotic stricture. Here, we will describe current knowledge and recent advances regarding the diagnosis and pathogenesis of intestinal fibrosis and will review potential future therapies.

CLINICAL AND PATHOLOGICAL DIAGNOSIS

The clinical presentation of stricturing disease is highly variable, depending on the location and degree of obstruction. Presentations range from non-specific symptoms including abdominal discomfort, poor appetite, energy and/or growth, to overt obstruction with abdominal pain, vomiting, and reduced bowel movements. Strictures may be inflammatory, fibrotic, or more commonly a

combination of both (8). Most strictures are in the small bowel, inaccessible to standard endoscopy, and even when accessible, endoscopy can only provide information (visual, histological) on the mucosa, while the fibrotic collagen deposition occurs submucosally. Capsule endoscopy can provide visual information but no tissue samples and is contraindicated in the presence of suspected strictures due to the risk of retention. Therefore, clinicians usually rely on radiological tools for the diagnosis of stenotic disease.

A fibrotic stricture is inferred by the presence of a narrowed lumen with proximal dilation. Computed tomography (CT) and magnetic resonance enterogram (MRE) are preferred to X-rays with enteral contrast [small-bowel follow through (SBFT)] as they allow assessment of extraintestinal as well as intestinal complications of CD and have similar sensitivity and accuracy. CT and MRE permit a transmural assessment of the bowel wall, enabling the classification of strictures as inflammatory, fibrotic, or mixed. Inflammation is suggested by avid enhancement and mesenteric inflammation (hypervascularity, fat stranding), whereas fibrosis is characterized by a thickened bowel wall with a featureless appearance, minimal or no enhancement and the absence of mesenteric inflammation. MRE has comparable (9–12) or superior (13) sensitivity and specificity to CT for the detection of fibrosis in the presence of inflammation in adult and pediatric patients, while MRE has greater sensitivity for the detection of fibrosis alone (10). A recent prospective pediatric study demonstrated 73% sensitivity and 81% specificity for the detection of strictures using MRE compared to 42% sensitivity and 68% specificity for CT enterograms (12). Due to its lack of ionizing radiation and its capacity to provide accurate diagnosis of both intra- and extraintestinal complications, MRE is the preferred modality in the pediatric population, although technical limitations (institutional access, need for general anesthesia) ensure a retained role for CT and SBFT. Recent advances in ultrasound elastography as a measurement of tissue stiffness have shown promising results in the differentiation between inflammatory and fibrotic strictures (14, 15), but have not yet reached routine clinical practice. As sensitivity does not reach 100% with any modality, clinical judgment is required in conjunction with imaging findings to decide about surgical exploration/intervention.

HISTOPATHOLOGY

Fibrosis is defined as the permanent and abnormal deposition of extracellular matrix (ECM), primarily collagen, within tissues, resulting in a distortion of structure and impeding normal tissue and organ function. It is understood to be an aberrant response to ongoing inflammation, where tissue remodeling in response to injury, through ECM deposition and subsequent breakdown, becomes self-perpetuating. In the normal small bowel, the mucosa consists of a single layer of epithelium, lamina propria, and basement membrane. Deep to the intact mucosa is the muscularis mucosa, a thin layer of smooth muscle cells, and then the submucosa. The submucosa is a loose connective tissue layer with fibroblasts as main cell type within an ECM traversed by blood vessels and nerves (16). The ECM is a complex biochemical structure where collagen types I and III predominate (17). Deep to the submucosa is the muscularis propria with its inner circular and outer longitudinal layers of smooth muscle cells. The collagen strands of the submucosa tend to be concentrated at its border with the muscularis propria.

Histological examination of CD strictures reveals abnormalities in both the submucosal space and muscular layers (**Figure 1**). The submucosa is increased in volume and density, with increased collagen deposition being concentrated around islands of smooth muscle cells and at the superficial margin of the muscularis propria. Studies have shown an increase in total protein and collagen content, especially collagen subtypes I, III, and V in these strictures. Although increased in absolute terms, the relative proportions of types I and III collagen appear to be comparable between strictures and healthy intestine, and although the proportion of type V collagen is relatively amplified, type I collagen remains dominant (16, 17). Myofibroblasts, differentiated from fibroblasts, are the primary source of ECM production and secretion. Increased numbers of local fibroblasts and myofibroblasts in fibrosis have been attributed to a variety of mechanisms including the proliferation of existing fibroblasts in the local area (18), the induction of epithelial-to-mesenchymal transition (19), the recruitment and differentiation of bone marrow-derived fibrocytes (20), as well as endothelial-to-mesenchymal transition (21), but the relative contribution of each process is unknown. Thickening of the muscularis mucosa and muscularis propria as well as smooth muscle cell proliferation within the submucosa itself have been observed. In some instances, the proliferation of smooth muscle cells within the submucosa can be so pronounced that it results in the obliteration of the submucosa (22). The combined overall effect of increased ECM deposition and muscular hypertrophy is transmural thickening and stiffening, which when occurring circumferentially causes narrowing and obstruction of the intestinal lumen.

SIGNALING AND THE FIBROTIC RESPONSE

Various growth factors and cytokines have been implicated in the development of fibrosis including IL-13, platelet-derived growth factor (PDGF), connective tissue growth factor, basic fibroblast growth factor, insulin-like growth factor, bone morphogenetic proteins (BMPs), and transforming growth factor-β (TGFβ) (23). The TGFβ family is secreted by a wide variety of cells throughout the body and its effects are highly varied and complex. As well as being responsible for cell proliferation and differentiation, anti-inflammatory control, wound healing, tumor suppression in healthy tissues and cancer progression in neoplastic tissues, TGFβ is the primary cytokine driving the development of fibrosis in tissues throughout the body. Differences in TGFβ subtype, receptors, cofactors, and signaling pathways allow this group of cytokines to display such versatility (24). TGFβ1 is the most common subtype and its role in the development of tissue fibrosis through the recruitment of fibroblasts, transdifferentiation to myofibroblasts, and stimulation of ECM secretion has been convincingly demonstrated in many organs including in intestinal fibrosis (25–28).

TGFβ1 is a homodimeric signaling molecule produced by myofibroblasts and inflammatory cells (e.g., macrophages),

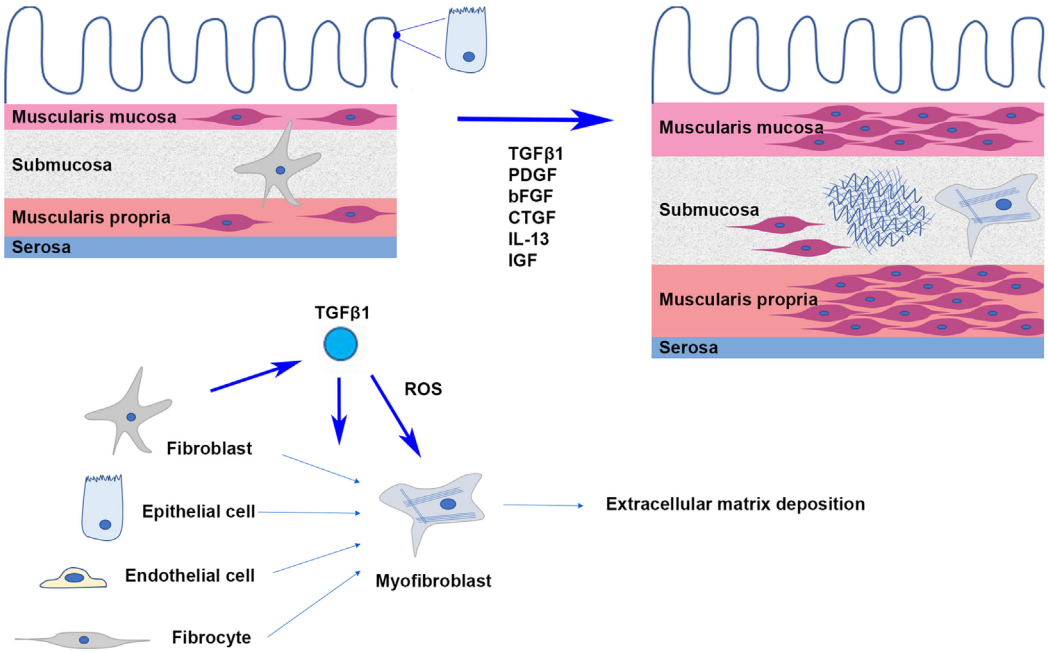

FIGURE 1 | In intestinal fibrosis, hypertrophy and hyperplasia of smooth muscle cells cause thickening of the muscularis mucosa and muscularis propria; smooth muscle cells proliferate in the thickened submucosa, where activated myofibroblasts secrete extracellular matrix proteins.

platelets, and parenchymal cells during the hemostasis and inflammation phases of tissue injury and healing. This para- and autocrine signaling is anti-inflammatory and supports wound healing but drives fibrosis. The TGFβ receptor is a transmembrane complex of two dimers: two type I receptors (ALK5, also called TGFBR1 or TβTI) and two type II receptors (TGFBR2); both are serine/threonine kinases and binding of TGFβ results in phosphorylation of ALK5 by TGFBR2 with subsequent binding and phosphorylation of downstream signaling proteins SMAD2 and SMAD3 by ALK5 (**Figure 2**). Once phosphorylated, SMAD2 and SMAD3 form a trimer with SMAD4, which translocates to the nucleus and binds to target DNA. The transcriptional targeting, nuclear translocation, and longevity of these SMAD transcription factors is modulated by the binding of a wide variety of effector molecules. SMAD2/3 binding to ALK5 is antagonized by SMAD7. SMAD7 antisense oligonucleotides (mongersen) showed promise in the treatment of inflammatory CD by restoring the TGFβ1–SMAD2/3 pathway (29); no associated increase in fibrosis has been observed but longer term follow-up is required. Several non-canonical TGFβ signaling pathways have been described (30). Phosphorylated TGFBR1 and TGFBR2 lead to activation of mitogen-activated protein kinases (MAPKs), which regulate multiple pathways including those leading to transcription. MAPKs also regulate the canonical TGFβ pathway by phosphorylating active SMAD to promote its proteasomal degradation. TGFβ activates phosphoinositide 3-kinase, leading to downstream activation of Akt, mTOR, and upregulation of protein translation. Akt interacts directly with SMAD3 to prevent its activation and indirectly inhibits SMAD-mediated transcription through phosphorylation of FoxO transcription factors, thus blocking their translocation to the nucleus. A more detailed review of the complexity of TGFβ signaling can be found in the study by Massague (24).

The pleiotropic and multifunctional effects of TGFβ1 signaling make it an unattractive therapeutic target in fibrosis. Substantial effort has been directed at characterizing downstream signaling pathways of TGFβ where fibrosis-specific pharmacologic intervention might be achievable. Reactive oxygen species (ROS) have been implicated as mediators of the profibrotic effect of TGFβ1. ROS can be produced non-enzymatically (e.g., by radiation, toxic chemicals) or enzymatically. Most cellular ROS is produced in the mitochondria during cellular respiration. Other ROS sources include the cytochrome P450 family, cyclooxygenases, peroxisomal oxidases, xanthine oxidoreductase, and the family of NADPH oxidases (NOX). The NOX family is unique as its only known biological role is ROS production, and NOX enzymes appear to be intimately involved in fibrogenesis. NADPH oxidase 4 (NOX4) is a constitutively active, transcriptionally regulated producer of H_2O_2. An accumulating body of evidence suggests that TGFβ1 upregulates NOX4 expression and that NOX4, through the production of H_2O_2, promotes myofibroblast differentiation and secretion of ECM proteins (31–33). In a rat model of renal fibrosis and in cell-based assay systems, TGFβ1 stimulation was associated with increased NOX4 expression and H_2O_2 production, while reduced NOX4 expression by siRNA-mediated knockdown decreased ROS production and expression of profibrotic proteins including collagen, α-SMA, and fibronectin (34). These findings have been replicated using cell-based assays, mouse models, and patient samples in the lung (35–38), skin (39), and liver (40–42). A study by Jung et al. using fibroblasts and a mouse model of renal

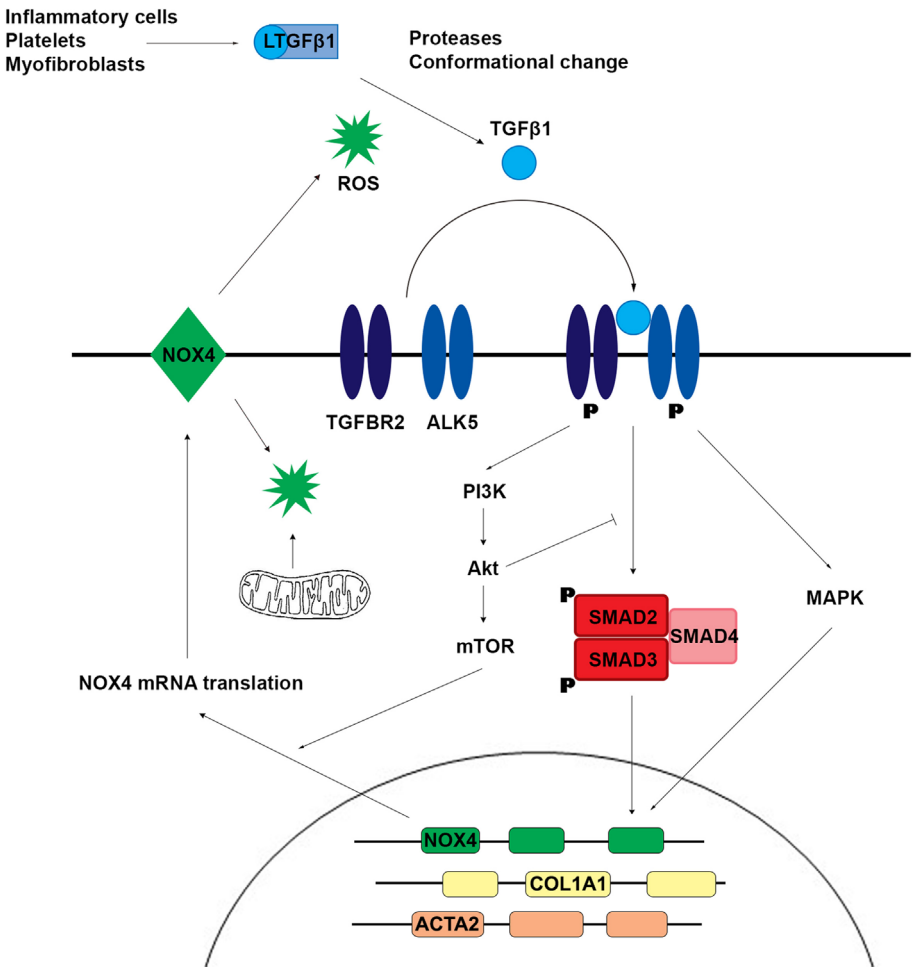

FIGURE 2 | TGFβ1 signaling induces SMAD2 and SMAD3 phosphorylation and activation of non-canonical mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways, which upregulate the expression of profibrotic genes including NADPH oxidase 4 (NOX4). Reactive oxygen species (ROS) generated by NOX4 and/or mitochondria promote further release of active TGFβ1.

fibrosis suggested that NOX4 upregulation by TGFβ1 depends on activation of SMAD2 and Akt (43). Latella and coworkers reported that SMAD3 was necessary for the development of intestinal fibrosis in a chronic TNBS model (44), suggesting that NOX4 mediates the effect of TGFβ1 through canonical and non-canonical pathways. Somewhat contradictorily, NOX4 was protective in a renal fibrosis model (45), and there is evidence from mice and patient data that NOX4 may be protective against atherosclerosis (46–49). Another mouse model showed preserved myofibroblast differentiation but impaired wound healing in NOX4−/− mice (50), suggesting that the requirement of NOX4 for myofibroblast differentiation and collagen secretion may be tissue and/or context dependent (acute versus chronic insult). Despite the growing evidence for a fundamental role of NOX4 in fibrosis, only limited studies exist for the intestine. Hotta and coworkers demonstrated a reduction in TGFβ1-dependent collagen I production by murine intestinal myofibroblasts treated with a pan-NOX inhibitor or NOX4 siRNA (51). Data from RNA-Seq analysis of intestinal fibroblasts showed variable upregulation of NOX4 transcription in three patients with CD compared to three healthy controls (52). BMPs belong to the TGFβ superfamily and signal *via* phosphorylation and complex formation of SMAD 1, 5, and 8 (53). BMP7 may protect against colitis and prevent fibrosis by antagonizing TGFβ signaling (54–56). Angiopoietin-like protein 2 (ANGPTL2) modulates BMP signaling and initial studies suggest that organ damage in ANGPTL2 knockdown mice is linked to NOX4 (57, 58).

TGFβ1 signaling stimulated the production of mitochondrial ROS (mtROS), possibly by inhibition of complex III and IV and *via* the mTOR signaling pathway, with subsequent increase in profibrotic gene expression. Reduction of mtROS by the antioxidant MitoQ reduced TGFβ1 expression, SMAD2 and SMAD3 activation, and collagen deposition in a liver fibrosis model (59). mtROS and NOX4 may interact through a positive feedback loop to promote TGFβ1-driven fibrosis. NOX4-derived ROS caused mitochondrial dysfunction and increased mtROS, while mtROS amplified the TGFβ1-mediated increase in NOX4 expression (60). Additionally, increased TGFβ1 inhibited the antioxidant

response, thereby exacerbating the prooxidant shift and further driving fibrosis (61).

Key enzymes in cross-linking and stabilizing the network of collagen fibrils are H_2O_2-generating lysyl oxidase (LOX) and the lysyl oxidase-like proteins (LOXL1–4), which are copper-dependent amine oxidases that oxidatively modify the ε-amino group of lysine side chains in collagen and elastin for formation of inter- and intrachain cross-links. Clinical and animal-based studies in the liver and myocardium demonstrate that LOXs promote tissue stiffening through cross-linking of existing collagen and elastin fibrils and that inhibition of LOXL2 may inhibit and even reverse fibrosis (62–65). Studies in rat and human lung fibroblasts, human trabecular cells, and human osteoblasts suggest that TGFβ1 upregulates LOXs, which in turn modify the actions of TGFβ1 (66–69). It is important to note that TGFβ1 is secreted and stored extracellularly bound to a latent TGF-β-binding protein and a latency-associated peptide. This inactive complex is bound to the ECM *via* integrins, and active TGFβ is released by protease cleavage or conformational changes caused by increased stiffness of the ECM (61, 70). This provides a possible explanation for TGFβ1's effects in the absence of active inflammation (71, 72) as well as the finding that a stiff matrix is required for myofibroblast differentiation (62, 73).

CURRENT TREATMENTS AND FUTURE POSSIBILITIES

Although improved management of CD inflammation by anti-TNFα therapy (infliximab, adalimumab) appears to reduce the rate of stricture development (74), there is currently no medical therapy directly targeting fibrosis in CD. Patients whose strictures fail to respond to anti-inflammatory therapies (aimed at any inflammation and edema coexisting with fibrosis) require surgical intervention. Endoscopic balloon dilatation (EBD) is an option for single, short, and uncomplicated strictures accessible by endoscopy, for instance, stricture recurrence at ileocecal anastomoses. Although technical success rates are high, with a low rate of complications, retrospective data from adult patients demonstrate that 42–70% of patients will require repeat EBD or surgical intervention by 5 years (75–77). Pediatric data are limited, but support the feasibility and safety of EBD (78, 79). Given the predominance of mixed fibrotic/inflammatory strictures over purely fibrotic strictures, intrastricture injection of corticosteroids has been proposed as an adjunct to balloon dilation. A prospective randomized control trial including nine adult patients showed no difference in stricture recurrence rates at 1 year (80), while a prospective RCT including 29 pediatric patients showed earlier stricture recurrence in patients treated with placebo (79). Due to the small trials and number of patients studied, the benefit of intralesional steroid injection has not been confirmed (81). Surgery is the mainstay of treatment for fixed Crohn's strictures. Simple, short strictures can be treated with bowel preserving strictureplasty; longer, multiple, or complicated strictures (e.g., significant inflammation, penetrating disease, and suspected cancer) are treated with resection and primary anastomosis. Resection carries its own risks, including anastomotic dehiscence and disease recurrence at the site of the anastomosis, malabsorptive issues following terminal ileal resection including vitamin B12 deficiency and bile salt malabsorption, and, in cases requiring repeated resections for recurrent strictures, short bowel syndrome leading to a dependence on parenteral nutrition.

Medical therapies to prevent and reverse fibrosis are eagerly sought and much of the focus for new therapies has been for pulmonary and hepatic disease. As our understanding of the pathophysiology of fibrosis improves, we are discovering more potential drug targets. Pirfenidone, a growth factor inhibitor, has been licensed for use in idiopathic pulmonary fibrosis (IPF), based on positive results from Phase 2 and 3 trials (82, 83). Nintedanib, a kinase inhibitor that acts on vascular endothelial growth factor receptors, PDGF receptors, and fibroblast growth factor receptors, showed efficacy and safety in Phase 2 and 3 trials and is licensed for use in IPF (83). Specific NOX4 inhibitors are still not available, albeit a NOX inhibitor (GKT137831) performed well in preclinical models (35, 42) and a Phase 1 clinical trial (84). A Phase 2 trial confirmed safety but not efficacy in the treatment of diabetic kidney disease; recruitment for a Phase 2 trial in patients with primary biliary cirrhosis is ongoing. Integrin αvβ6 is another target of interest; it mediates the conformational release of active TGFβ from its latent complex (72) and Phase 2 trials of a monoclonal antibody in the treatment of IPF are ongoing (85). Given the complex interaction between TGFβ secretion and tissue stiffness mediated by myofibroblast contraction, ECM deposition, and cross-linking with further TGFβ release, it is possible that the effective treatment of fibrosis will require combination therapy. For example, both Simtuzumab, a monoclonal antibody to LOXL2, and Relaxin (an inhibitor of myofibroblast contraction) have individually lacked efficacy for fibrotic disease in Phase 2/3 trials (86). However, a recent preclinical trial demonstrated a reduction in airway fibrosis using Relaxin and anti-LOXL2 antibody together (87).

Clinical trials assessing antifibrotic efficacy of currently available drugs in CD have not yet been initiated. Three times daily oral doses of pirfenidone commenced at time of transplantation and continued for 7 days reduced TGFβ expression and intestinal fibrosis in an intestinal transplant mouse model (88), suggesting that it may prevent fibrosis in CD. Animal models of intestinal fibrosis will provide an opportunity for preclinical testing of future drugs that target signaling pathways (23, 89), and therapies to reverse as well as prevent fibrosis will be required (35). A separate but related challenge still to be addressed will be the development of biomarkers for the accurate categorization of patients at risk of, or in the early stages of fibrosis, in order to intervene in CD at an early stage with existing or future antifibrotic drugs.

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ES: conception and drafting of article. ES, BB, and UK: revision and final approval of article.

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CASE REPORT
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Pneumocystis jirovecii Pneumonia in Pediatric Inflammatory Bowel Disease: A Case Report and Literature Review

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Immunosuppressive therapy is a known risk factor for opportunistic infections. We report the first case of severe *Pneumocystis jirovecii* infection requiring intensive care in a pediatric patient with inflammatory bowel disease (IBD). The literature was reviewed and there were 92 reported cases of *Pneumocystis* pneumonia (PCP) in patients with IBD. Most sources were case reports and there was likely reporting bias toward patients receiving immunomodulators, anti-tumor necrosis factor (anti-TNF) therapy, and those who died. Overall, 56% of patients were males and 58% had Crohn's disease. The median age was 45 years (interquartile range 30–68, range 8–78) and 86% of patients were lymphopenic. The case-fatality rate was 23%. Corticosteroids were used as IBD treatment in 88% of patients who subsequently developed PCP, 42% received thiopurines, 44% used anti-TNF therapy, and 15% received either cyclosporine or tacrolimus. Rates of mono, dual, triple, and quadruple immunosuppression therapy were 35, 35, 29, and 2%, respectively. This report highlights the importance of considering PCP in immunosuppressed lymphopenic pediatric IBD patients who present with unusual symptoms. Moreover, it should give gastroenterologists the impetus to limit immunosuppressive therapy to its minimal effective dose and consider options such as exclusive enteral nutrition wherever possible. Although there is no place for global PCP prophylaxis in IBD given the low incidence, in an era when there is increasing use of biologic agents with combination immunosuppressive therapy, the risk-benefit profile of PCP chemoprophylaxis should be revisited in selected cohorts such as patients on triple immunosuppression with corticosteroids, thiopurines, and a biological agent or calcineurin inhibitor, especially in lymphopenic individuals.

Keywords: *Pneumocystis jirovecii*, pneumocystis pneumonia, inflammatory bowel disease, pediatric, opportunistic infection, immunosuppressive therapy, lymphopenia

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INTRODUCTION

Immunosuppressive therapy is a known risk factor for opportunistic infections (1). We report a case that highlights the importance of considering opportunistic infection in immunosuppressed pediatric patients with inflammatory bowel disease (IBD) who present with unusual symptoms. We present the first case of severe *Pneumocystis jirovecii* (*P. jirovecii*) infection requiring intensive care in a pediatric patient with IBD.

CASE REPORT

A 12-year-old Caucasian girl was diagnosed with gastric and ileocolonic Crohn's disease (CD), Paris classification L3, L4a, B1, G1, having presented with growth failure and abdominal pain and having undergone endoscopic and magnetic resonance enterography assessment. She failed exclusive enteral nutrition (EEN) and was treated with oral prednisone tapered over 3 months with maintenance azathioprine (AZA) (2 mg/kg/day). Treatment resulted in resolution of clinical symptoms and improved biochemical markers. She had recurrence of symptoms after 9 months with abdominal pain and diarrhea. Infective work-up was negative and she was commenced on oral budesonide 9 mg and her AZA dose was increased (2.5 mg/kg/day). Thiopurine metabolites at this stage were normal [6-TGN 278 pmol/8 × 10^8 (normal range 230–400), 6-MMPN 734 pmol/8 × 10^8 (normal range <5,700)].

Three months later, she presented with a 3-day history of fever, dry cough, and progressive dyspnea. On examination, she was hypoxic with SaO_2 90% despite 15 l/min high flow oxygen. She had severe lymphopenia (0.0 × 10^9/l on manual count), elevated white cell count (10.8 × 10^9/l), raised C-reactive protein (268 mg/l), and lactate dehydrogenase was 763 U/l. Chest X-ray showed bilateral interstitial infiltrates (**Figure 1**). She required intensive care for bi-level positive airway pressure respiratory support. AZA and budesonide were discontinued and she was commenced on piperacillin–tazobactam, clarithromycin, and oseltamivir to provide empiric coverage against bacteria (including atypical) and influenza, with minimal improvement in symptoms. An induced sputum sample was negative for bacterial and viral pathogens [culture and broad panel polymerase chain reaction (PCR)] but revealed *P. jirovecii* on silver stain (**Figure 2**). Intravenous (IV) trimethoprim–sulfamethoxazole (TMP–SMX) 20 mg/kg TMP/100 mg/kg SMX and methylprednisone 1 mg/kg twice daily were initiated, which resulted in improvement in respiratory status and weaning of respiratory support over 7 days.

Prior to discharge, she was investigated for an underlying primary immunodeficiency. Human immunodeficiency (HIV) testing was negative and immunoglobulin levels were normal. B and T cell panel revealed a low absolute count of CD3, CD4, and CD8 (0.47, 0.36, 0.14 × 10^9/l respectively) with normal number of B cells (0.43 × 10^9/l), normal CD4/CD8 ratio (2.43), and a good response to previous diphtheria and tetanus vaccines. She remained lymphopenic during admission, but this slowly improved. Notably, at IBD diagnosis, she had normal lymphocyte levels [lymphocyte count 1.7–2.6 (normal range 0.9–3.5 × 10^9/l)]; however, after AZA initiation, she had intermittent lymphopenia (0.4–1.2 × 10^9/l). Thiopurine methyltransferase activity testing was unfortunately unavailable at diagnosis.

She was discharged on oral TMP–SMX for 21 days, having had 3 days of IV therapy. She received 3 days of methylprednisone followed by 5 days of oral prednisone, which was tapered over 2.5 months. AZA was restarted at a reduced dose (1.5 mg/kg/day). Within 2 months, she had normal lymphocyte numbers with a normal absolute CD4 count (0.56 × 10^9/l) and normal T-cell numbers. She developed an urticarial rash thought to be secondary to TMP–SMX 4 days post discharge and was changed to oral clindamycin and primaquine to complete the 21-day treatment course. She was then started on prophylactic oral dapsone, which was discontinued when she developed arthralgia. She has not subsequently taken PCP prophylaxis.

One year post-PCP admission, she was commenced on adalimumab monotherapy due to ongoing poor growth, abdominal pain, and an elevated fecal calprotectin (>1,800 μg/g). This resulted in resolution of symptoms, catch-up growth, and progression through puberty. She has had no further significant infections in the last 5 years and her lymphocyte count has remained stable.

LITERATURE REVIEW AND DISCUSSION

Overview of *P. jirovecii* Pneumonia

Pneumocystis jirovecii (formerly known as *Pneumocystis carinii*) is a ubiquitous opportunistic fungus, which causes

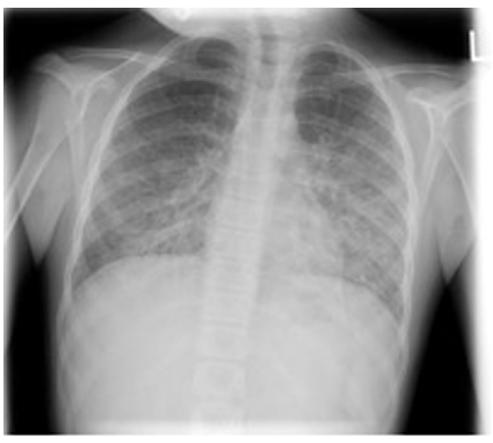

FIGURE 1 | Chest X-ray demonstrating bilateral pulmonary infiltrates caused by pneumocystis pneumonia.

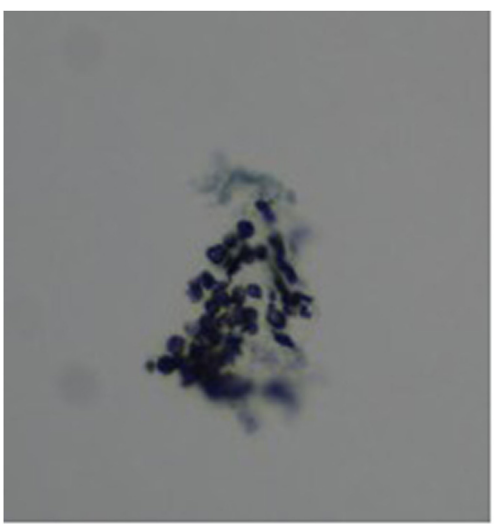

FIGURE 2 | Grocott–Gomori's methenamine silver stain of sputum specimen showing "cup shaped" *Pneumocystis jirovecii* cysts in small aggregates.

pneumonia [*Pneumocystis* pneumonia (PCP)]. It was first described in 1940s malnourished infants (2). In the 1980s, it was associated with HIV-infected patients with low CD4 counts (3). PCP continues to be an acquired immune deficiency syndrome-defining illness although the advent of antiretroviral therapy has resulted in incidence reduction (4). PCP has been recognized as a disease in children with primary cell-mediated immunodeficiency, patients receiving chemotherapy for hematological malignancies, solid organ, and bone marrow transplant recipients and in patients requiring immunosuppressants (5). Notably, the incidence in the immunosuppressed non-HIV population is increasing with the escalating use of immunosuppressive agents (4, 6, 7). Inflammatory autoimmune conditions such as IBD account for up to 20% of PCP in HIV-negative patients (8).

Pneumocystis jirovecii exposure appears to occur early in life and is often asymptomatic (9, 10). Reinfection of the immunosuppressed host through environmental or person-to-person transmission rather than reactivation from latency appears to be the major mode of acquisition in immunosuppressed patients (10). Effective host defenses against *P. jirovecii* are mediated by the innate, T-cell, and to a lesser extent, humoral immune responses. In immunosuppressed patients, the infection results in a dysfunctional immune response, composed of mononuclear cells, CD8 lymphocytes, and activated macrophages, which causes diffuse lung damage (11). Moreover, the inhaled *Pneumocystis* trophozoites inhibit epithelial repair processes within the alveoli resulting in severe lung damage (12).

Adult data show that unlike in HIV-infected individuals where presentation can be slow and insidious, non-HIV infected immunocompromised individuals can have an aggressive course often culminating in respiratory failure over several days (4, 13–15). PCP is characterized by a non-productive cough, fever, and dyspnea. On chest X-ray, diffuse bilateral interstitial pulmonary infiltrates are most common (4). In equivocal cases, high resolution chest computed tomography is more sensitive and commonly shows diffuse ground glass opacity (16).

Pneumocystis jirovecii cannot be cultured, therefore, definitive diagnosis is made by visualization of the organism's cysts and trophozoites. This can be achieved using induced sputum, bronchioalveolar lavage specimens, or lung tissue stained with Grocott–Gomori's methenamine silver or visualized using immunofluorescence. Unfortunately, sensitivity is <67% in the non-HIV infected patient due to low numbers of *P. jirovecii* (17). PCR testing of *P. jirovecii* nucleic acid in respiratory samples is more sensitive for diagnosis; however, discrimination between colonization and actual disease has been challenging. Quantitative PCR is thought to help differentiate between these two entities better than qualitative PCR with recent research focusing on oral washes as a less invasive diagnostic tool (18, 19).

First line treatment of PCP is IV TMP–SMX (15–20 mg/kg/24 h of the trimethoprim component in three divided doses) due to excellent tissue penetration, rapid response, and low cost (4). Adverse reactions include leukopenia, thrombocytopenia, and rashes including Stevens–Johnson syndrome (5). However, reactions appear less common in children (20). Alternative therapy includes clindamycin plus primaquine, pentamidine, and dapsone (21). Atovaquone has been used for mild disease as it is less effective than TMP–SMX, but better tolerated (22). Corticosteroids have been used as an adjuvant treatment in severe disease, reducing respiratory failure and the length of intensive care unit stay (23).

PCP mortality rates are between 20 and 60% in immunocompromised non-HIV infected individuals in contrast to rates of 10–20% in HIV-infected patients (4, 7, 13, 15, 24, 25). The more severe course is thought to be secondary to a more disseminated pulmonary inflammatory response and diagnostic delay (4, 26).

PCP and IBD

A retrospective cohort study determined that IBD patients were at an elevated risk of PCP compared to the general population with an increased relative risk (hazard ratio 2.96; 95% CI 1.75–4.29) but low absolute risk (0.03%) (26). The incidence of PCP in immunosuppressed IBD patients [thiopurines, methotrexate, calcineurin inhibitors, anti-tumor necrosis factor (anti-TNF) agents, or steroids] was 32/100,000 patient-years (PY) compared to 5.5/100,000 PY for non-immunosuppressed IBD patients (26). The risk appeared greater in CD compared with ulcerative colitis (UC) (26). In another population-based IBD cohort, double immunosuppression resulted in a higher risk of PCP than monotherapy (0.6/100 vs. 0.3/100 PY). There were few patients on triple therapy making risk analysis challenging (27).

There is only one published case of a child with IBD developing PCP. The 8-year-old CD patient, on infliximab monotherapy, developed PCP with concurrent disseminated histoplasmosis after 15 months therapy. His disease course was not severe. He did not require respiratory support and responded to TMP–SMX. He was maintained on budesonide with no PCP prophylaxis (28). PCP has been described in adult IBD patients on corticosteroids, calcineurin inhibitors, thiopurines, and anti-TNF agents (8, 26, 29–33).

We performed a literature review of PCP in IBD. A database search for studies on MEDLINE, EMBASE, and the Cochrane Controlled Trials Registry was performed. References of included articles were searched for further studies. **Table 1** summarizes the details of published IBD studies reporting patients with PCP. Most sources were case reports [evidence level 5 (34)], and there was likely reporting bias toward immunomodulators, anti-TNF therapy, and patients who died. There were 92 reported cases of PCP in patients with IBD in the English literature, of which 56% were males and 58% had CD. The median age was 45 years [interquartile range (IQR) 30–68, range 8–78]. There was little documented information about CD4 counts at PCP diagnosis; however, 86% (12/14) of patients were lymphopenic. The case-fatality rate (CFR) was 23% (7/31) based on the reported outcome data. Where medication was documented, rates of mono, dual, triple, and quadruple immunosuppression therapy were 35% (18/52), 35% (18/52), 29% (15/52), and 2% (1/52), respectively. The numbers were too small to comment on the effect of incremental risk with increasing numbers of immunosuppressants. Corticosteroids, as mono, dual or triple therapy, were used as IBD treatment, in 88% (46/52) of patients who developed PCP. 42% (22/52)

TABLE 1 | Summary of published literature of inflammatory bowel disease patients who developed pneumocystis pneumonia ($n = 92$).

Reference	No of patients	Disease subtype	Gender	Age (years)	Medication at time of PJP	Single (S), dual (D), triple (TR), quadruple (Q) immunosuppression	Outcome
Khatchatourian and Seaton (30)	1	UC	M	68	CS + T	D	Died
Lee et al. (35)	1	UC	M	21	CS + T	D	Survived
Takenaka et al. (36)	3	UC (100%)	F (67%)	26–68	CS + T (67%), CS (33%)	S (33%) D (67%)	Survived (100%)
Bernstein et al. (29)	2	UC (100%)	M (100%)	32–73	CS (100%)	S (100%)	Died (50%)
Escher et al. (32)	2	UC (100%)	M (100%)	72–74	CS + Tac, CS + Tac + T	D (50%) TR (50%)	Died (100%)
Art et al. (37)[a]	3	UC (100%)	M	32	CS + CSA + T (100%)	TR (100%)	Died (33%)
Quan et al. (38)	1	UC	M	63	CS + CSA	D	Died
Scott et al. (31)	1	UC	M	43	CS + CSA	D	Survived
Smith and Hanauer (39)	1	UC	M	32	CS + CSA	D	Survived
Desales et al. (8)	1	CD	M	36	CS + T + anti-TNF	TR	Survived
Lawrance et al. (40)	2	CD (100%)	F (50%)	18–32	CS + T + anti-TNF, CS + MTX + MMF + anti-TNF	TR (50%) Q (50%)	Survived (100%)
Cotter et al. (27)	3	UC (67%)	M (100%)	63–78	MTX + anti-TNF, CS + anti-TNF, T	S (33%) D (67%)	Survived (100%)
Tschudy and Michail (28)	1	CD	M	8	Anti-TNF	S	Survived
Iwama et al. (41)	1	CD	M	51	Anti-TNF	S	Survived
Velayos and Sandborn (42)	1	CD	M	19	T + anti-TNF	D	Survived
Kaur and Mahl (43)	1	CD	M	59	CS + anti-TNF	D	Died
Stratakos et al. (44)	1	CD	F	77	CS + anti-TNF	D	Survived
Estrada et al. (45)	1	UC	M	45	CS + T + anti-TNF	TR	Survived
Sharma and Rao (46)	1	CD	F	36	CS + T + anti-TNF	TR	Survived
Seddik et al. (47)	1	CD	M	29	CS + T + anti-TNF	TR	Survived
Itaba et al. (48)	1	CD	F	57	CS + T + anti-TNF	TR	Survived
DeFilippis et al. (49)	1	CD	F	56	CS + MTX + anti-TNF	TR	Survived
Long et al. (26)	38	CD (55%) UC (40%) ND (5%)	F (55%)	43–57 IQR	CS: 11/38 T: 1/38 T + CS: 5/38 CS + 2IM: 2/38 CS + IM + anti-TNF: 2/38	S: 12/38 (32%) D: 5/38 (13.2%) TR: 4/38 (10.5%)	ND
Kaur and Mahl (33)	16	CD (88%)	ND	ND	Anti-TNF (100%)	ND	ND
Fillatre et al. (14)	1	ND	ND	ND	ND	ND	ND
Bienvenu et al. (7)	4	ND	ND	ND	ND	ND	ND
Roblot et al. (50)	2	ND	ND	ND	ND	ND	ND

CS, corticosteroids; T, thiopurines (azathioprine/6-Mercaptopurine); Tac, tacrolimus; CSA, cyclosporine; anti-TNF, anti-tumor necrosis factor therapy (adalimumab or infliximab); MTX, methotrexate; MMF, mycophenolate mofetil; IMs, immunomodulators (thiopurine, tacrolimus); ND, not documented; CD, Crohn's disease; UC, ulcerative colitis; IQR, interquartile range.

[a]*Data on gender and age only available for the one patient who died.*

received thiopurines, 44% (23/52) took anti-TNF therapy, and 15% (8/52) used either cyclosporine or tacrolimus.

PCP and Corticosteroids

Corticosteroids are known to reduce CD4 lymphocytes, which predisposes to PCP development (51). Corticosteroids have emerged as a major contributor to PCP in the non-HIV immunosuppressed population and the risk is particularly increased at or above 16 mg of prednisolone (50–52). The median therapy duration prior to PCP was 8–12 weeks (50, 51). Interestingly, in some studies, the disease only became apparent when corticosteroids were tapered (24, 50, 51).

PCP and Thiopurines

Thiopurines (AZA and 6-mercaptopurine) can inhibit cell-mediated immunity, which influences PCP development. In the literature, two IBD patients on thiopurine monotherapy developed PCP, 10 were on dual therapy, as was the case with our patient, and 10 were on triple therapy. The CFR in this group was 19% (3/16), where outcome data were available.

PCP and Calcineurin Inhibitors

Cyclosporine works by inhibiting production of IL-2 by helper T-cells and by affecting T-cell, B-cell, neutrophil, and mast cell function. In the literature, 50% (4/8) of IBD patients with PCP died (32, 37, 38). Such cases have prompted a discussion regarding the role of prophylactic antibiotics in patients on cyclosporine; however, the limited cases and reporting bias needs to be considered (37, 53, 54).

PCP and Methotrexate

Three patients in the IBD literature developed PCP while on methotrexate in combination with corticosteroid and anti-TNF therapy. Methotrexate has been implicated in the development of PCP in rheumatoid arthritis with 28 documented cases, 25% of whom died (55, 56).

PCP and Anti-TNF Agents

Cytokines inhibited by anti-TNF agents are involved in the host response to PCP resulting in reduced PCP clearance. Moreover, anti-TNF therapy can lower CD4 counts making patients more susceptible to PCP (57). Review of the Food and Drug Administration Adverse Event Reporting System data between 1998 and 2003 identified 84 patients with PCP associated with infliximab, with a mean age of 55 years; 19% of patients had IBD. Concomitant immunosuppressive agents included immunomodulators (66%), corticosteroids (50%), and cyclosporine (5%). The CFR was 27% (33). PCP has been reported to occur 9–14 weeks after the first infliximab induction dose (33, 57, 58). Notably, Colombel et al. did not report any cases of PCP associated with 3,160 patients (in six global clinical trials) on adalimumab (59). However, there are two case reports of PCP in IBD patients on adalimumab (8, 40). In total, 162 cases of PCP are reported in the IBD and rheumatology literature associated with anti-TNF therapy, which includes pediatric patients. Of the 138 patients with outcome information available, 20% died. Unfortunately, studies do not always specify whether single, double, or triple immunosuppression was used (8, 26–28, 33, 40–49, 56, 58, 60, 61). Data on the incidence of PCP in patients on anti-TNF agents are largely based on rheumatoid arthritis studies and most are population based. The majority of studies report incidence rates <50 cases per 100,000 PY (62). Results are hampered by heterogeneity in the method of PCP diagnosis, moreover, discrimination between *P. jirovecii* colonization and actual disease can be challenging.

There are no published data on PCP associated with vedolizumab, ustekinumab, or other novel IBD treatments (63, 64). More long-term data are required to assess safety profiles of these agents.

Other Risk Factors for PCP in Non-HIV Infected Immunosuppressed Patients

The use of multiple immunosuppressive agents incrementally increases the risk of opportunistic infection in IBD; moreover, malnutrition and surgery can play a role, although this has not been specifically addressed in PCP (1, 27, 40, 51, 52, 62). As documented in our case, lymphopenia (especially CD4 count <300 cells/mm^3) has been associated with increased risk of PCP in 60–95% of cases (5, 24, 50, 65). Notably, not all immunosuppressed patients will present with lymphopenia (24, 65).

Long et al. reviewed a case series of 38 IBD patients who developed PCP and showed that rates of hospitalization at some stage within 60 days of PCP were high (50%) (26). Moreover, patients had higher rates of comorbidities, principally, lung disease and diabetes mellitus, compared to the background population. Advanced age >65 years was found to be an additional risk (26).

PCP in Other Pediatric Non-HIV Immunosuppressive Conditions

A population-based cohort study in juvenile idiopathic arthritis patients reported an incidence of 7/100,000 PY (66). Multiple immunosuppressive agents and lymphopenia are important risk factors in rheumatic diseases (67). The risk of PCP in pediatric cancer depends on the malignancy type and chemotherapy category. Lymphoid malignancies have the highest risk with rates of 22–45% (68). The overall risk of PCP post solid organ transplant has been estimated to be 5–15% in the absence of PCP prophylaxis (5). Risk factors include malnutrition, previous cytomegalovirus infection, and underlying lung disease. Medications such as steroids, antilymphocyte agents, calcineurin inhibitors, and biological agents such as alemtuzumab (anti-CD52 monoclonal antibody) have also been implicated (5).

Prophylaxis against PCP

In a 2012 survey of PCP prophylaxis practice by gastroenterologists, 11% prescribed prophylaxis to their patients with IBD on combination therapy. Gastroenterologists were more likely to prescribe if they had previous practical experience of PCP, or practiced in an academic center (69).

The effects of PCP can be severe, however, prevention entails using drugs with adverse effects that may counterbalance the benefits, as occurred in our patient. The guidelines for PCP prophylaxis in HIV-infected patients have been universally adopted, but there is a lack of consensus on prophylaxis with TMP–SMX in IBD patients. A recent Cochrane meta-analysis of prophylactic treatment with TMP–SMX in non-HIV infected patients included 1,412 patients, of which 520 were children. The authors were unable to find published literature addressing prophylaxis in IBD. In patients with hematological cancers and transplant recipients, they reported an 85% reduction in PCP incidence with prophylaxis and PCP-related mortality reduced by 83% with few adverse events reported (20). No children in the included studies had a severe adverse event compared to 3.1% of adults suggesting a lower probability of harm in children.

There is insufficient evidence to recommend PCP prophylaxis for all IBD patients on immunosuppressive monotherapy; however, with increasing number of immunosuppressive agents, the risk of opportunistic infection is known to increase (1, 27, 62). Large studies addressing the specific incremental increased risk associated with multiple immunosuppressive agents especially triple therapy have not been done. This needs to be considered, as the use of multiple immunosuppressive agents are increasing. A recent study showed that approximately half of patients started on anti-TNF therapy were already on two immunosuppressive agents (40). Dietary therapies (EEN, partial enteral nutrition, and exclusion diets) in CD are increasingly being used and investigated and are attractive options to limit immunosuppression and improve nutrition in this population (70–72).

A study using simulation modeling to address cost and comparative effectiveness of PCP prophylaxis in CD concluded that at the present incidence, routine chemoprophylaxis was not cost effective but, based on limited data, it may be effective in triple immunosuppressive therapy (73). The 2014 European Crohn's and Colitis opportunistic infection guidelines recommend PCP prophylaxis in IBD patients on triple immunosuppression including either a calcineurin inhibitor or anti-TNF therapy. This recommendation is based on expert gastroenterology and infectious disease opinion (74). Other practical approaches proposed for patients on high-dose steroids and multiple immunosuppressants include measurement

of CD4 counts in those patients with a total lymphocyte count of <600 cells/mm^3 as a means of identifying patients at risk of infections, although not all patients who develop PCP have lymphopenia, therefore, precluding its use as an isolated risk identification guide (24, 43, 65, 75). Evaluating the utility of measuring CD4 counts in IBD patients on multiple immunosuppressants should be considered.

CONCLUSION

We described a case of severe PCP in an immunosuppressed lymphopenic pediatric IBD patient. This case reiterates the importance of limiting immunosuppression to its minimal effective dose. Although the incidence of PCP in the IBD population is low, it is an aggressive condition that has a higher relative risk in IBD patients compared to the general population. Moreover, it is associated with significant morbidity and mortality. PCP should be considered in the differential diagnosis of immunosuppressed pediatric IBD patients who develop respiratory symptoms, with a low threshold for treatment. Although there is no place for global PCP prophylaxis in IBD, in an era when there is increasing use of biologic agents with combination immunosuppressive therapy, the risk-benefit profile of PCP prophylaxis should be revisited in selected cohorts such as patients on triple immunosuppression with corticosteroids, thiopurines, and a biological agent or calcineurin inhibitor especially in lymphopenic individuals. Further studies are required to guide definitive PCP prophylaxis in high risk subgroups of IBD patients.

INFORMED CONSENT

Written informed consent for publication of the case report and figures was obtained from the patient and parents.

AUTHOR CONTRIBUTIONS

SL performed the literature review and wrote the manuscript. MS and KJ critically reviewed the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CASE REPORT
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The Adjunctive Role of Nutritional Therapy in the Management of Phlegmon in Two Children with Crohn's Disease

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Crohn's disease may be complicated by the development of penetrating (fistulizing) or structuring complications. The presentation of an intra-abdominal phlegmon or abscess with or without an associated fistula has traditionally required surgical intervention. This series of two cases illustrates a beneficial role of non-surgical management, with parenteral and then enteral nutrition playing central roles. This report further elaborates the potential adjunctive role of enteral nutrition in the management of this complication of CD.

Keywords: children, Crohn disease, exclusive enteral nutrition, nutritional treatment, phelgmon

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INTRODUCTION

Crohn's disease (CD), one type of inflammatory bowel disease (IBD), may present at any age, with up to a quarter of cases diagnosed in childhood or adolescence (1). CD may be complicated by the development of fistulae or penetrating disease, which may lead to an extra-intestinal collection, or phlegmon. Generally, fistulizing disease is uncommon at diagnosis in children, with most children having uncomplicated inflammatory disease at that time (2). Typically, the management of a phlegmon might include drainage and/or early resection.

Exclusive enteral nutrition (EEN) is a well-established therapy to induce remission in pediatric-onset CD (3). This intervention comprises a period of a liquid diet, using a polymeric or elemental formula, with the exclusion of normal foods during this time. EEN induces remission in more than 85% of individuals and is associated with numerous other benefits, including high rates of mucosal healing, nutritional improvements, and enhanced bone health (3).

We have previously reported the use of EEN in an adolescent with an entero-vesical fistula (4) and in the management of children with peri-anal fistulae (5). A recent report indicates that EEN may have an adjunctive role in the management of phlegmon in adults with CD (6). This report illustrates the beneficial role played by EEN, in conjunction with other interventions, in two cases of children with phlegmon development early in the course of their CD.

REPORT OF CASES

Case 1

A 12-year-old boy presented to a peripheral hospital with a 12-month history of increasingly severe abdominal pain over a 4-week period prior to his hospital admission. He was noted to have had poor growth and anorexia for approximately 12 months, with recent weight loss (estimated at 2 kg) and lethargy. He had otherwise been well in the past, and there was no family history of note.

On initial examination, he was found to be mildly febrile but not tachycardic, with a tender mass palpable in the lower central abdomen. Weight was 26.7 kg with height of 143.7 cm and body mass index (BMI) of 12.9. Initial bloods showed a microcytic anemia, with normal white cell and platelet counts. CRP was elevated (237 mg/l) and albumin was at the lower range of normal (35 g/l). Abdominal CT scan with oral contrast showed circumferential wall thickening over the distal 17 cm of the ileum, with an adjacent loculated collection of fluid and gas measuring at least 4 cm in diameter. There was mild proximal distention in the ileum, but no other small bowel lesions were seen, although sigmoid colon wall thickening was also evident. The child was then transferred to a tertiary pediatric center (Christchurch Hospital) for further and ongoing care.

Upon arrival in Christchurch, he was commenced on intravenous cefuroxime and metronidazole (based on his weight). Oral intake was ceased and he was started on parenteral nutrition (PN), with progressive increases in fat and protein delivery over the following 3 days. A peripherally located central venous catheter (PICC) was placed on the second day. Given the recent completion of the CT scan, cross-sectional imaging was not repeated: however, an ultrasound scan (USS) was undertaken on the day of arrival. This imaging confirmed the phlegmon, with suggestion of a fistula from the involved ileum to the collection.

During the rest of his stay, he underwent serial USSs. On Day 13, the collection was resolving, with improved ileal wall thickening. By day 19, the collection was felt to have reduced to a volume of 1 ml, with normal peristalsis of the persistently mildly thickened ileal wall. By day 25, the USS showed resolution of the collection, with further reduction in mural thickening. No new changes were evident.

He continued PN and antibiotics for 25 days. During this time, he progressively improved clinically and biochemically. The course was, however, complicated by the development of a drug reaction on day 20 (thought most likely secondary to cefuroxime). On day 25, he commenced enteral nutrition with Fortisip (Nutricia), which was well tolerated. He progressively increased his oral intake with this polymeric formula, until day 29 at which time he was on full EEN (8 × 200 ml fortisips daily, providing 2400 kcal/day) whereupon his PN was ceased. On day 33, he was discharged home, with ongoing EEN for 8 weeks in total. He was also commenced on oral azathioprine prior to discharge following standard protocol. Approximately 2 months post presentation, he underwent diagnostic upper gastrointestinal endoscopy and ileocolonoscopy. Endoscopically, there were scattered colonic ulcers with mild chronic inflammatory changes seen histologically.

Subsequently, he was seen on a regular basis with serial measurement of inflammatory markers. Three months after diagnosis, he was noted to be well, with no symptoms, had no positive examination findings, and had gained approximately 6 kg. CRP at this time was 2, along with normal albumin, platelets, and ESR. Sixteen months post diagnosis, he was well on maintenance azathioprine, with no symptoms, satisfactory growth, normal examination findings, and with normal inflammatory markers (CRP 3, albumin 36, platelets 326, and hemoglobin of 119). Ongoing follow-up continues.

Case 2

This girl presented at 12 years of age with a history of several months of diarrhea and abdominal pain. She had anorexia with weight loss of 3 kg in the preceding weeks. At presentation, she weighed 49.25 kg and was 158.1 cm tall (with BMI of 19.7). Abdominal examination was unremarkable with no mass evident. Initial blood tests showed elevated CRP but normal platelet count. Abdominal USS showed ileal wall thickening with no extra-intestinal abnormalities. She proceeded to undergo an endoscopic assessment, with no specific abnormalities seen. Histologically, focal active gastritis and colonic eosinophilia was seen (unfortunately, ileal biopsies were not obtained).

Given her disease location, and presentation pattern, EEN was recommended and commenced on the day following her endoscopy. She started this therapy without concerns and was able to be discharged home 2 days later.

However, she re-presented with increased abdominal pain 3 days after discharge. She was found to have localized tenderness in the right iliac fossa, but no clear palpable mass evident. She proceeded to have an USS, which suggested a phlegmon. Repeat blood tests showed an elevated CRP (268 mg/l) and neutrophilia (15.1×10^9/l). After reviewing these results, she was commenced on intravenous antibiotics (metronidazole, gentamicin and amoxicillin) and placed on full intravenous fluids (nil by mouth). Magnetic resonance enterography (MRE) with oral and intravenous contrast was booked and PICC line placement was arranged for the following day. MRE confirmed the USS impressions of a phlegmon, with marked ileal wall thickening extending proximally from the ileocecal valve for 20 cm. The small phlegmon was seen lying anterior to the most severely involved area of small bowel with no fistulous tract evident and no other abnormality evident on the imaging.

She was managed with PN for a fortnight, during which time her inflammatory markers fell (CRP 4 g/l at day 11). Repeat USS imaging after 2 weeks of PN showed marked improvement of the phlegmon but showed continued ileal wall thickening. She then slowly recommenced EEN and was able to establish full EEN intake orally by day 17 (at which point PN was ceased). She was also commenced on azathioprine in standard fashion and discharged home on day 18.

Over the following period of time, she was followed as an outpatient. She completed 8 weeks of EEN and resumed normal diet subsequently. After this, she remained well, with maintenance EN and azathioprine (dose at 2.5 mg/kg/day with satisfactory 6-thioguanine nucleotide levels). Follow-up included subsequent USS imaging, which showed no recurrence of collection, and routine review of serum inflammatory markers (with no increase in CRP).

Unfortunately, 18 months following diagnosis, she represented with abdominal pain and was found to have a palpable tender mass in the right iliac fossa. She was admitted and underwent a further USS followed by a second MRE, along with

routine blood testing. Imaging showed recurrence of phlegmon without evidence of fistula or any other changes. She was again commenced on PN, with prompt improvements (clinically, biochemically, and radiologically) in the following 2 weeks. She restarted EEN at this time orally and quickly reached required daily volumes (7–8 fortisips daily, providing 2,100–2,400 kcal daily).

Given her recurrent presentation, surgical review was requested during this admission and arrangements were made for elective ileocecal resection. In the interim, she continued EEN. Resection was completed approximately 2 months later, with 17 cm of ileum resected along 8 cm of colon and primary re-anastomosis completed. Post-operatively she was managed with 3 months of oral metronidazole and continued oral azathioprine. Routine surveillance colonoscopy 6 months after her operation showed >6 aphthous ulcers at and distal to the anastomosis, with no stricture or other changes. She was subsequently commenced on adalimumab (standard dosage) in combination with azathioprine. Subsequently, over the following 5 years, she has been well, with no recurrent fistulizing disease, or other complication of CD.

DISCUSSION

These two cases illustrate the potential adjunctive role of nutritional interventions in the management of ileal CD complicated by the development of phlegmon. Both children had radiological features of a collection adjacent to the distal ileum, with a fistula demonstrated in one instance. Both children were managed in the same fashion with initial gut rest and intravenous nutrition, antibiotics, and subsequent EEN. Although the second child had recurrence of disease many months later, the acute management on this second occasion was similar and again successful. Case 1 has, however, had no further disease complications over more than 18 months of further observation. In addition to the previous case reports of EEN having roles in the management of fistulizing CD in other locations (4, 5), and a report of the benefits of EEN in the management of phlegmon in 33 adults (6), these two cases further illustrate that EEN (as a part of a managed package of conservative measures) may also have a role in this complication of CD.

The benefits of EEN in the induction of remission of active CD have been described over the last decades, with the first comparative data coming from O'Morain and colleagues in 1986 (7). Numerous studies have clearly shown that this intervention is safe and effective, with particular benefits in children and adolescents (3, 8). EEN has also been shown to enhance quality of life in children (9, 10). Consequently, EEN is now recommended as the first line management for active IBD in consensus statements (11, 12) and used in many pediatric centers in this manner.

In addition to the roles of inducing remission, EEN also clearly leads to high rates of mucosal healing, along with macro- and micro-nutritional benefits and enhanced bone health (3, 8). Furthermore, this therapy is associated with few side effects. Although the understanding of the mechanisms by which EEN acts are incomplete, data do demonstrate that EEN leads to substantial changes in the patterns of the intestinal microbiome and enhanced epithelial barrier function (3).

The adult and pediatric studies evaluating the roles of EEN in active CD have typically included subjects with luminal disease, with exclusion of those with fistulizing complications (8, 13). Previous short reports demonstrated that EEN had a role in the management of an entero-vesical fistula in an adolescent boy with active CD (4) and also in the management of two children with peri-anal fistulae (5).

Adult reports have also illustrated the benefits of EEN for fistulizing CD. A Chinese report showed that 30 of 48 adults with entero-cutaneous fistulae had complete healing after up to 3 months of EEN (14). In that series of patients, CRP level and BMI scores were associated with outcome. Similarly, EEN was successfully utilized in the management of inflammatory strictures in a series of Chinese adults (15). Thirty-five of 50 patients who completed 12 weeks of EEN achieved radiological remission, while 42 had clinical remission. Another recent report demonstrated that EEN was helpful in a group of Chinese adults with intra-abdominal abscess ($n = 33$) or stenosis ($n = 10$): EEN lead to resolution or improvement in the size of phlegmon (6). Furthermore, the use of EEN in a group of 51 adults with structuring or penetrating CD resulted in a reduction in the need for surgical intervention: one quarter of this group did not subsequently require operation (16).

The management of a phlegmon or intra-abdominal abscess in the context of CD has variously included peripheral drainage, antibiotics, and early surgical intervention (17–19). While there are no direct comparisons between these managements in the current report, the conservative nutritional approach outlined in these two cases suggests that this may be a valid option to consider. This report is limited as it involves just two children, who were assessed in slightly different manners and their outcomes reviewed retrospectively. Further careful prospective evaluation of this approach in children with this complication of CD is now required.

ETHICS STATEMENT

This case report does not include any identifying features throughout. Accordingly, formal approval from the University of Otago Ethics Committee was not required. Written informed consent was obtained from Patient 2 and the parents of Patient 1 with their agreement to the publication of this case report.

AUTHOR CONTRIBUTIONS

AD prepared the initial draft of the manuscript and participated in the drafting of the work. SB participated in the drafting of the manuscript. Both authors approved the final version of the manuscript.

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REVIEW
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Pancreatic Involvement in Pediatric Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) is a chronic condition that includes two clinical entities: Crohn's disease and ulcerative colitis. Although both entities mainly affect the gastrointestinal tract are considered multisystemic diseases and may present extraintestinal manifestations involving other organs and systems. Pancreatic involvement in Pediatric IBD includes a heterogeneous group of clinical entities like acute pancreatitis, chronic pancreatitis, autoimmune pancreatitis, asymptomatic exocrine pancreatic insufficiency, increased pancreatic enzyme levels, structural abnormalities, and granulomatous inflammation. Although the mechanism for pancreatic involvement in IBD is not clearly elucidated, is important to keep in mind the association of these two entities in order to perform a prompt diagnosis and establish an appropriate treatment. The objective of this review is to update the available evidence on pancreatic involvement in children with IBD.

Keywords: inflammatory bowel disease, pancreatitis, pancreatic enzyme, extraintestinal manifestations, children

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BACKGROUND

Inflammatory bowel disease (IBD) is a chronic and recurrent condition that encompasses two clinical entities: Crohn's disease (CD) and ulcerative colitis (UC), which occur in a genetically susceptible individual. Numerous environmental factors influence the microbiota, giving rise to an excessive immune response, intestinal barrier function impairment and, ultimately, inflammation with subsequent tissue damage (1). Although both entities preferentially affect the gastrointestinal tract, the conditions can involve other organs and systems (skin, eyes, mouth, osteoarticular, hepatobiliary system, etc.), given that they are multisystem diseases (2).

We can find extraintestinal manifestations (EIM) that are the result of inflammatory or autoimmune phenomena and extraintestinal complications, secondary to metabolic abnormalities, adverse effects of drugs or anatomical abnormalities (3). The incidence of EIM in adult patients with IBD is around 30–40%. Two pediatric studies have shown similar results. The PediIBD Consortium Registry with a cohort of 1,649 patients showed that up to 29% of the patients had at least one EIM during the 15 years of follow-up (4), with 6% of them presenting before the diagnosis of IBD. Similar results were obtained from a series by Dotson et al. in the Pediatric IBD Collaborative Research Group Registry (5). This series included 1,009 patients, 28.2% of them experienced at least one EIM episode. It is noteworthy that those with more extensive or more severe disease had a greater risk of developing an EIM. Up to 130 EIMs were reported, but fortunately most of them were rare. The most common EIMs are musculoskeletal (axial and/or peripheral arthritis), mucocutaneous (erythema nodosum, pyoderma gangrenosum, or aphthous stomatitis), ocular (episcleritis and uveitis), and hepatobiliary. Pancreatic involvement in IBD may be due to EIM with a broad spectrum of presentations including

acute pancreatitis (AP), chronic pancreatitis (CP), autoimmune pancreatitis (AIP), asymptomatic exocrine pancreatic insufficiency, increased pancreatic enzyme levels, structural abnormalities, and granulomatous inflammation (6). The pancreas can also be affected during the course of the disease due to drugs used for the treatment of IBD (drug-related pancreatitis or pancreatitis associated with total parenteral nutrition). The objective of this review is to update the available evidence on pancreatic involvement in children with IBD.

ACUTE PANCREATITIS

Acute pancreatitis is the most common pancreatic process associated with IBD. In fact, IBD is one of the five most important causes of AP in children (7). In adults, the incidence rate of AP is 1.2% for UC and 3.1% for CD and is 2.1 and 4.3 times more common in UC and CD, respectively, when compared with the general population (3). There are scarce published data on its actual incidence in children with IBD. In a retrospective study of 124 patients (97 cases of CD, 16 of UC, and 11 of IBD-U) by Le Large-Guiheneuf et al. (8), 18 patients (14.5%) presented symptomatic pancreatitis and 15 (12.5%) presented asymptomatic pancreatitis. The authors found an association between the inflammatory activity, the severity of the flare and the onset of AP. There was also an association with the drugs used (25%), the duodenal location of the CD (18%), and the presence of hepatobiliary complications (15%). In another study that included 101 children (79 cases of UC and 22 of CD), 4.5% of the patients with CD and 5.1% with UC developed AP (9). In a recent multicenter Italian study that recruited 649 patients (302 cases of UC, 297 of CD, and 50 of unclassified IBD), 27 patients (4.1%) showed increased levels of amylase and lipase, meeting only 11 (1.7%) the criteria for AP. The increased levels were more common in girls with colon involvement and active disease.

The most common situation is that AP appears once IBD has been diagnosed; however, in a small group of predominantly adult patients, AP was reported as the first clinical manifestation of IBD. Broide et al. (10) reported this condition in a multicenter study conducted in seven hospital centers in Israel. The study only included patients who had developed AP before the IBD diagnosis. The authors identified 30 episodes of pancreatitis in 12 patients (7 males, 10 children and 2 adults) from a cohort of 3,960 patients with IBD (3,500 adults and 460 children). The incidence rate in children was 2.17% at a mean age of 13 ± 4.8 years versus 0.06% for the adults. The mean time between the first AP episode and the IBD diagnosis was 24 (range 1–156) weeks. Of the 12 patients, 6 developed UC (4 cases of pancolitis), and 6 developed CD (2 cases of colonic, 2 of ileocolonic, and 2 of small intestine CD). Despite the fact that 9/12 (75%) patients had moderate-severe disease, the subsequent course was favorable, with a mean hospital stay of barely 3.5 days (range 0–18 days).

Etiology

The causes of AP in patients with IBD can be summarized as follows: pharmacological causes, hepatobiliary diseases [cholelithiasis, duodenal obstruction and primary sclerosing cholangitis (PSC)], idiopathic causes, and granulomatous inflammation of the bile duct or ampulla of Vater (11).

Pharmacological Causes

Most of the drugs used for treating IBD could be responsible for the onset of AP (azathioprine, mercaptopurine, cyclosporine, sulfasalazine, 5-ASA, metronidazole, and steroids) mediated by different mechanisms (direct toxicity, hypersensitivity, dyslipidemia, and secondary hypercalcemia). Of the drugs included by Bai et al. (11), the American Gastroenterology Association has classified aminosalicylates and 6-MP (does not reference azathioprine) as drugs that are definitely associated with pancreatitis. The rest of the drugs were previously listed as probably associated (12). Toxic pancreatitis occurs in the first weeks of treatment. The symptoms are typical of AP, although they are usually mild and resolve after discontinuing the treatment (13). The risk of thiopurine-induced AP is low (14) and is more prevalent in children with CD (4.9%) than in those with UC (1.1%). The onset of AP with azathioprine does not represent an absolute contraindication for the use of mercaptopurine (13, 15). Before establishing the diagnosis of drug-induced pancreatitis, other causes for the AP must be ruled out. The definitive diagnosis of drug-induced pancreatitis requires three additional criteria to the International Study Group of Pediatric Pancreatitis: In Search for a Cure (INSPPIRE) criteria (16): a temporal sequence between the introduction of the drug and the onset of AP, the cessation of symptoms after discontinuing the treatment and a reappearance of AP after re-exposure. In practice, reintroduction of the drug is not standard practice (17).

Hepatobiliary Disease

The incidence of cholelithiasis in IBD is higher than in the general population, particularly in CD (there is no higher risk in UC than in the general population), although this complication is extremely rare in children. The risk of cholelithiasis is higher in patients with extensive ileal involvement and increases after ileal resection due to a reduction in enterohepatic circulation. The risk factors described for developing cholelithiasis in adults with CD include the following: previous intestinal resection (>30 cm), age (>50 years), involvement of the ileum and colon, duration of the disease (>10 years), number of hospitalizations (≥3), number of relapses (≥3), total parenteral nutrition, and length of hospital stay.

Duodenal involvement in CD is a rare cause of AP in adults (0.5–4% of all cases) and can be due to papillary obstruction, reflux of the duodenal content toward the pancreatic ducts or to the presence of duodenal-pancreatic fistulae or between duodenum and duct of Wirsung (1, 2, 14).

Most patients with PSC have UC (75–80%), and 3–10% of patients with IBD have associated PSC. Although patients with PSC can develop bile duct stricture, the onset of AP in these patients is extremely rare.

Idiopathic Causes

The association between colon involvement and AP development remains unexplained in many cases, and there are several

hypotheses on this subject. A possible explanation could be the fact that the colon is the main source of bacteria responsible for pancreatic necrosis and that subtotal colectomy prior to the development of AP in rats decreased their mortality (10). In the study by Martinelli et al. (18), 3 of the 11 patients who presented AP did so at the onset of the disease. The systemic inflammatory nature and the hypercoagulability state of active IBD can predispose patients to developing AP, which would explain the correlation with the extent and severity of the flare-up (17). In addition, there is a risk in IBD of thromboembolic phenomena secondary to a hypercoagulability state due to an imbalance between procoagulant and anticoagulant factors and genetic determinants (17, 19). These thrombi can affect the gastrointestinal circulation, favoring pancreatic ischemia and the development of AP. Controlling the IBD through anti-TNF and the subsequent blocking of the TNF-mediated inflammatory cascade decreases the risk of AP (17, 20). The protein associated with pancreatitis (PAP-1), with anti-apoptotic and anti-inflammatory effects, inhibits NFkB activation, cytokine production and the expression of adhesion molecules in the inflamed tissue. An increase in the mRNA of PAP has been confirmed in active IBD, as well as high plasma PAP levels in patients when compared with the controls, both of which correlate with the clinical and endoscopic severity. Experimental data suggest that PAP-1 overexpression in the pancreas during trinitrobenzenesulfonic acid-induced colitis in murine models is the result of inflammatory stress that occurs in the pancreas of mice during experimental colitis. The failure of the intestinal mucosal barrier function plays a vital role in the course and development of AP, favoring bacterial translocation and development of infectious complications. Furthermore, changes in pancreatic epithelial tight-junctions are one of the earliest events that occur in AP in murine models. The *MYO9B* gene encodes for an unconventional myosin compound that can activate the domain of the GTPase protein (Rho-GAP), which regulates the tight-junction assembly and maintains the selectivity of the paracellular pathway in enterocytes (17, 21, 22). Genetic abnormalities in the MY09B gene have been found in both IBD and celiac disease, two entities where impairment of intestinal patency plays an important role. Polymorphisms of a single nucleotide in the MYO9B gene could explain the relationship between the two entities (IBD and AP) (17, 21, 22). It has also been postulated that abnormal MUC1 expression could be responsible for the pancreatic involvement in patients with IBD (3, 23). MUC1 is a transmembrane glycoprotein that is expressed on the apical membrane of the cells of the ductal epithelium of various organs and is also present in the colon epithelium of patients with IBD. MUC1 is abnormally expressed in innate immune cells. These MUC1-specific cells migrate not only to the colon but also to the pancreas of mice with IBD, which suggests that pancreatic inflammation could be the result of abnormal and proinflammatory MUC1 expression (23). Despite the fact that approximately 27–39% of patients with CD and 0–5% of those who have UC have high levels of anti-pancreas antibodies, the role of these antibodies in IBD-associated pancreatitis has not yet been elucidated and does not appear to be related to either the disease activity or with the onset of pancreatitis.

Diagnosis

According to the Atlanta criteria and the INSPPIRE definitions (16), the diagnosis of AP is established with the presence of at least two of the following three criteria:

1. Clinical symptoms: abdominal pain, nausea, vomiting, and back pain.
2. Increase serum amylase/lipase levels at least three times the normal value.
3. Radiological abnormalities: pancreatic edema in ultrasonography or computed tomography.

In asymptomatic patients, increased pancreatic enzyme levels are not sufficient to establish the diagnosis of AP. Martinelli et al. (18) reported 16 cases (68.8% boys) of increased amylase/lipase levels without the patients meeting the AP criteria. This finding was correlated with the extent of the CD and the degree of inflammatory activity in the intestinal mucosa. The elevation of pancreatic enzymes (serum amylase/lipase levels expressed as the number of times the levels were above the normal value) was significantly lower in the IBD group without pancreatitis than in those of the AP group: amylase 1.6 (0.6–3.2) vs. 2.48 (1.5–4.1), $p = 0.009$; lipase 2.3 (0.6–8.4) vs. 13.6 (8.5–42.7), $p = 0.001$. A noteworthy fact is that, unlike what happened in those with AP, the results of the imaging tests were normal in the patients with increased pancreatic enzyme levels. The mechanism underlying this EIM is not well known but could be due to direct damage to the pancreas or perhaps to the increase in the passage of pancreatic enzymes from the intestinal lumen to the bloodstream due to an increase in intestinal permeability. The presence of macroamylasemia must be ruled out as the cause for increased plasma amylase levels (24). Regardless of the underlying mechanism for this impairment, pancreatic involvement must be screened, because 25% of patients who presented increased pancreatic enzyme levels at the onset developed AP in the following 6 months (3, 18). The prognosis was satisfactory in most cases.

Treatment

The treatment for AP in patients with IBD does not differ from that of other causes of pancreatitis with the exception of the need for withdrawing the responsible drug (2, 25).

CHRONIC PANCREATITIS

Chronic pancreatitis is a chronic inflammatory process resulting from the destruction and fibrosis of exocrine pancreatic tissue and, in some cases, loss of endocrine pancreatic function. Unlike its acute form, which is defined by clinical criteria, CP is defined by morphological criteria. In adults, the incidence rate for IBD-associated CP varies (depending on the diagnostic criteria employed) between 1.2 and 1.5% (1:3 in women with UC and 1:1.3 in women with CD). IBD-associated CP is an extremely rare condition in children. Only two cases have been well documented, both after recurrent episodes of AP. One of the patients had the F1052V mutation in heterozygosity in the

CFTR gene (18). In the other case, the results of the sweat test and the mutation study of the PRSS and SPINK1 genes (26) were normal. While AP can be considered an event, CP is a process and is occasionally the end result of repeated episodes of AP (27). The diagnosis of CP is based on a combination of clinical findings (abdominal pain, weight loss, and diabetes mellitus), functional impairment (documented exocrine pancreatic insufficiency), and imaging studies. Despite the fact that the etiological study was insufficient for ruling out the most common causes of recurrent pancreatitis, it is worth highlighting the importance of pancreatic function follow-up in these patients.

AUTOIMMUNE PANCREATITIS

Autoimmune pancreatitis is a rare entity and is most common in adults in whom two well-differentiated types are known. Type 1 AIP or lymphoplasmacytic sclerosing pancreatitis is the most common form, mostly affecting men in their 60s, and is currently considered the pancreatic manifestation of IgG4-related disease accompanied by other systemic manifestations such as sclerosing cholangitis, sclerosing sialadenitis and retroperitoneal fibrosis (3). Type 2 AIP or idiopathic duct centric pancreatitis is not characterized by increased IgG4 levels and affects adults in their 40s and 50s, has no predominance by sex and is the form frequently associated with IBD. The prevalence of IBD in patients with AIP is higher than in general population, mostly in UC patients.

In children, only 48 cases of AIP have been reported from a systematic review and cases collected from a multicenter group (INSPPITE) and those from Cliniques St-Luc (28–31). The mean age at diagnosis was 13 years (range 2–17). Both types of AIP can present as painless jaundice, weight loss, diabetes, and mild abdominal pain (3). Abdominal pain (43/47, 91%) and obstructive jaundice (20/47, 42%) were the most common symptoms at diagnosis. Diagnostic criteria for AIP include histology, imaging studies, serology (IgG4), involvement of other organs and response to treatment to steroids. IgG4 levels, one of the distinctive elements of AIP in adults, was high in only 9/40 (21%) cases. The results of the imaging tests were abnormal for all patients, with the following findings: overall or focal hypointense pancreatic hypertrophy (39/47, 83%), irregularity of the main pancreatic duct (29/43, 67%), and common bile duct narrowing (25/43, 58%). For those patients who underwent pancreatic biopsy, the most common findings consisted of a combination of lymphoplasmacytic inflammation, pancreatic fibrosis and ductal granulocytic infiltration (18/25%). The response to steroid treatment was fast. Twenty-seven percent of the patients developed other autoimmune phenomena: nine developed IBD (two CD and seven UC).

The AIP complications include diabetes (3/27, 11%) and exocrine pancreatic insufficiency with the need for replacement therapy with pancreatic enzymes (4/25, 16%). The findings recorded in this series suggest that AIP in children is a different subtype of pancreatitis than that described in adults and has the following characteristics:

1. High rate of abdominal pain at diagnosis.
2. Low rate of high IgG4 levels.
3. Ductal or parenchymal abnormalities in imaging tests.
4. Lymphoplasmacytic, granulocytic infiltrate with fibrosis.
5. Good response to steroid treatment.

SILENT PANCREATIC DISORDERS

There have been reports of histological changes, pancreatic duct abnormalities and exocrine pancreatic insufficiency in patients with IBD but no pancreatic symptoms. These patients were likely underdiagnosed due to the symptoms (abdominal pain, diarrhea, etc.) being attributed to poor control of the inflammatory disease. Necropsy studies published in the 1950s showed that 38% of the 39 patients with CD showed pancreatic fibrosis and that 53% of the 86 patients with UC had chronic interstitial pancreatitis. Some 16.4% of the patients with UC and no previous history of alcohol consumption or previous episodes of AP showed pancreatic ducts disorders in the magnetic resonance cholangiopancreatography. Regardless of the imaging test findings or increased pancreatic enzyme levels, 4–18% of the patients with IBD presented exocrine pancreatic insufficiency, although two-thirds of the patients returned to normal fecal elastase levels at 4–6 months of progression, suggesting that the condition involved transient pancreatic insufficiency (1).

CONCLUSION

Pancreatic involvement in IBD encompasses a heterogeneous group of clinical entities that can occur prior, together with or after the onset of IBD. The mechanisms responsible for pancreatic involvement in patients with IBD are not clearly elucidated.

Acute pancreatitis is the most common presentation but the increase in pancreatic enzyme levels is not sufficient to establish the diagnosis of AP in asymptomatic patients. It is important to follow diagnostic criteria for the different entities, as well as to establish the correct treatment in each case. We should reliably document drug-induced pancreatitis to avoid unnecessarily discontinuing the treatment, given the limited therapeutic options available.

AUTHOR CONTRIBUTIONS

All the authors have contributed to the review of published literature regarding the topic, writing the article, and the manuscript review.

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Outcomes of a National Cohort of Children with Acute Severe Ulcerative Colitis

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Aim: All Irish children with ulcerative colitis (UC) attend the National Centre for Paediatric Gastroenterology at Our Lady's Children's Hospital, Crumlin. The aim of this study was to determine the outcomes of children with acute severe ulcerative colitis (ASC) and the impact of infliximab on these outcomes following its introduction for this indication in 2011.

Methods: A retrospective chart review of all patients admitted with ASC between January 1, 2009 and December 31, 2015 was undertaken. Patients were identified from the departmental database cross-referenced with the hospital inpatient enquiry system. Inpatients with a paediatric ulcerative colitis activity index (PUCAI) of ≥65 were included. Data collected included baseline demographic and laboratory data, concomitant treatments, PUCAI scores on days 3 and 5, second-line treatments, surgery, and discharge outcomes. Infliximab dose, frequency, and available therapeutic drug monitoring results were recorded, along with clinical response outcomes (remission, primary, and secondary loss of response). The cohort was sub-analysed to determine if there was any era effect pre- and post-introduction of infliximab (2009–2010 and 2011–2015, respectively).

Results: Fifty-five patients (M:F = 1.4:1) were treated for acute severe colitis over the study period (8 in the pre-infliximab and 47 in the post-infliximab era) and 46/55 (86%) had steroid-refractory disease. Of these, 7/8 (88%) required colectomy in the pre-infliximab era, compared with 15/47 (36%) in the post-infliximab era. The remission rate with second-line infliximab was 61% at maximal follow-up. There were no identifiable factors that predicted likely success or failure of infliximab, including gender, CRP, day-3 and day-5 PUCAI scores. Of the 33 patients treated with infliximab, dose increase was required in 23/33 (70%); 21/33 (64%) received an accelerated dose schedule, and 9/33 (27%) eventually needed colectomy. Primary and secondary loss of response to infliximab was seen in one and nine patients, respectively.

Conclusion: This is the first population-based study of the outcomes of severe UC in Irish children, and suggests a higher burden of steroid-refractory disease compared with previous international studies. While infliximab treatment has led to reduction in colectomy rates, a significant proportion of patients lose therapeutic effect.

Keywords: acute, severe, ulcerative, colitis, infliximab, children, paediatric, steroid

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INTRODUCTION

Ulcerative colitis (UC) is a chronic relapsing inflammatory disease of the colon affecting both adults and children that extends variably from the rectum to the caecum. The incidence of paediatric inflammatory bowel disease (IBD) in the UK and Ireland has been reported as 5.2 per 100,000 per year (1). The highest rates of UC internationally have been reported in northern Europe, Canada, Unites States, and Australia (2). While a recent systematic review showed an overall stable or decreasing incidence of IBD in North America and Europe, an increasing incidence has been reported in Africa, Asia, and South America (3). There is evidence of a global increase in the incidence of paediatric-onset IBD (4). While there are many similarities between adult-onset and childhood-onset UC, higher admissions rates for acute severe exacerbations along with higher colectomy rates have been reported in the paediatric population (5, 6). In children, 15–30% of those with UC experience an acute severe attack at some point (7). Other considerations that are unique to the paediatric population include disease and treatment impacts on growth, pubertal development, bone health, educational impact, and psychosocial wellbeing, making timely optimal treatment a priority.

The paediatric ulcerative colitis activity index (PUCAI) is a validated scoring system used to assess the severity of paediatric UC that is incorporated in the joint ECCO and ESPGHAN guidelines for management of paediatric UC (8–10). The index includes six variables (abdominal pain, rectal bleeding, stool consistency, frequency of stools, nocturnal stools, and limitation of activity) that are individually scored to give a total PUCAI of between 0 and 85. Mild disease is defined as a PUCAI of 10–35, moderate disease as PUCAI of 40–60, and severe disease as a PUCAI of 65 or above.

Acute severe ulcerative colitis (ASC) is considered a medical emergency requiring immediate management, in order to prevent complications such as intestinal perforation, peritonitis, sepsis, and even death. Since the 1950s, intravenous corticosteroid (IVCS) therapy has been shown to reduce mortality in ASC and has become the mainstay of treatment (11). Steroid-refractory cases make up a significant proportion of the paediatric population, as it is estimated to occur in one-third of ASC cases (12). Recently, published data on a large cohort of children with treatment naive UC, who were initially managed with IVCS, showed remission rates of 40%, need for anti-TNF treatment in 24% and a 3% colectomy rate at 4 weeks post-commencement of treatment. Strong predictors for the need for additional medical treatment or colectomy in this same group of children included, high-total Mayo clinical and endoscopic severity score, decreasing serum albumin, rectal biopsy eosinophil count, and rectal biopsy surface viliform changes (13). Effectiveness of first-line treatment with IVCS can be measured using PUCAI on day 3 and day 5 of treatment (14). At day 3 of treatment, a PUCAI of >45 is considered indicative of steroid-refractory disease and at this time second-line treatment options should be considered (10). Colectomy was historically the second-line treatment option for steroid-refractory ASC in many centres. While this may still be appropriate in certain cases, current European guidelines also propose the use of second-line medical management with agents such as infliximab, tacrolimus, and cyclosporine (10).

Infliximab is a chimeric monoclonal antibody to human TNF-α, which has been shown to be effective in modulating intestinal inflammation in UC (15). A landmark prospective study of paediatric ASC reported reduced colectomy rates in patients receiving infliximab by discharge (9%) and 1-year follow-up (19%) (14). The use of infliximab in children with ASC has been reported in six-case series, with a pooled short-term response rate of 75% and a long-term response rate of 64% (12, 14, 16). Infliximab was first introduced for the treatment of ASC in children at Our Lady's Children's Hospital, Crumlin (OLCHC) in 2011. The aims of this study were to determine the outcomes of Irish children with severe UC; the impact of infliximab on these outcomes following its introduction; and the outcomes of infliximab therapy in children with UC since its introduction.

MATERIALS AND METHODS

The study was carried out at the National Centre for Paediatric Gastroenterology, OLCHC, Dublin, which is the only tertiary referral centre for paediatric gastroenterology in the Republic of Ireland. A retrospective chart review was carried out of all patients admitted with an episode of ASC between January 1, 2009 and December 31, 2015. Patients were identified using the hospital discharge coding system and the department's patient database. Patients were eligible for inclusion in the study if they had a new or pre-existing diagnosis of UC *and* required acute admission *and* had a PUCAI score of ≥65 and/or a physician global assessment rating of severe disease activity. Although the PUCAI score was still being developed at the beginning of the study period, the elements of the score were being recorded as part of standard clinical care of the time.

Patients were phenotyped according to the Paris classification (17) following diagnostic work up according to the Porto criteria (18). Data collected included gender, age at diagnosis of UC, age at episode of ASC, and endoscopic disease location and histological findings. PUCAI scores on days 3, 5 and at discharge were also recorded along with laboratory values including CRP, ESR, and albumin values. Clinical outcomes recorded included remission (defined as PUCAI ≤10), colectomy, adjunctive medications, adverse treatment events, and post-colectomy complications.

A subgroup analysis compared outcomes in patients treated prior to and after the introduction of infliximab at OLCHC. The standard induction protocol with infliximab for ASC involved doses of 5 mg/kg at weeks 0, 2, and 6 (9, 10). Standard maintenance protocol involved doses of 5 mg/kg every 8 weeks from week 14. Depending on clinical response, modifications to the regimen were made at clinician discretion accordingly. Patients were considered to have undergone accelerated dosage when either infliximab dose/kilogram was increased and/or if infliximab was administered sooner than outlined in the standard protocol. Outcomes of all UC cases managed with infliximab were analysed including clinical response and progression to colectomy.

Statistical analysis was carried out using IBM SPSS Statistics version 24 (IBM, Armonk, NY, USA). Continuous variables were analysed using Mann–Whitney or *t*-test and categorical variables analysed using χ^2 test or Fisher's exact test. Kaplan–Meier survival

curve was used to show the colectomy free interval from infliximab commencement. Multivariate logistic regression was used to evaluate the associations between remission and predictor factors.

RESULTS

A total of 55 patients with an episode of ASC were identified over the 7-year study period with a median follow-up of 29 months (IQR, 16.5–48.5). Patient characteristics are described in **Table 1**. Females accounted for 58% (32/55) of the population. The average age at episode of ASC was 11.7 years (SD ± 3.9 years), with an average age at diagnosis of UC of 11.1 years (SD ± 3.7 years). Endoscopic disease location was documented as left-sided colitis in 2% (1/55), extensive colitis in 7% (4/55), and pan colitis was present in 91% (50/55) of cases. Extra-intestinal manifestations of IBD were present in 6% (3/55) of cases. Steroid-dependent disease was reported in 40% (22/55). Prior to commencement of treatment for ASC, 64% (35/55) of the patients were on maintenance medical therapy, including oral 5-ASA's (62%; 34/55), rectal 5-ASA's (24%; 13/55), and oral steroids (46%; 25/55). A further 5% ($n = 3$) subsequently commenced adalimumab. The mean duration of IVCS treatment was 7.3 days (SD ± 3.8 days), with average doses equivalent to 2.3 mg/kg/day of methylprednisolone. The mean PUCAI scores on day 3 and day 5 IVCS were 57 (SD ± 15.1) and 51 (SD ± 20.2), respectively.

Thirty-three patients were subsequently treated with infliximab as second-line medical therapy, with 18/33 (55%) receiving our standard induction protocol. The remaining patients had protocol modifications in response to their clinical status, including increased dosage and reduced intervals between infusions. Most patients (69%; 23/33) subsequently required an increase in dose to 10 mg/kg. The mean number of infusions before dose escalation was four doses (SD ± 2.8 doses). A similar proportion, 64% (21/33), required a reduction in interval time between infusions. Fifteen patients required escalation prior to week 14 (i.e., during the induction period). Six of these 15 patients ultimately needed a colectomy within a median time of 2.6 months (IQR, 1.2–2.9), 6 in remission as at maximum follow-up, 1 patient switched to adalimumab, and the remainder had some degree of ongoing disease activity. Conversely, 18 patients completed the standard induction, 3 of whom eventually had a colectomy within 2.6 months (IQR, 2.3–20.5) and 14 of whom reached remission on infliximab. The mean number of days from infliximab commencement to dose escalation in the 23 patients was 111 days (SD ± 130.2 days). Of the 23 patients, 52% (12/23) were in remission as at last date of follow-up and 30% (7/23) had required a colectomy. The mean duration of time from diagnosis of UC to starting infliximab was 9.8 months (SD ± 12.5 months) and the mean duration of subsequent infliximab therapy was 2.4 years (SD ± 1.1 years) from the first infusion.

There was an apparent increase in the mean annual number of cases of ASC from 2011, from 4.0 to 9.4 cases per year, in tandem with the increased incidence of IBD in the Irish population, as previously reported (19).

Following first-line treatment with IVCS, only 16% (9/55) of the study population achieved remission. The remaining 86% (46/55) required further treatment in the form of either repeat course of IVCS, colectomy, or infliximab. These were divided into two groups: those managed prior to the introduction of infliximab in 2011 ($n = 7$) and those managed from 2011 onwards ($n = 39$). Of those treated prior to 2011, 86% (6/7) had a colectomy as second-line treatment. The remaining patient had a second course of IVCS but ultimately required a colectomy. In those treated from 2011 onwards, 15% (6/39) underwent colectomy as second-line therapy (including cases whereby parents declined infliximab), while the remaining 85% (33/39) were commenced on infliximab. Of the 47 patients managed after 2011, 14 underwent a colectomy. **Figure 1** gives a breakdown of reasons necessitating colectomies.

Twenty of 33 patients on infliximab (60%) entered remission following induction, 8 (24%) underwent a colectomy within a median time of 1.9 months (IQR, 3–5) from the commencement of infliximab. Of the remaining five patients, two lost infliximab response and switched to adalimumab and three remained on infliximab without fully achieving remission. Of the 20 patients who entered remission on infliximab, 80% (16/20) were still on infliximab by the end of the study period, 15% (3/20) were successfully weaned off infliximab following prolonged sustained remission and one patient discontinued infliximab due to severe periorbital cellulitis subsequently required a colectomy. Nine patients were commenced on thiopurines after starting infliximab. Of the total 33 patients on infliximab, only 3 patients had adverse events, 1 episode of flushing and acute desaturations, which recovered, 1 liver injury and 1 severe periorbital cellulitis. The latter two patients required discontinuation of infliximab.

There were 14 patients treated after 2011 who required a colectomy, 6 as second-line treatment for steroid-refractory ASC and 8 as third-line management, following treatment failure on infliximab. Gender, age at diagnosis, CRP, PUCAI on days 3 and 5,

TABLE 1 | Characteristics of patients with ASC.

Total patient number	***N* = 55**
Male: female	1:1.4
Average age at ASC	11.8 years (SD ± 3.9)
Average age at diagnosis of UC	11.1 years (SD ± 3.7)
Endoscopic disease location	
– Left-sided colitis	2% ($n = 1$)
– Extensive colitis	7% ($n = 4$)
– Pancolitis	91% ($n = 50$)
Extra-intestinal manifestations of IBD	6% ($n = 3$)
Treatment at time of admission	($n = 35$)
– Topical/rectal 5-ASA	24% ($n = 13$)
– Oral steroids	46% ($n = 25$)
– Oral 5-ASA	62% ($n = 34$)
PUCAI score:	
– Day 3 of IVCS	57 (SD ± 15.1)
– Day 5 of IVCS	51 (SD ± 20.2)
Colectomy	($n = 22$)
– Acute/urgent setting	20% ($n = 11$)
– Elective/semi-elective	20% ($n = 11$)
Remission status at time of maximal follow-up	56% ($n = 29$)

ASC, acute severe ulcerative colitis; IBD, inflammatory bowel disease; IVCS, intravenous corticosteroids; UC, ulcerative colitis.

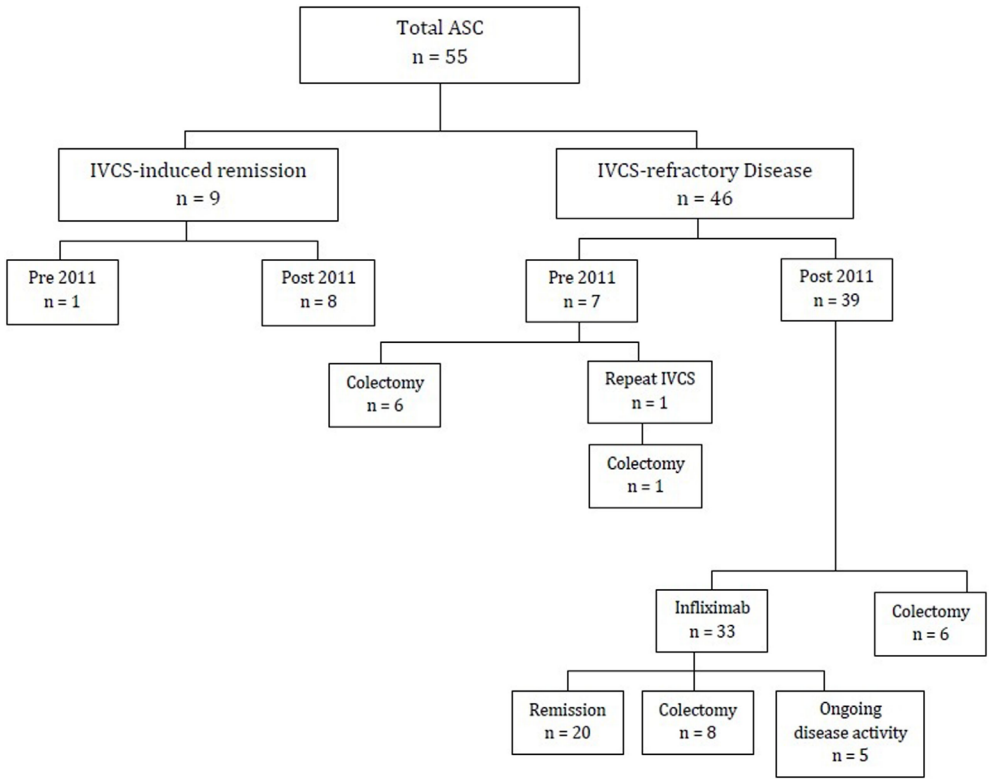

FIGURE 1 | Flow diagram of patients diagnosed with ASC (2009–2015). ASC, acute severe ulcerative colitis; IVCS, intravenous corticosteroids.

along with receiving standard induction protocol did not significantly increase the odds of a colectomy (**Table 2**). Kaplan–Meier survival estimates of the cumulative probability for colectomy in patients treated with infliximab were 24% at 1 year (**Figure 2**), with 50% undergoing colectomy by week 10 following treatment with infliximab. The 20 patients who achieved remission on infliximab were further examined in order to identify any variables, which may predict successful remission. The variables of gender, age at diagnosis, CRP, PUCAI on days 3 and 5, receipt of standard induction protocol, and first episode of ASC were all analysed. None of these variables showed a statistical significance in predicting outcome (**Table 3**).

Age of disease onset, based on the Paris classification, did not have a significant impact on the outcome of infliximab therapy in our group. Most infliximab-treated patients were over the age of 10 years at diagnosis (79%; 26/33). There were fewer girls aged less than 10 years at diagnosis ($n = 3$), but fewer boys ($n = 7$) aged over 10 at diagnosis and the mean age of diagnosis was 7 years (SD ± 2.5 years) and 14 years (SD ± 1.6 years) in the respective groups.

TABLE 2 | Predictive factors for requiring a colectomy post 2011.

Variable	Odds ratio	CI	*P*-value
Gender	1.1	0.016–73.868	0.965
Age at diagnosis	1.3	0.637–2.442	0.520
CRP	1.0	0.986–1.045	0.318
PUCAI D3	1.0	0.844–1.11	0.646
PUCAI D5	0.3	0.907–1.374	0.300
Standard induction	0.1	0.002–2.642	0.152

CRP, C reactive protein; PUCAI, paediatric ulcerative colitis activity index.

DISCUSSION

This is the first report of the outcomes of ASC in the Irish paediatric population, spanning the time immediately before and since the introduction of infliximab as second-line therapy for ASC. This was a single-centre retrospective cohort study, but as Ireland has a single paediatric IBD centre, the data are representative of the Irish population. In this study, the overall colectomy rate of 40% is in keeping with data from studies of a similar era. A 2007 Canadian study reported colectomy rates at discharge, 1 and 6 years of 42, 58, and 61%, respectively (5). Since the introduction of infliximab in the study centre for ASC rescue therapy, the colectomy rate has declined compared with the preceding epoch, although the periods were neither of equivalent time nor patient number. Previous adult and paediatric studies have shown infliximab to significantly reduce colectomy rates in ASC, reducing surgical rates to 19% at 1-year follow-up in one large paediatric study (14, 20, 21). The impact of infliximab rescue therapy on longer-term outcomes of ASC is less clear. Data from this current study are limited in this regard, but the long-term colectomy rate in published studies remains considerable (13, 22).

The clinical response to infliximab therapy in this study was broadly in keeping with previously published reports, with 70% of

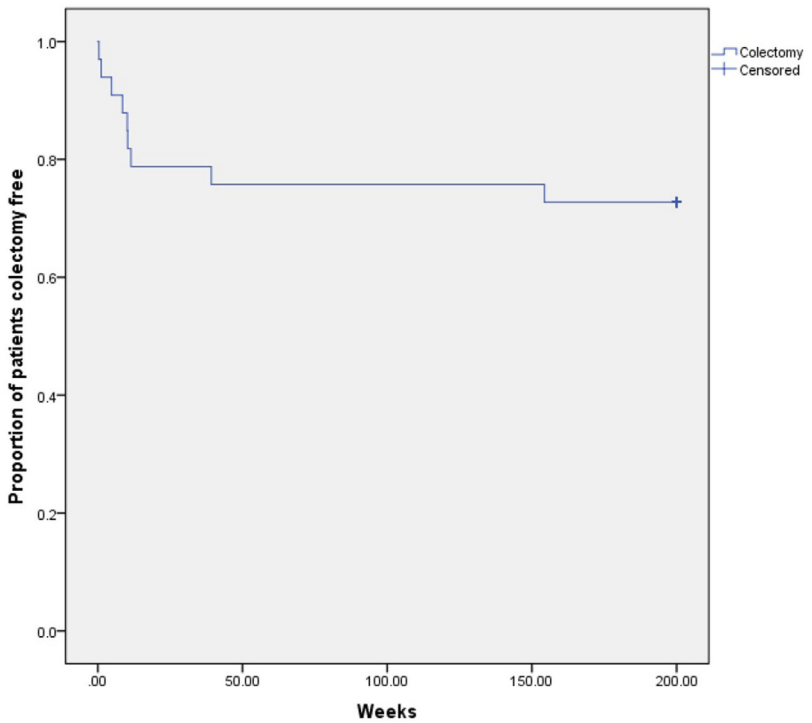

FIGURE 2 | Kaplan–Meier cumulative time to colectomy for infliximab patients.

TABLE 3 | Predictive factors for achieving remission on infliximab.

Variable	Odds ratio	CI	*P*-value
Gender	4.381	0.273–70.189	0.297
Age at diagnosis	0.813	0.536–1.235	0.332
CRP	0.993	0.971–1.015	0.510
PUCAI D3	1.095	0.962–1.248	0.171
PUCAI D5	0.911	0.805–1.03	0.137
Standard induction	6.355	0.479–84.347	0.161
First episode of ASC	0.311	0.009–10.722	0.518

CRP, C reactive protein; PUCAI, paediatric ulcerative colitis activity index; ASC, acute severe ulcerative colitis.

patients showing sustained clinical response. A 2011 systematic review showed a 1-year response rate of 64% (12). Other studies have demonstrated long-term response rates from 57 to 61% (16, 23) in paediatric UC. In contrast, data from this study also suggest a less favourable response to steroids in Irish children with ASC. The reasons for this are not clear from this particular study, but are under investigation in an ongoing prospective study of paediatric IBD in Ireland. While patient numbers are small, even the IVCS response rates in the infliximab era are less than reported in previous studies (5). The mean day 3 PUCAI scores of patients requiring second-line therapy in this study were consistent with prediction models previously published by Turner et al. (8). The impact of international paediatric ASC management guidelines on reported disease course and outcomes in this study is difficult to objectively quantify, but they have undoubtedly influenced the reported natural history of ASC in the immediate hospitalisation period. Evidence-based guidelines now advocate prompt planning and initiation of second-line therapy, guided by PUCAI score dynamics. It is no longer conscionable that children with unresponsive ASC, continue on indefinite IVCS therapy, as may have happened in the pre-infliximab era, and IVCS-refractory ASC is now diagnosed within days rather than weeks of admission.

This study includes the earliest patients with ASC in our centre that had protocol acceleration of infliximab treatment for UC. Recent reports have described potential benefits of accelerated infliximab induction regimens for optimal disease control and reducing the early colectomy rate in severe UC (24, 25). Shapiro et al. suggest that an initial intensive regimen is necessary in extensive disease (90% of the patients in this study) to maintain sustained efficacy (26). Therapeutic drug monitoring may be a useful adjunct to clinical judgment to identify which patients need therapy escalation with infliximab (27). In the current study, such monitoring was not available across the entire study period so we were unable to reach conclusions in this regard.

This study is a descriptive account of the outcomes of ASC in our national paediatric cohort over a 7-year period, but substantive extrapolation is tempered by the limitations of its retrospective design. It was not possible to extend the study before 2009 due to our resource limitations. The resulting small patient numbers and inequitable "era" groups curtailed the generation of more robust statistical findings and comparative analysis. The study period coincided with a significant and sustained increase in the incidence of paediatric IBD in Ireland, which may account in part for the increased occurrence of ASC over time (19, 28). The advent of the PUCAI score and published

guidelines for modern management of ASC also evolved during the study, adding to the potential heterogeneity of the clinical management observed. Small case numbers may account for the seemingly disproportionate numbers of IVCS refractory disease in our population. However, IVCS refractory ASC was substantial across the entire time period and objective measures including day 3 PUCAI scores were consistent with the published literature regarding need for second-line therapy. It is tempting to speculate that delayed access or delayed presentation of children from areas remote to our hospital may have contributed to disease burden by the time of their admission for IVCS treatment, but elucidating these data were not possible retrospectively.

This is the first study of paediatric ASC in the Irish population. It provides further supportive evidence of the beneficial impact of infliximab on the otherwise natural history of this condition in children, although a considerable proportion of children required escalation of infliximab therapy beyond a standard induction regimen. More robust prediction models for early identification of patients at risk of steroid unresponsiveness or needing infliximab escalation are keenly awaited from ongoing collaborative prospective studies.

ETHICS STATEMENT

The Research Ethics Committee of Our Lady's Children's Hospital, Crumlin approved the DOCHAS study. The committee does not require prior patient consent for endeavours that only involve retrospective chart reviews undertaken for the purpose of anonymised clinical data collection, audit, and analysis. The current study falls into the latter category.

AUTHOR CONTRIBUTIONS

AA, TR, RO, and SH contributed to the conception and design of this work along with the statistical analysis of this work. All authors contributed to the generation and organisation of the database. AA, RO, and SH wrote sections of the manuscript. All authors have read and approved the submitted version.

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CASE REPORT
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Anorexia Nervosa Complicating Pediatric Crohn Disease—Case Report and Literature Review

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Crohn disease and anorexia nervosa share common symptoms of weight loss and reduced oral intake. The prevalence of both disorders has increased over time. Symptoms of Crohn disease and anorexia nervosa can mimic each other leading to a delayed diagnosis and requiring complex, multidisciplinary management. Here we present a case of a 15 year old girl with Crohn disease who subsequently developed anorexia nervosa, and review the published literature on the occurrence of both diagnoses.

Keywords: Crohn disease, adolescent, anorexia nervosa, BMI, weight loss

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INTRODUCTION

Crohn disease (CD) is a chronic idiopathic inflammatory bowel disease characterized by transmural inflammation and granulomatous lesions in the bowel (1). Its prevalence is increasing worldwide (1). Presenting childhood features include anorexia, weight loss, diarrhea, abdominal pain, perianal disease, growth, and pubertal delay, with most children being diagnosed after age 10 years (1).

Anorexia nervosa (AN) is an eating disorder diagnosed by the following DSM V Criteria: restriction of energy intake relative to requirement leading to a significantly low body weight in the context of age, sex, developmental trajectory, and physical health; an intense fear of gaining weight or becoming fat, or persistent behavior that interferes with weight gain even though at a significantly low weight even though underweight; disturbance in the way one's body weight and shape is experienced, undue influence of body weight or shape on self- evaluation, or persistent lack of recognition of the seriousness of current low body weight (2). As per Swanson et al. (3), the lifetime prevalence of AN is 0.3% in both adolescent males and females and increasing in the developed world (3, 4). The prevalence of subthreshold AN- cases that appear similar but do not meet full diagnostic criteria- is 1.5% in females vs. 1% in males (3). The incidence of CD amongst adolescent males is 7.4 per 100,00 and 6 per 100,000 females per year. Benchimol et al. (5) Crohn disease should be considered in the differential diagnosis of AN, especially in younger patients (6, 7).

CASE

An 11 year old girl originally presented with a 3 month history of diarrhea, weight loss, perianal skin tags and a labial abscess. Her centiles for weight and height were <3rd and 25th centile respectively. Her diagnostic endoscopy and biopsies revealed ileocolonic ulceration and granulomatous inflammation, consistent with CD.She responded well to exclusive enteral nutrition (EEN) induction treatment and had sustained remission on thiopurine therapy for 18 months.

She then had a symptomatic relapse, including a 10 kg weight loss, and was commenced on infliximab, an anti-TNFα monoclonal antibody, which re-induced and maintained remission until age 15 years. Her weight had improved from the 3rd to the 25th centile. Infliximab dosage and infusion intervals were optimized according to therapeutic drug monitoring results. At clinical review aged 15 1/2 years, she reported excellent health without any symptoms.Notably, her weight was < 3rd centile, and lab results included raised inflammatory markers and hypoalbuminemia. There was deep ileocolonic ulceration on repeat endoscopy but neither fibrostenotic nor fistulating disease on radiologic imaging. The patient was admitted, adalimumab was commenced instead of infliximab and EEN was commenced at 2,400 kcal per day. By day 10 of admission her weight had continued to fall and her BMI was 12.4 kg/m2. Biochemical work up revealed a hyponatremic, hypokalemic metabolic alkalosis. Due to concerns of non-compliance with EEN, 24-h "one-on-one" supervision was commenced. Within days her weight increased. Following multidisciplinary team engagement, she divulged she was "terrified of being overweight," "hated" when she was in remission and felt "uncomfortable" when her weight was over 45 kg. She had been restricting her food intake and "idolized thin women" according to her mother. Following an adolescent psychiatry assessment, a diagnosis of AN was established. She ultimately required short-term parenteral nutrition until her disease stabilized and adequate feeding was re-established. She was discharged after 2 months of intensive child psychiatry and psychology input and has remained stable as an outpatient.

DISCUSSION

Crohn disease and anorexia nervosa share certain indistinguishable clinical features at diagnosis. A number of published reports highlight delays in diagnosis of CD due to an initial presumptive diagnosis of anorexia nervosa (8–10). Guidelines from the American Psychiatric Association now advise consideration and exclusion of gastrointestinal disorders when reaching the diagnosis of anorexia nervosa (3).

The literature regarding delayed diagnosis of AN in patients with CD is limited. Just 6 case reports, 6 case series and 2 cohort studies (pediatric and adult) were eligible for inclusion in a recent systematic review of inflammatory bowel disease and eating disorders (6). This included patients with an initial diagnosis of either an eating disorder or inflammatory bowel disease. Anorexia nervosa was more commonly associated with CD than ulcerative colitis. Of a total of 17 patients with detailed information, 5 "used" inflammatory bowel disease activity to exacerbate their weight loss. Notwithstanding the limited patient numbers involved, the prognosis of this dual pathology appears grim. Five patients required intestinal resection, 2 developed toxic megacolon and 1 died from intestinal perforation. It is tempting to speculate how inflammatory bowel disease may be a risk factor for eating disorders, given the focus on diet, fear of abdominal pain, poor body image and poor emotional wellbeing, factors also noted in the patient in this report (7).

There are just 3 pediatric reports to date of anorexia nervosa complicating CD. The first case is of an 11 year old girl who developed AN following corticosteroid treatment of CD (11). Another patient presented with both AN and CD leading to a complex and delayed diagnosis (12). The third case had significant weight loss despite good control of her underlying CD (13). Management included admission to psychiatric units in 2 cases, and psychotherapy in the third (11–13). It is possible that changing body habitus during disease course may be a potential trigger factor for the development of AN.

Gastroenterologists should maintain an index of suspicion for development of AN in pediatric IBD patients. Anorexia nervosa is classically more prevalent in adolescent than pre- adolescent patients, and among females rather than males (3). Obsessional, conforming and perfectionistic personality traits are common amongst patients with AN (14, 15). Ongoing weight loss out of proportion with disease activity is a clinical "red flag," especially among patients reporting satisfaction with current weight, normal dietary intake and good medication compliance (13). Reviewing weight and height centile charts at each clinic visit, regardless of reported clinical symptoms is essential. Concerns regarding changes to body image with treatment should also be anticipated and explored (10). Access to multidisciplinary team assessment of potential contributory biopsychosocial factors is essential for pediatric gastroenterologists. Factors including interpersonal relationships, bullying, family dynamics, and personality traits can influence the onset of AN (11, 12).

Inflammatory bowel diseases are associated with other mental health disorders (16). Eating disorders have also been associated with other auto-immune diseases (17). Rates of depression and anxiety in patients with CD are higher than in the reference population, even more than 5 years after initial diagnosis (16). Pediatric patients with inflammatory bowel disease have higher rates of depression, anxiety, and phobic disorders than either healthy controls or patients with other chronic pediatric illnesses (18). Multidisciplinary input and support for children and adolescents with inflammatory bowel disease is vital for elucidating such complex presentations and ongoing patient support.

Crohn disease is a complex and highly variable disease. The current case highlights the importance of considering eating disorders in patients with poor treatment responses, and of the challenge of discerning co-morbid AN in patients with significant inflammatory disease burden. Multidisciplinary input and support for children with CD disease is necessary, even while in remission. Concerns regarding non-compliance with treatments, abnormal eating habits or unexpected weight loss should prompt early multidisciplinary engagement.

ETHICS STATEMENT

Local requirements did not mandate Ethics Committee approval as this was a retrospective anonymised case report. Parental informed consent was obtained and permission given to include patient details.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Regulation of Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells (NF-κβ) in Inflammatory Bowel Diseases

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Inflammatory bowel diseases (IBD), encompassing both Crohn Disease (CD) and ulcerative colitis (UC) are globally prevalent diseases, impacting children of all ages. The hallmark of IBD is a perturbed immune system that leads to continuous inflammation in the gut and challenges optimal treatment. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ), a nuclear transcription factor, plays a major role in gut homeostasis and contributes significantly toward a balanced, homeostatic immune system. Dysregulation in the NF-κβ pathway and factors that regulate it lead to a state of uncontrolled inflammation and altered immunity, as typically observed in IBD. Levels of proinflammatory cytokines that are regulated through NF-κβ are increased in both CD and UC. Genes known to activate NF-κβ, such as, Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) and Interleukin 23 (IL-23), are associated with IBD. Factors involved in inhibition of NF-κβ, such as A20 and TOLLIP, are also affected in IBD, resulting in failed inflammation suppression/regulation. NOD-2 and A20 have specifically been found to be strongly associated with pediatric IBD. Gut commensals are known to exert anti-inflammatory activities toward NF-κβ and can have a potential role in attenuating inflammation that likely occurs due to microbial dysbiosis in IBD. Failure to terminate/downregulate NF-κβ signaling results in chronic inflammation in IBD. Well-regulated control of inflammation in children with IBD can help better control the disease and suppress immune responses. Better understanding of factors that control NF-κβ can potentially lead toward discovering targeted therapeutic interventions for IBD. Suppression of NF-κβ can be achieved through many modalities including anti-sense oligonucleotides (ASOs), siRNA (small interfering RNA), factors regulating NF-κβ, and microbes. This review focuses on the role of NF-κβ, especially in pediatric IBD, and potential therapeutic venues for attenuating NF-κβ-induced inflammation.

Keywords: nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ), inflammatory bowel diseases (IBD), Crohn Disease (CD), ulcerative colitis (UC), immunity, microbes, homeostasis

BACKGROUND

The immune system of the gastrointestinal tract is normally well-tuned with the gut microenvironment, which enables the existence of a steady homeostatic state. The gut environment is continuously exposed to various exogenous materials, including food, xenobiotics, and microbial pathogens. Eradication of pathogens with simultaneous survival of gut commensals that are beneficial for maintaining homeostasis is a major challenge faced by the intestinal immune system. Nature has many protective mechanisms in place that help maintain this stable environment in the gut. This stability is disrupted in disease conditions affecting the gastrointestinal system, such as inflammatory bowel diseases (IBD). IBD, including both Crohn disease (CD) and ulcerative colitis (UC) have a debilitating impact on the lives of children and adults alike (1, 2). Some of the major features distinguishing between adult and pediatric IBD are nutritional challenges, poor bone health, delayed puberty, and growth failure, all of which are linked to inflammation (3, 4). Given the complex complications of IBD in children, it is critical to understand the basic factors that trigger inflammation and modulate treatment regimens accordingly.

Although many factors have been associated with IBD, the etiology is still not clearly understood, but seems to involve integrated mechanisms of uncontrolled immune response to various environmental/microbial stimuli in genetically susceptible hosts. Recent research has highlighted the importance of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ) in regulating immune responses in the gut. This review will focus on the role of NF-κβ in IBD and potential therapeutic mechanisms that can control NF-κβ-mediated inflammation in IBD, highlighting aspects especially relevant to children (5).

NF-κβ, a nuclear transcription factor, is a central player of sustaining a stable state of innate immunity in the gut. Disruptions and imbalances in the NF-κβ pathway lead to chronic inflammation, dysregulation of natural immune responses (6, 7), and altered immunity in IBD (8). Pathogenesis in both CD and UC is heavily marked by expression of multiple proinflammatory cytokines (9), many of which are regulated through NF-κβ. In fact, several of the key genes associated with IBD, such as Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) and Interleukin 23 (IL-23), drive NF-κβ activation. On the flip side, dysregulation of NF-κβ inhibitory pathways, such as reduced expression of A20 (tumor necrosis factor α-induced protein 3; TNFAIP3) or TOLLIP (10), could also contribute to unremitting inflammation, as seen in NF-κβ essential modulator (NEMO) epithelial cell-specific knockout mice (8, 11). Thus, better understanding of factors that drive and control NF-κβ could lead to targeted therapeutic interventions for inflammatory conditions, including IBD.

As we hypothesize that dysregulation, or the inability to terminate NF-κβ signaling is critical for the persistence of chronic inflammation in IBD, we would argue that this is especially important in children as microbe-driven persistent inflammation cannot be turned off in this setting and is likely to drive chronic inflammation at its early stages. We have recently shown that A20, a negative regulator of NF-κβ, is specifically disrupted in pediatric IBD; despite an observed increase in A20 gene expression, protein levels and associated signaling are reduced, suggesting a pediatric-specific dysregulation of A20 and NF-κβ (12). In contrast, adult studies have shown variable expression of A20 (13).

Early life factors can define the immune milieu and microbial interactions and predispose for immune-mediated conditions, such as IBD; regulation of NF-κβ is likely to be critical for this process. For all these reasons, better defining how NF-κβ is regulated in children is likely to provide important insight into pathogenesis and guide future therapies. It is quite likely that as more therapies will target NF-κβ regulation, physicians and scientist caring for children with IBD would benefit from deeper understanding of this complex pathway.

STRUCTURE OF NF-κβ

NF-κβ structure consists of multiple protein subunits: p52, RelA (p65), p50, c-Rel, and RelB, which are coalesced in the cytoplasm bound to Iκβ proteins (**Figure 1**). N-terminal Rel homology domain (RHD) is shared by all subunits and is essential for dimer formation. The subunits are conjoined in the cytoplasm in the resting state and remain inactive while attached to Iκβ proteins. Gene transcription can be regulated by RelA, RelB, and c-Rel as they have a transcriptional activation domain (TAD). The transcriptional activity of the p50 and p52 subunits depends upon binding with proteins that have TAD, for example, RelA, RelB, and c-Rel (14).

The IKK complex is the major factor that activates the NF-κβ pathway. It consists of IKKγ (a non-catalytic protein) and the kinases IKKα and IKKβ. Phosphorylation of Iκβ by IKK results in its proteasomal degradation and thus activates NF-κβ, releasing NF-κβ, and resulting in NF-κβ subunits being translocated into the nucleus and leading to proinflammatory gene transcription.

NF-κβ SIGNALING PATHWAYS

The NF-κβ pathway is activated either through the canonical or the non-canonical pathway (**Figure 1**). Initiation of the canonical pathway occurs through a process of receptor-ligand binding. Binding and stimulation of TLRs with antigens leads adaptors, such as TRADD, TRAF 2, cIAP1, and cIAP2 and RIP1, to bind to NF-κβ receptor's cytoplasmic domain. K63-linked polyubiquitination of RIP1 aids the IKK complex in recruitment of NF-κβ to the activated receptor. This activates the IKK complex, which in turn phosphorylates Iκβ and causes it to degrade (15). Activation of NF-κβ via the canonical pathway triggers production of cytokines in IBD, such as TNF-α and IL-6 (16).

The non-canonical pathway depends upon activation of NF-κβ inducing kinase (NIK); this involves phosphorylation and subsequent activation of the IKKα complex by NIK. Activation leads to phosphorylation of p100 by IKKα, which in turn results in the formation of the p52/RelB active heterodimer. The p52/RelB active heterodimer is then translocated to the nucleus. Induction of the non-canonical pathway occurs through multiple

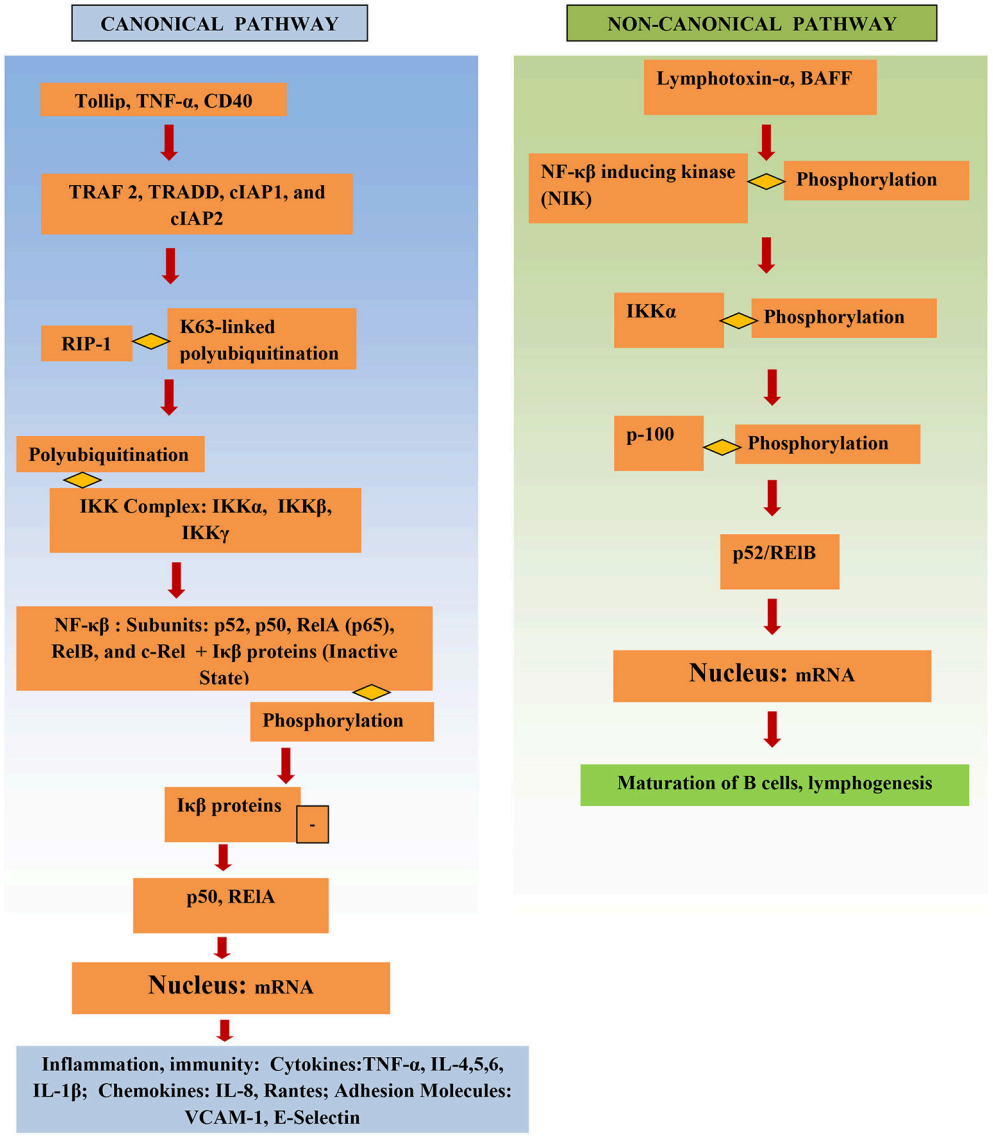

FIGURE 1 | Structure and activation of NF-κβ.

factors, such as, IL-1β lymphotoxin-α, and BAFF. IκBα mediates the turn-off inhibitory signal for NF-κβ by binding with nuclear NF-κβ complexes and transferring them back to the cytoplasm (6). Several genes involved in the non-canonical pathway were significantly higher in diseased tissue of IBD patients vs. adjacent healthy areas and healthy controls (17).

Microbe-associated molecular patterns (MAMPs), damage-associated molecular pattern molecules (DAMPs), cytokines, oxidative stress, bacteria, viruses, and ischemia stimulate and activate the NF-κβ pathway (6).

NF-κβ IN IBD

There is a pathological shift in gut homeostasis in IBD that activates NF-κβ, which in turn further propagates inflammation (8). IBD patients had high levels of NF-κβ, and biopsies of inflamed regions showed a significantly higher number of NF-κβ positive cells compared to normal regions (18). NF-κβ activation has been linked to disease phenotype in CD patients, with high NF-κβ levels correlating with increased ileocolonic and less perianal involvement (19). While NF-κβ is closely linked to IBD, much remains unknown about the specific mechanisms of involvement in disease pathogenesis. Below are a few examples of what is known and how this could impact IBD.

Cell-Specific Factors Affecting NF-κβ in IBD

Expression and activation of NF-κβ is greatly increased in the gut of IBD patients and is largely cell-specific. NF-κβ subunit p65 levels were found to be higher in the lamina propria of

biopsy specimens from CD patients as compared to UC patients and controls (20). NF-κβ is involved in the induction and regulation of many cytokines, including IL-6, TNF-α, IL-1β (21), and IL-12 (9). IL-12 plays an important role in augmenting the differentiation of Th-1 helper cells, and other cytokines, such as TNF-α and IL-23 are also involved in this phenomenon that is critical for inflammation propagation (22, 23). Thus, the effect of NF-κβ on IBD is paramount, as TNF-α is involved in exerting extensive damage to the mucosa and extracellular matrix by being involved in the regulation of, and increasing levels of matrix metalloproteinase (24). In addition, it was found that NF-κβ was induced by IL-6 in colonic epithelial cells and caused an increase in the expression of intercellular adhesion molecule-1 in epithelial cells, which is essential for recruiting neutrophil granulocytes to places of inflammation (25). CD40L induced NF-κβ activation in fibroblasts of colonic epithelial cells, which in turn augmented the expression of IL-6 and IL-8 (26). The IL-6-STAT3 pathway is activated in pediatric IBD (27).

Several cytokines that are increased in IBD and contribute toward inflammation are especially relevant to children. For example, IL-6 is increased in intestinal lamina propria biopsies and serum of pediatric IBD patients (28). In children with IBD, low bone mineral density is attributed to high levels of IL-6 (29). Similar to adults there is an increase in TNF-α levels in the terminal ileum of pediatric CD patients (12). IL-7, IL-1β, IL-5, IL-16, interferon (IFN)-γ-inducible protein-10, leukemia inhibitory factor, monokine induced by IFN-γ, IFN-α2, and IFN-γ were also found to be increased in serum of pediatric IBD patients as compared to healthy control patients, whereas, IL-17, macrophage inhibitory protein-1β, and IL-2 were decreased; many of these cytokines are regulated by NF-κβ-related pathways (30). This imbalance in cytokine regulation indicates the need for further exploration of NF-κβ-related inflammatory pathway in pediatric IBD, as their role in propagating inflammation remains unclear.

Along with its association with inflammation, evidence suggests that NF-κβ has an anti-inflammatory role as well, as seen by increased intestinal inflammation, apoptosis, and reduced antimicrobial peptides in a NEMO-deficient epithelial cell mouse model (31). Similarly, NF-κβ RelA intestinal cell conditional knock-out mice were susceptible to develop DSS-induced colitis (32). These examples highlight the multifaceted, complex nature of innate immune control in the gut.

Regulation of NF-κβ Through A20

A20 is a cytoplasmic protein that acts as a significant inhibitor/regulator of NF-κβ-induced inflammation (33). A20 plays an important role in counter-regulating inflammation in the gut, as shown by the presence of damaged intestinal epithelium and increased apoptosis after intestinal epithelial cells-specific A20 knock-out mice were treated with TNF-α (34). A20 is an important inhibitor of TNF-α-induced NF-κβ inflammation (35). A20 also suppresses CD40 and IL-1, and pattern recognition receptors induced NF-κβ-mediated inflammation (36). A20 expression is increased in pediatric IBD patients with a simultaneous reduction in A20 protein levels, possibly due to destabilization of A20-chaperone factors in IBD (12). Genome-wide association studies (GWAS) have shown linkage between A20 and IBD (11). In adult IBD patients, A20 profiling has shown varying correlations with disease phenotype and severity. A20 expression was low in the colonic and terminal ileum (TI) mucosa (13) and was found to be high in colonic biopsies of adult UC, but not CD patients (37).

Microbial Regulation of NF-κβ

Gut commensals are integral to homeostasis and many regulatory functions, interacting with the mucosal immune system. As such, commensals are heavily involved in anti-inflammatory activities targeted toward NF-κβ in the gut, such as inhibition of NF-κβ activity through peroxisome proliferator activated receptor-γ (PPAR-γ) by *Bacteroides thetaiotaomicron*, which in turn suppresses transportation of the NF-κβ subunit RelA into the nucleus (38). *Bifidobacterium infantis* downregulates NF-κβ activity induced by LPS (39). *Lactobacillus Casei* counteracts inflammation induced by *Shigella flexneri* infection that causes increased transcription of inflammatory cytokines by acting on pathways that stabilize Iκβ and hence prevents translocation of NF-κβ to the nucleus (40). Microbial dysbiosis is a prominent factor involved in IBD and contributes toward inflammation.

Specific Relevance of NF-κβ to Pediatric IBD

IBD in children has been subclassified into different categories by age; above or below 10 years, very early onset IBD (VEOIBD) in children <6 years, and infantile IBD in children <2 years (41). Pediatric IBD differs from adult IBD in many aspects. Positive family history of IBD is more often the case in pediatric cases vs. in adults. Most often, at the initial stages, IBD occurs in the colon in young children, whereas in adults, small bowel is usually involved. Young children with Crohn disease have more colonic involvement than adults do. Pediatric IBD is more often refractory to medical and surgical treatments commonly used for management of IBD in older patients (42). The proportion of monogenetic causes of IBD-like presentation is highest in the VEOIBD and infantile groups and genetic defects that control NF-κβ, such as variations in TRIMM22, appear to be especially relevant in children (5). Defects and variations in IL-10 and IL-10 receptor are also significant in children with VEOIBD (43, 44). Alterations in other genes, such as, *LRBA* (45), *XIAP* (46), *and TTC7A* (47) is associated with a high risk of developing IBD mostly in childhood (48), but also in adults.

INVOLVEMENT OF CURRENT IBD TREATMENTS IN NF-κβ PATHWAY

Changes in expression of NF-κβ and associated factors have been observed with several treatments for IBD. In IBD patients treated with corticosteroids, colonic epithelial, mononuclear, and endothelial cells had significantly less nuclear NF-κβ-p65 levels than cells from untreated patients. Corticosteroids increase the expression of IκBα, which retains NF-κβ in the cytoplasm and interacts physically with p65, thus preventing the activation of NF-κβ (49). However, prolonged use of corticosteroids affects

linear growth and has the potential to cause hypertension, osteopenia, and increased susceptibility to infection (50).

Sulfasalazine was found to suppress IKKα and IKKβ, which in turn inhibit NF-κβ (51)

In vitro experiments revealed that when NF-κβ activation induced by TNF was suppressed by methotrexate, it appeared to be through prevention of phosphorylation and degradation of IκBα, which retains NF-κβ in the cytoplasm and interact physically with p65, thus preventing the activation of NF-κβ (52).

Infliximab treatment caused an increase in IκBα and IκBγ in colonic biopsies; this then inhibits NF-κβ activation and helps in maintaining remission in pediatric patients (53).

Exclusive enteral nutrition (EEN) has been proven to induce clinical remission and lead to mucosal healing in pediatric CD with matching or even superior efficacy to that of corticosteroids (54, 55). Although the use of EEN has been adopted across the globe (56), the mechanism of action remains unclear (54). Increased attenuation of inflammation was also observed in murine models of DSS-colitis along with suppression of TNF-α, IL-6, and IL-8 in colonic biopsies with administration of a novel nutritional polymeric formula. *In vitro* experiments also showed suppression of genes associated with the NF-κβ pathway including, TNF, TNFSF10, NF-κβ1, and RELB with polymeric formula (57). Arginine and glutamine present in polymeric formula suppress phosphorylation involved in the NF-κβ and P38 pathways preventing NF-κβ activation (58). Curcumin, glutamine, and arginine together suppressed IL-8, raising the option of addition of curcumin to polymeric formula to suppress inflammation in IBD (55).

Thus, although current standard therapies for IBD do exert an effect on NF-κβ to some extent, through its associated factors, additional therapies are required for better control of NF-κβ-associated inflammation, Furthermore, much of the published work was done in *in vitro* settings, which is a controlled environment and is starkly different than the actual gut microenvironment where multiple factors are simultaneously at play. Therefore, it is important to focus on the development of a translational approach to develop additional therapies that impact NF-κβ, as described in the following section.

POTENTIAL TREATMENTS TARGETED TO MODIFY NF-κβ REGULATORY AND ASSOCIATED FACTORS

Given the pathogenic role of NF-κβ in IBD pathogenesis, it would be attractive to reduce NF-κβ activity by manipulating its regulation. This could be achieved by either directly suppressing NF-κβ or indirectly, by enhancing factors that regulate it. The function of proteasomes differs amongst CD patients and healthy individuals as evident by an increased conversion of the precursor p105 toward its active form, p50 in CD patients (59). Proteasome inhibitors designed for proteasomes that convert the p105 precursor toward active p50 and enhance NF-κβ activation can be beneficial in breaking this pathway. Animal studies have reported successful treatment of TNBS-induced colitis with p65 antisense oligonucleotides that directly target NF-κβ proteins and block them, thus inhibiting the activation of NF-κβ (60). Similar studies have not yet been done in humans, highlighting the importance of early stages of developing drugs focused on enhancing immune regulation, in contrast to current mostly immunosuppressive approaches.

Alterations in gut microbial composition in IBD has been reported by many studies (61) and focus on future targeted microbe-altering therapies is under consideration (62). Fecal microbial transplant (FMT) has been used as a treatment for IBD, but results reported by different studies are variable (63, 64). While studies have shown that gut microbes do exert an inhibitory action on NF-κβ, experimental approaches for studying the mechanism of action of microbes toward NF-κβ in models of IBD and gut environment, such as, organoids derived from IBD patients, need to be developed. As "designer microbes" have been developed to induce immune regulation (through secretion of IL-10, for example) (65), it would be attractive to use microbes to directly regulate the NF-κβ pathway.

Treatments to prevent A20 degradation and administration of factors that stabilize it might be beneficial. A striking correlation was found between A20 and anti-TNF therapy within a Danish cohort of IBD patients, where functional polymorphisms in A20 were predictive of response to anti-TNF therapy (66). Probing another cohort revealed a correlation between *A20* SNPs and response to anti-TNF therapy (67). These findings suggest the possibility of using polymorphisms in *A20* as a genetic biomarker, a venue that should be explored further for practical application. Post-translation modification highly impacts the stability of A20. IKKβ, a kinase required to activate the NF-κβ pathway is also involved in the phosphorylation of A20 at the serine 381 (S381) site, and thus helps A20 to stabilize and stop NF-κβ signaling. As phosphorylation of A20 by IKKβ occurs in response to LPS and TNF (68), presence of IKKβ in inflammation could be a counteractive action to combat NF-κβ induced inflammation too, in addition to stimulating it. Perhaps the key is in studying the conditions that help IKKβ stimulate NF-κβ and altering them.

Paracaspase MALT-1 regulates T cell receptor signaling to NF-κβ and is essential for T cell activation. A20 is directed to a complex of MALT-1 and Bcl-10 upon T-cell receptor stimulation, resulting in its cleavage and rendering it unable to stop the NF-κβ signal (69). Thus, drugs that target MALT-1 can also have an indirect affect on A20, by removing MALT-1 from the cellular environment.

Interaction of A20 with other proteins, such as Tax1 binding protein 1 (TAX1BP-1) and A20-binding inhibitor of NF-κB activation 1 (ABIN-1) helps in attenuating inflammation. The ABIN family of proteins negatively regulate NF-κβ; they are ubiquitin binders and attach to NEMO (NF-κβ essential modulator complex; the IKK complex) (70). ABIN-1 aids A20 to attach to the IKK/NEMO complex and exert its deubiquitinating process (71). The expression of ABIN-1 is dependent upon NF-κβ (72). TAX1BP-1 is also involved in inhibition of NF-κβ-induced inflammation (73) and recruits A20 to the polyubiquitin chains, where A20 breaks and interrupts the IKK complex (74). Ensuring constant presence and stability of TAX1BP-1 and ABIN-1 therefore can play a very important role in suppressing NF-κβ induced inflammation.

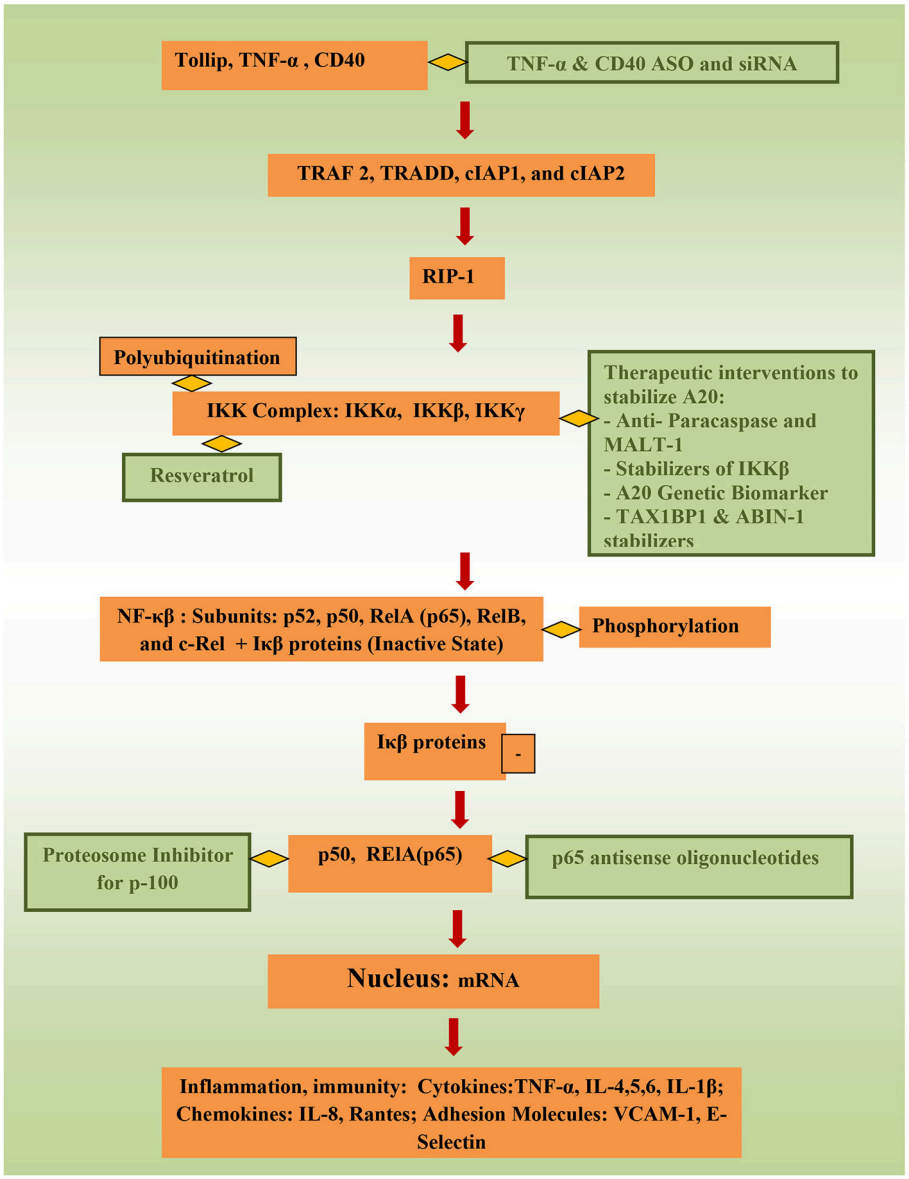

FIGURE 2 | Potential therapeutic pathways for NF-κβ attentation (therapeutic interventions are shown in green).

As it was reported that CD patients with high NF-κβ levels had more ileocolonic disease and less perianal involvement than those with normal NF-κβ activity (19), it is important to confirm in larger cohorts whether NF-κβ activity is indeed site-specific and correlates with disease status. This could especially be important for pediatric patients, as IBD can possibly be better controlled in early stages of diagnosis and at an earlier age, without the presence of other co-morbidities.

Targeting controllers of NF-κβ to attenuate its activity directly has tremendous potential to suppress inflammation. Stability of tollip, protein that inhibit inflammation by preventing IL-1 interaction with IL-1Rs, inhibit IRAK phosphorylation and inhibit TLR-2 and TLR-4 mediated inflammation could be of benefit as well (75). In animal models, TNBS-induced colitis and DSS-induced colitis were attenuated through targeting NF-κβ 65 (60, 76), and NF-κβ 65 antisense oligonuceotides (ASO) are undergoing clinical trials currently. Better control of TNF-α (6), which activates the canonical pathway through Toll-like receptors (TLRs), through ASOs and siRNA (small interfering RNA) can be of significance important in precision therapy. Using phosphorothioate ASOs for CD40 also has anti-NF-κβ potential (77, 78). Resrevatrol, an immunomodulator and anti-cancer agent has been found to suppress p65 and IKKβ (79). **Figure 2** illustrates the connection between factors affecting NF-κβ and potential therapeutic models that could be relevant to pediatric IBD.

TABLE 1 | Current IBD Treatments Affecting NF-κβ.

Treatment	Mechanisms of Action	References
Corticosteroids	Increase the expression of IκBα and prevent the activation of NF-κβ	(49)
Sulfasalazine	Suppresses IKKα and IKKβ and inhibits NF-κβ	(51)
Methotrexate	Prevents phosphorylation and degradation of IκBα, retaining NF-κβ in the cytoplasm and preventing its activation	(52)
Infliximab	Increases production of IκBα and IκBγ, which inhibit NF-κβ activation	(53)
Exclusive enteral nutrition (EEN)	Suppression of cytokines TNF-α, IL-6, and IL-8; suppression of related genes: TNFSF10, NF-κβ1, and RELB; prevention of phosphorylation of NF-κβ and p38 pathways	(55, 57, 58)

TABLE 2 | Potential future treatments for IBD, related to NF-κβ.

Treatments	Mechanism of Action	References
Proteasome inhibitors	Targeting proteasomes that convert the p105 precursor to active p50 and enhance NF-κβ activation	(59)
p65 antisense oligonucleotides	Directly target NF-κβ proteins and block their action	(60)
Microbial therapy	Use microbes to directly regulate the NF-κβ pathway	(62–65)
A20 stabilizers	Targeting MALT-1; stabilizing IKKβ (phosphorylates A20) and A20 chaperone proteins (ABIN-1, TAX1BP1)	(68–71, 73)

Current IBD therapy is mostly aimed at sustaining immunosuppression. A definite cure is yet to be found. The aim of IBD treatment is induction and maintenance of remission, and prevention of flares and complications. Balance between drug safety and efficacy is a therapeutic challenge, as current medications have serious side effects, emphasizing the need of development of precision therapy for IBD patients. Current IBD treatments affecting NF-κβ are described in **Table 1**. Potential future treatments for IBD related to NF-κβ are described in **Table 2**.

FUTURE OF ANTI- NF-κβ DIRECTED INTERVENTIONS

Treatments directed toward suppressing NF-κβ have a huge therapeutic potential against diseases such as IBD, cancer, and other inflammatory conditions. This is especially relevant in children as controlling the inflammatory cascade at early stages can prevent the devastating long term effects of uncontrolled inflammation as seen in IBD. Systematic *in vitro* and *in vivo* studies in animal models need to be conducted to analyze factors that antagonize/attenuate NF-κβ, and results need to be interpreted with caution; however, in contrast to almost all IBD treatments used today, which suppress the immune response, modulating pathways, for example by enhancing NF-κβ regulating molecules, such as A20, could allow the gut environment to return to homeostasis, possibly without an increase in infection risk. Obviously, before translating potential treatments to treat humans, safety and efficacy of potential therapeutic regimens needs to be established. Development of medications specifically targeting NF-κβ to control its inflammatory activity can be of tremendous benefit to children with IBD, such as early control of symptoms and low risk of immunosuppression that is often associated with current IBD medications.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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CASE REPORT
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Central Venous Sinus Thrombosis in a Boy With Acute Severe Ulcerative Colitis

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Cerebral venous sinus thrombosis (CVST) in childhood is uncommon. Certain diseases predispose patients to CVST, such as inflammatory bowel disease (IBD), which is considered a risk factor for developing thrombosis, which in turn is considered an extraintestinal manifestation of IBD. The use of prophylaxis in certain patients is a controversial topic. We present the case of a 5-years-old child with ulcerative colitis, who presented with transverse sinus thrombosis immediately after colectomy. Considering the recent recommendations on prophylaxis in this disease, our patient and probably many others would benefit from establishing treatment with low-molecular-weight heparin. We believe that these recommendations should be known, with our case serving as an example, given that we are heading in a direction that has so far been controversial.

Keywords: cerebral venous thrombosis (CVT), prophylaxis, inflammatory bowel disease, pediatrics, cerebral venous sinus thrombosis (CVST)

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BACKGROUND

Pediatric cerebral venous sinus thrombosis (CVST) has an incidence rate of 0.67 cases per 100,000 children and mainly occurs during the neonatal period (1). The diagnosis of CVST has increased due to greater diagnostic suspicion, improved neuroimaging techniques and increased survival of children with diseases that predispose them to the development of CVST. The most common risk factors in older children are head and neck infections and chronic systemic diseases such as chronic inflammatory and hematological diseases and cancer.

CASE REPORT

We present the case of a child aged 5 years and 2 months who was diagnosed at the age of 3 years with moderate (E4S0) ulcerative colitis (UC). After the initial presentation, the patient was started on treatment with oral mesalazine. In the following months, treatment with oral steroids was started, which was subsequently changed to intravenous, along with azathioprine, tacrolimus, and infliximab, without success. At 20 months from the disease onset, the treatment proceeded to the implementation of a total proctocolectomy with J-pouch reconstruction. The immediate postoperative period elapsed without incident and early total parenteral nutrition was started while he was fasting. However, 96 h after the surgery, the patient presented right hemicranial headaches, left arm distal monoparesis, a complex partial seizure, and subsequently two secondarily generalized seizures that ceased after the introduction of intravenous phenytoin.

Based on the suspicion of a stroke, the patient underwent cranial Computed Tomography (CT), the results of which were normal. Magnetic Resonance Imaging (MRI) and Magnetic Resonance Angiography (MRA) for venous intracranial system (**Figures 1**, **2**) revealed a right occipital venous infarction secondary to partial thrombosis of the right transverse sinus. The decision was made to start anticoagulant therapy with intravenous heparin and subsequently with low-molecular-weight heparin (LMWH). The patient presented no known cardiovascular risk factors, although a subsequent hypercoagulability study revealed that the patient was a homozygous carrier of the methylene tetrahydrofolate reductase (MTHFR) gene C677T mutation and that the parents were heterozygous carriers of this MTHFR mutation; the rest of the results of the study were normal. One month after the episode, the thrombosis image persisted in the MRA. The patient therefore underwent long-term therapy with acenocoumarol for 3 years. The patient's subsequent progress was satisfactory and no further treatment was necessary. The patient is now asymptomatic from the neurological and gastrointestinal point of view.

DISCUSSION

Strokes are 2 to 4 times more common in patients with inflammatory bowel disease (IBD) compared to the general population. It was documented that 1–7.7% of patients presented some event, while post-mortem studies reported that the figure can reach 40% of patients (2). Deep vein thrombosis and pulmonary embolism are the most commonly associated entities, the risk of which is especially high in young patients, women, smokers, those with S-protein deficiency, and patients with colonic disease (3). In particular, venous thrombotic phenomena are three times more common in patients with IBD than in healthy controls (3–5), with a prevalence of ∼4% (6).

The relationship between venous thromboembolism and IBD is based mainly on the inflammatory component of IBD, which produces a hypercoagulability state with coagulation cascade activation, platelet activation, and fibrinolysis anomalies (4, 6, 7). IBD could be considered a risk factor *per se* for the development of venous thrombosis, which would be an extraintestinal manifestation of IBD (4).

The role of other risk factors in the development of thrombosis has been studied. The classic risk factors are also valid for this group, although, no differences in the prevalence of hereditary disorders predisposing to the development of thrombosis were shown compared to healthy controls. The study by Alatri et al. (6) found a statistically significant association between surgery and UC but not with Crohn's disease (CD), probably due to the greater inflammatory burden in patients with UC who undergo surgery compared with patients with CD who undergo surgery for fistulas or stenosis. No relationship with drugs was found, but a positive correlation with disease activity and hospitalization was reported (4).

The Canadian registry of ischemic infarction in the pediatric population identified a risk factor in 98% of children, a prothrombotic state in 41%, and a chronic systemic disease in 36% (1). In a meta-analysis of case-control studies published in 2010 including more than 200 cases of pediatric and neonatal venous sinus thrombosis and 1,200 controls, the prevalence of the factor V Leiden mutation and the prothrombin gene G20210A mutation was higher in cases than in the controls with a greater risk of developing CVST (8). In contrast, the value of the MTHFR gene 677CT mutation is controversial. A 2010 meta-analysis of case-control studies in adults reported comparable frequency of the MTHFR gene C677T mutation in 382 adult patients with CVST and in 1,217 controls (9) while, a 2011 meta-analysis reported a positive association between CVST and the MTHFR gene C677T mutation (10). Our patient's thrombophilia study documented that he is a homozygous carrier of the MTHFR gene C677T mutation, without presenting a factor V Leiden mutation or the prothrombin gene G20210A mutation. In our case, we can consider as risk factors the surgery in a patient with chronic IBD with a homozygous MTHFR gene C677T mutation, the insertion of a central venous access (for parenteral nutrition) and immobility.

From the clinical standpoint, venous thrombosis of the transverse sinus usually starts with headaches, which are frequently severe, and precede the other neurological signs. Focal abnormalities and seizures should alert physicians to the possibility of venous thrombosis. Although the clinical spectrum is variable, venous thrombosis should be suspected upon the onset of any neurological sign in patients with IBD, mainly in those with UC especially during the postoperative period (7).

CVST treatment should start as soon as possible after the diagnosis has been confirmed and should aim at the following: (a) reversing the predisposing factor when is known, (b) controlling the seizures and intracranial hypertension, (c) administering antithrombotic treatment to recanalize the sinus obstruction, (d) preventing the propagation of the thrombus, and (e) treating the prothrombotic state to prevent recurrences. Antithrombotic treatment during the acute phase of CVST in children is similar to that used in adults but has weaker evidence due to the lack of randomized studies in pediatric populations. The presence of a haemorrhagic venous infarction is not a contraindication for anticoagulant therapy. For children with CVST without significant intracranial hemorrhage, the evidence-based guidelines of the American College of Chest Physicians recommend initial treatment with unfractionated heparin or LMWH, followed by treatment with LMWH or vitamin K antagonists for at least 3 months (Grade 1B). Anticoagulation for 3 more months is recommended if the cerebral-sinus occlusion persists or if the symptoms progress (Grade 2C). For children with CVST and recurrent risk factors (e.g., nephrotic syndrome and undergoing treatment with asparaginase), antithrombotic prophylaxis is recommended due to the recurrence risk (Grade 2C). Thrombolysis, thrombectomy, and surgical decompression is reserved for cases of severe CVST that do not improve with initial treatment with unfractionated heparin (11). The American Heart Association and American Stroke Association guidelines published in 2011 on diagnosing and managing CVST, recommended treatment with full doses of LMWHs (even in the presence of cerebral hemorrhage) for

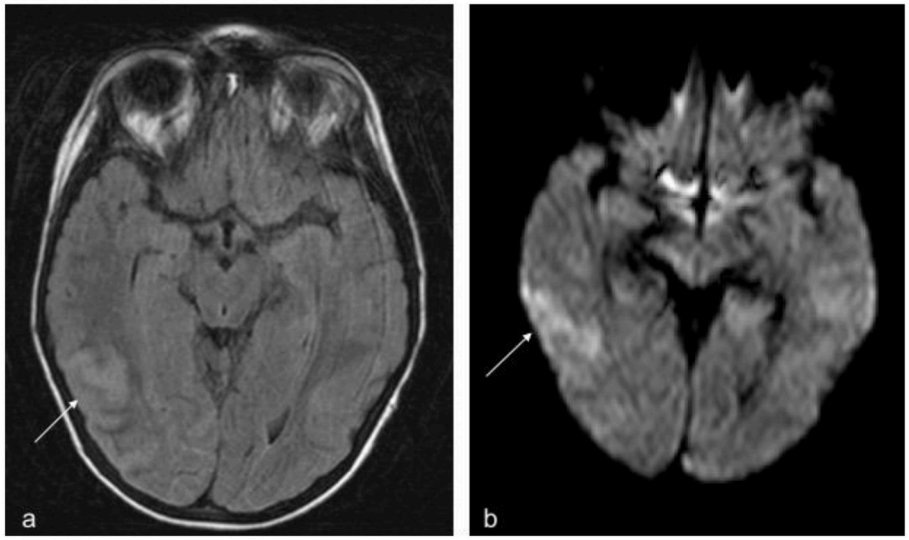

FIGURE 1 | (a) Transverse plane, FLAIR sequence, movement artifacts. The arrow shows the cortical-subcortical lesion compatible with a non-haemorrhagic infarction in a venous territory. **(b)** Transverse plane, b1000 diffusion sequence. The arrow shows parenchymal shine due to diffusion restriction indicative of acute vascular disease (Not shown ADC map with signal drop in the area).

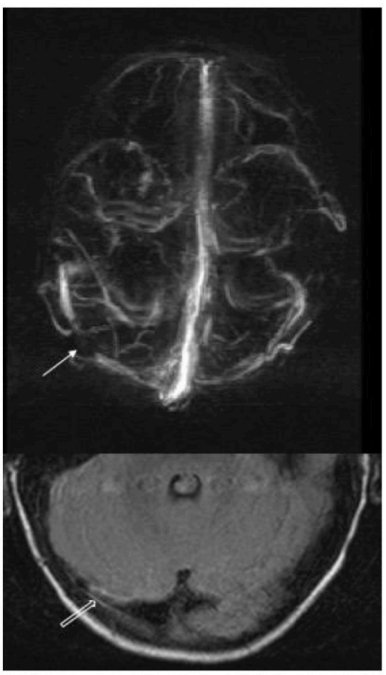

FIGURE 2 | Venous Magnetic Resonance Angiography with contrast. 3D reconstruction in the transverse plane and magnification of the baseline sequence in the occipital region. The arrow shows the repletion defect caused by the thrombus in the middle third of the right transverse sinus, and the hollow arrow shows the peripheral wall contrast that delimits the luminal thrombosis of the sinus.

pediatric patients older than 28 days followed by the treatment with LMWHs or oral vitamin K antagonists from 3 to 6 months (12).

The goal of long-term anticoagulant therapy is to prevent the recurrence of CVST (which can occur in 2–4% of patients) and of venous thrombosis in other locations (4–7%). A European cohort study of 396 patients with CVST, with a median age of 5 years and a 36-months follow-up, reported that venous thrombosis reoccurred in 22 children (6%) with 13 CVST (3%) at a mean of 6 months (range, 0.1–85). The recurrence of CVST was observed only in children with an initial CVST diagnosed after the age of 2 years. The independent factors associated with the recurrence of systemic or cerebral venous thrombosis in this pediatric population were the absence of anticoagulant therapy before the relapse, the persistence of the thrombosis in the neuroimaging control study and the presence of heterozygous prothrombin gene G20210A mutation (13). A mortality of 3% (12 patients) was observed in the first 2 weeks of the event.

There is controversy regarding the application of drug prophylaxis for the thrombosis. Various studies and guidelines have recommended prophylaxis with LMWH to prevent venous thromboembolism in adults with severe acute colitis (14, 15). This prophylaxis is not currently used routinely for pediatric patients. Although the absolute risk of venous thromboembolism is lower in children than in adults, it appears that the risk of the disease is greater compared to healthy pediatric controls (16). Lazzerini et al. (17) in a systematic review found that up to 50% of IBD patients who suffered an episode of thrombosis had at least one risk factor. A recent retrospective study, where data from 34,000 children who underwent abdominopelvic surgery were analyzed, showed an increasing rate of venous thrombotic episodes in IBD patients (0.98 vs. 0.19 %). Despite the identification of potential risk factors for the development of thrombosis, there are no evidence-based guidelines about the screening or prophylaxis of thrombosis in pediatric patients who are going to undergo abdominal surgery (18).

Zitomerskyet et al. (19) and, more recently, Turner et al. recommended prophylaxis with heparin for this patient group; prophylaxis with LMWHs is recommended for patients with IBD if they present at least two of the following risk factors: tobacco use, oral contraceptives, complete immobility, central venous catheters, obesity, concomitant infection, previous venous thromboembolic event, family history, and known prothrombotic disease (20). A systematic review of the literature (21) on the need for preoperative thromboprophylaxis in patients with IBD, recommends with grade III quality of evidence (according to the GRADE system, i.e., evidence from non-experimental descriptive studies, such as comparative studies and case–control studies), the need for pre and postoperative thromboprophylaxis in patients with IBD who are not at risk of bleeding, in order to prevent the risk of thrombosis. Previously, the Canadian Gastroenterology Association (22) had recommended against routine thromboprophylaxis in pediatric patients hospitalized for a flare of IBD, but recommended prophylaxis only in obese adolescents with severe acute colitis undergoing surgery and in hospitalized patients with a previous history of thrombosis.

These recommendations are similar to those proposed by Turner et al. (23) since they combine more than one risk factor for the development of thrombosis. Patients with IBD and severe acute colitis, would probably benefit from screening prior to the surgery, considering that acute colitis *per se* is a risk factor for the development of thrombosis and that the presence of a genetic prothrombotic factor is unknown, although cost-benefit studies are necessary. If prophylaxis is established, enoxaparin 1 mg/kg/day (100 IU/kg/day) in one daily dose is the most recommended drug (23).

In our case, the patient presented two risk factors for developing thrombosis: central venous catheter (for total parenteral nutrition) and prolonged immobility. Considering the new criteria proposed by the authors, prophylactic treatment should have therefore been started but this is not what happened with our patient who revealed at his subsequent examination, the presence of a homozygous MTHFR gene C677T mutation.

Several studies have been conducted in the past in order to evaluate the risk of bleeding and other complications derived from thromboprophylaxis. Some studies, carried out in pediatric population, concluded that thromboprophylaxis is safe and well tolerated with a bleeding rate of 1–5% (24, 25). In previous studies (1, 26, 27), patients with venous sinus thrombosis who received heparin did not have more bleeding episodes or worsening, compared with the placebo group, while mortality was also lower in the treatment group (28–30). Finally, a systematic review and meta-analysis of the literature, with more than 2,000 pediatric patients included, suggested that thromboprophylaxis in pediatric age is safe and effective (31).

Although CVST is a rare (even more so in childhood) but potentially severe complication, we believe it is important to understand the association, especially in patients who undergo surgery, because the neurological prognosis will depend on early diagnosis and early initiation of treatment.

Although the use of prophylaxis is not currently standardized, the implementation of the new guidelines and further studies are expected to change the clinical practice. Although thromboprophylaxis is safe and effective, the risk of thrombosis and bleeding requires individual assessment to optimize the treatment and minimize the complications, costs, and outcome of the disease.

ETHICS STATEMENT

Written informed consent was obtained from parents before manuscript preparation and submission.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Stress Triggers Flare of Inflammatory Bowel Disease in Children and Adults

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Inflammatory bowel disease (IBD) is an idiopathic inflammatory disease characterized by chronic and relapsing manifestations. It is noteworthy that the prevalence of IBD is gradually increasing in both children and adults. Currently, the pathogenesis of IBD remains to be completely elucidated. IBD is believed to occur through interactions among genetics, environmental factors, and the gut microbiota. However, the relapsing and remitting course of IBD underlines the importance of other modifiers, such as psychological stress. Growing evidence from clinical and experimental studies suggests that stress acts as a promoting or relapsing factor for IBD. Importantly, recent studies have reported an increasing incidence of anxiety or depression in both children and adults with IBD. In this article, we review the mechanisms by which stress affects IBD, such as via impaired intestinal barrier function, disturbance of the gut microbiota, intestinal dysmotility, and immune and neuroendocrine dysfunction. With regard to both children and adults, we provide recent evidence to describe how stress can affect IBD at various stages. Furthermore, we emphasize the importance of mental healing and discuss the value of approaches targeting stress in clinical management to develop enhanced strategies for the prevention and treatment of IBD.

Keywords: inflammatory bowel disease, stress, pediatrics, gut microbiota, brain-gut axis, treatment

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INTRODUCTION

Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC), and Crohn's disease (CD), is a chronic, relapsing, and remittent intestinal inflammatory disorder (1) affecting millions of people worldwide (2). Notably, IBD is gradually becoming a global disease with rapidly increasing incidence in emerging industrial countries in the twenty-first century (3). Although IBD can occur at any age, ∼25% of patients are diagnosed with IBD before 20 years of age (4). The incidence of IBD in children varies among different countries, but the overall trend is increasing globally. The incidence is about 0.5–23/1,00,000 for IBD, 0.1 to 13.9/1,00,000 for CD, 0.3 to 15.0/1,00,000 for UC (3, 5–7). In addition to the common gastrointestinal symptoms (abdominal pain, diarrhea, hematochezia, and weight loss) similar to those of adults, children may present with unique manifestations, including poor growth and delayed puberty (8). IBD is considered to be an immune-mediated intestinal disorder, resulting from complex interactions among genetics, environmental factors, and gut microbiota (9). Various factors, such as genetic transmission, intestinal immune disruption, gut microbiota disturbance, diet, infection, lifestyle, psychological stress, sleep disorders, smoking, and early life exposure to antibiotics, have been found to influence the progress of IBD on the basis of studies in recent decades (10, 11). However, the exact pathophysiological mechanism of IBD remains to be inadequately understood. Its complex and multifactorial pathogenesis, severity of symptoms, uncertainty of the condition and prognosis, and

adverse reactions to medication and cancer risk bring multiple challenges to the cure of IBD. Owing to these challenges, patients' quality of life may be significantly affected, particularly by increasing psychosocial burdens and inducing psychological disorders. For children and adolescents, IBD can even threaten the healthy psychosocial development.

Stress may cause abnormalities of behavior and/or mentality, such as anxiety and depression, and also influence the function of visceral organs, especially the digestive system. Psychological comorbidities, especially depression, have similar pathophysiological mechanisms to IBD. Pro-inflammatory cytokines and plasma acute phase protein C increased in depression patients (12). Elevated levels of malondialdehyde, a fatty acid peroxide, in the serum of depressed patients suggests that mental disorders may be associated with oxidation and oxidative stress (13). Meanwhile, autoimmune changes and bacterial translocations have also been observed in depression (14, 15). Thus, depression and IBD share a common pathway that seems to explain the interaction between the two diseases. In recent years, a growing number of studies have indicated that the prevalence of mental disorders in both children and adults with IBD is higher than that of healthy people (16). Significant progress has been made in elucidating the pathophysiological mechanisms of IBD, which indicates that stress is closely correlated with IBD. Accumulating evidence suggests that there is a bidirectional influence between IBD and stress. The underlying mechanisms consist of immune dysfunction, intestinal microbiota disturbance, impaired intestinal barrier function, and neuroendocrine system alterations (17). In addition, whenever stress occurs, whether in early life or adulthood, the development of IBD can be affected to some extent.

This paper provides an overview of recent literature focusing on the connection between stress and IBD in children and adults. Both experimental and clinical evidence illustrating the importance of stress in the pathogenesis of IBD is presented in this review. Finally, insights into comprehensive approaches for managing IBD and potential therapeutic implications of psychological interventions are provided.

STRESS: PATHWAYS AND PATHOPHYSIOLOGY

More than 80 years ago, Hungarian endocrinologist Hans Hugo Bruno Selye first defined the medical term "stress" as the physiological adaptive responses of organisms to adverse threats (stressors), which are endogenous or exogenous, psychological or physical, real or perceived (18). To maintain homeostasis under threat, organisms have evolved an extremely complex system, called the stress system, which involves physiological and behavioral adaptations via appropriate central and peripheral neuroendocrine responses. When exposed to long-term or severe stress, the organisms may reach a state called cacostasis, in which many vital physiological functions are impaired, and may develop many acute and chronic diseases (19). Stress-induced disorders occur in multiple systems throughout the body, among which the gastrointestinal tract is a sensitive system.

When the brain receives stress input, multiple pathways containing the autonomic nervous system and hypothalamic-pituitary-adrenal axis (HPA axis) are activated (20). Stress from different sources results in modifications of the brain-gut axis, which eventually leads to the progression of a wide range of gastrointestinal disorders. The frequently involved diseases include IBD, irritable bowel syndrome (IBS), peptic ulcers, food antigen allergic reactions, and gastroesophageal reflux disease. The potential mechanisms are summarized in the following sections (**Figure 1**).

HPA Axis

Corticotrophin-releasing factor (CRF) is considered to be a pivotal component in the HPA axis. It is produced by central and peripheral tissues in response to stress. CRF plays an important role in translating stimuli into physical responses in the brain (21). Stress directly activates the hypothalamus (mainly paraventricular nucleus of the hypothalamus system) to release CRF, inducing the anterior pituitary gland to secrete adrenocorticotrophic hormone, which further stimulates the adrenal cortex to secrete cortisol. Ultimately, cortisol acts on all tissues in the body via the circulation of blood. CRF receptors, as well as their ligands, which can be modulated by stress, are expressed in the gut as well as in the brain (22).

CRF acts on enteric peristalsis, secretion, and the mucosal barrier, playing a role in functional and organic disorders, such as IBS and IBD (23). In experimental animal studies, CRF has the opposite effect on upper and lower digestive transit, such as gastric emptying inhibition, reduction of small intestinal transit and increase of colonic transit and defecation (24, 25). In addition, CRF can induce mast cell degranulation and increase mucosal permeability (26), which is the key mechanism of intestinal disorders. Blocking CRF restrains the development of IBD by inhibiting mast cell degranulation and reducing tumor necrosis factor-alpha (TNF-α) and protease production (27).

The results from clinical studies are consistent with the above findings. In one study, healthy volunteers were asked to give a public speech to induce psychological pressure. The pressure induced by public speaking enhanced the permeability of the gut only in subjects with significantly elevated cortisol levels, suggesting that HPA axis activation is affected. Furthermore, peripheral injections of CRF were administered to reproduce stress-induced disorder. The results showed that exogenous CRF, as well as psychological stress, can increase the ratio of urine excretion rates of milk fructose and mannitol, showing signs of increased permeability in the small intestine. Furthermore, the mechanism appears to rely on intestinal mast cells because it can be abrogated by a mast cell stabilizer (28).

During the stress-induced changes of the gut, effectors cells, including mast cells (28), neutrophils (29) and lymphocytes, as well as pro-inflammatory cytokines, are placed in a pivotal position. Mast cells play an important role in the transmission of stress signals to the gut. Animal experiments have demonstrated that stress can damage the gut barrier function in a mast cell-dependent manner, which may facilitate the development of IBD

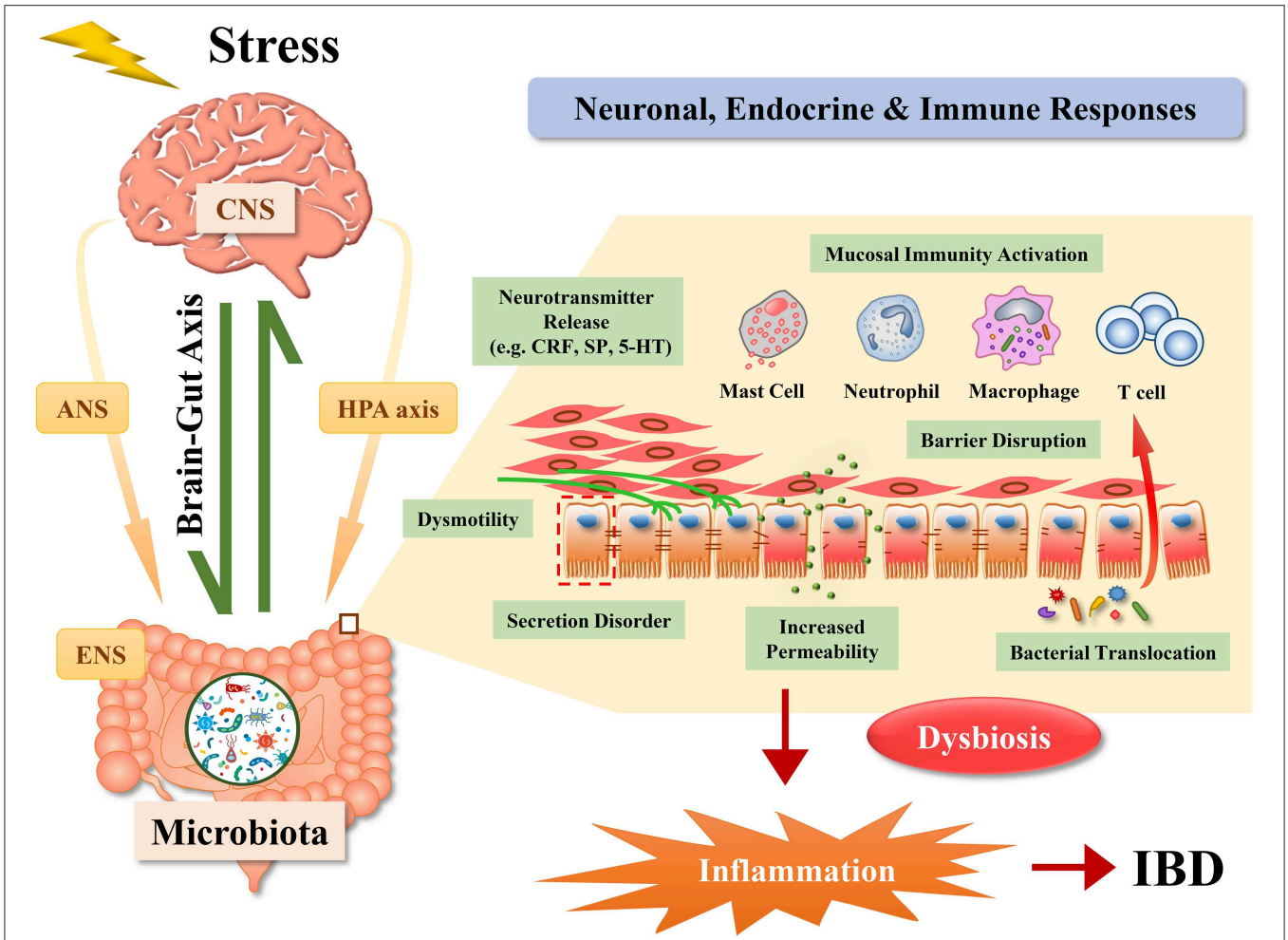

FIGURE 1 | Pathways by which stress affects inflammatory bowel disease. Effects of stress on the gut involve comprehensive reactions among neuronal, endocrine, and immune systems. Stress induces the activation of the brain-gut axis, the hypothalamic-pituitary-adrenal axis (HPA axis), the autonomic nervous system (ANS) and the enteric nervous system (ENS), contributing to the development of inflammatory bowel disease (IBD) via dysbiosis, alterations of secretion and mobility, disruption of the intestinal barrier and the release of inflammatory mediators. CNS, central nervous system; CRF, corticotrophin releasing factor; SP, substance P; 5-HT, 5-hydroxytryptamine.

(30, 31). In wild-type rats, chronic stress can induce intestinal barrier dysfunction, inflammatory cell infiltration, ultrastructural changes in epithelial cells, and mast cell proliferation and activation. In contrast, the intestinal epithelial function and morphology are not damaged in mast cell-deficient rats, and there is no evidence of inflammatory cell infiltration, which highlights the regulatory role played by mast cells (32).

The Autonomic Nervous System

The sympathetic and parasympathetic autonomic nervous systems serve the entire gastrointestinal tract and are closely connected with the enteric nervous system (ENS). Together, these systems govern secretion, motility, sphincter control, and microcirculation in the gut (33). Under stress conditions, the ENS produces large neuropeptides, which in turn affect intestinal immunity and inflammation. Geboes et al. found that there are mixed abnormalities in all CD and UC patients for different cell types of the ENS (34). Another study showed that patients with UC have markedly lower autonomic functions in comparison to those with CD and healthy controls (35).

Stress can activate the sympathetic autonomic system, leading to increased production of major adrenal medulla hormones, mainly catecholamines, such as epinephrine and norepinephrine. Catecholamines mediate increases of central and peripheral inflammatory cytokines and activation of the inflammatory nuclear factor κB signaling pathway in response to stress (36). In addition, the vagus nerve, which has anti-inflammatory effects, is inhibited by stress, leading to an increased systemic inflammatory response to endotoxin and intestinal inflammation (37, 38).

In addition, changes in tissue levels of neurotransmitters have been demonstrated in patients with IBD. Stress can also affect the follicle-associated epithelial barrier via vasoactive intestinal polypeptide (VIP) and its receptor on mucosal mast cells. These findings highlight an important effect of VIP-bacterial-epithelial interactions on regulating intestinal barrier function (39). In a mouse model of chronic restraint stress, substance P (SP) and its

receptors enhanced CRH expression and release in eosinophils, resulting in epithelial barrier dysfunction mediated by mast cells (40). Another study revealed that water avoidance stress (WAS)-induced colonic hypermotility is probably dependent on the upregulation of the neurokinin-1 receptor (NK1R) in the colon and increased serum SP levels, suggesting a potential mechanism for diarrhea in IBD patients with anxiety or depression (41).

The Microbiota Brain-Gut Axis and the Immune System

The effects of the gut microbiota on IBD have attracted much attention in the last decade. Microbiota communicates with the brain-gut axis through mucosal cells, immune cell, and neural endings (42). Published data from animal and clinical studies indicate that stress causes dysbiosis. Stress-induced dysbiosis is characterized by a decrease in the abundance of Lactobacillus and aggravated bacterial translocation. Notably, reduced *Lactobacillus* abundance contributes to opportunistic infections of *Campylobacter jejuni* and *Shigella flexneri* in monkeys (43). The gut microbiota of male mice exposed to chronic social defeat is characterized by reduced richness and diversity. The predicted functional profile shows reduced functional diversity. In particular, the lower prevalence of pathways involved in the synthesis and metabolism of short-chain fatty acids and neurotransmitter precursors has been described (44). A study showed that exposure to stress inhibits the NOD-like receptor, pyrin domain containing (NLRP)-6 inflammasome, altering the constitution of the gut flora, thus leading to inflammation of the intestine. Interestingly, transmissible intestinal inflammation, accompanied by upregulated CRF and reduced NLRP6, was observed after the mice were cohoused (45).

Stress can also break the established tolerance and augments immune responses in chronic intestinal inflammation. Increased intestinal permeability caused by stress allows microbiota to cross the gut epithelial barrier to trigger the mucosal immune reactions (42) and then transfer to secondary lymphoid organs (46) to activate the innate immune system. A recent study based on a dextran sulfate sodium (DSS)-induced colitis model provided evidence that chronic stress increases sensitivity to colitis via dysbiosis and immune system dysfunction. Under chronic stress conditions, the colonic lamina propria showed B cell, neutrophil, and pro-inflammatory ly6C^{hi} macrophage infiltration. Mesenteric lymph node (MLN) changes were also discovered with a significant change in the proportion of MLN-associated immune cells. The results of this study further showed marked activation of IL-6/STAT3 signaling in response to stress. Interestingly, the detrimental effects of stress were not terminated in *IL-6*$^{-/-}$ mice, indicating that the hyperinflammatory response is not the real culprit. In contrast, when the intestinal microbiota was shared by cohousing or was destroyed by antibiotics, the severity of DSS-induced colitis was indistinguishable between the stressed and control groups, unequivocally suggesting that the gut microbiota is responsible for the deleterious effects of stress. In general, stress disturbs the gut microbiota, triggers immune system dysfunction and facilitates DSS-induced colitis (47). A novel phenomenon has been revealed, showing that stress restrains the suppressive action of intestinal regulatory T cells (Tregs), instead of changing their quantity. It was found that prolactin, a stress-related mediator, can transform the phenotypes of intestinal Tregs, thus contributing to intestinal inflammation (48).

It has been shown that stress-induced flora disturbance has a vital impact on IBD by influencing host-microbiota crosstalk and regulating the neuro-immune-endocrine system (49–51). There is a complex network among the gut microbial landscape, immune system and nervous system. Microbiota-targeted therapies have been highlighted as a novel approach to treat systemic inflammation diseases, such as IBD, multiple sclerosis, systemic inflammatory arthritis, and asthma (52). Generalized microbiota-targeted therapies include antibiotics, antibacterial conjugate vaccines, probiotics, fecal microbiota transplants (FMTs) and other interventions that alter the community composition (53). In conclusion, these therapies might be beneficial to both physical and psychological recovery in IBD patients.

INTERACTION BETWEEN STRESS AND IBD

In the 1950s, IBD was considered a psychosomatic disorder (54), and previous studies have demonstrated a close association between IBD and stress. Specifically, IBD patients are often exposed to stress, which induces mood swings or even leads to mental complications. Meanwhile, increased emotional disorders can exacerbate symptoms such as abdominal pain, and can enhance the severity of IBD in turn.

Prevalence of Psychological Comorbidities in IBD

Psychological Comorbidities in Adult IBD

Most clinical studies have shown that mood disorders are associated with an increased risk of a variety of chronic diseases, such as IBD, arthritis, asthma, and diabetes mellitus (55, 56). In an IBD cohort, patients exhibited a high incidence of psychological distress and comorbidities, including depression, anxiety disorders, and bipolar disorder (57). Research from Canada examined the prevalence of depression in two typical surveys in a large sample. Statistical data indicated that the 12-month depression incidence rates of people with IBD and similar intestinal disorders in the survey mentioned above were 14.7 and 16.3%, respectively. Furthermore, IBD patients showed a three-fold higher incidence of depression than healthy people (58). In the National Health and Nutrition Examination Survey (NHANES) of Americans, the relationship between IBD and depression was examined. In this big data study, IBD hallmarked by chronic and recurrent disease, was found to act as an independent risk factor for depression (59). The Canadian Community Health Survey in 2012 reported that IBD is strongly associated with generalized anxiety disorder. Generalized anxiety disorder was identified by the WHO-CIDI lifetime criteria. The results revealed that IBD patients were prone to generalized anxiety with a two-fold increased incidence (60). Neuendorf et al.

screened 171 articles, including a total of 158,371 participants, to conduct a comprehensive systematic review. The findings showed that 35% of IBD patients develop anxiety symptoms and 21% develop anxiety disorders; 22% of IBD patients develop depression symptoms; and 15% develop a depressive disorder. Furthermore, this study pointed out that this condition is more prevalent during the active period of the disease (61).

With the objective of exploring the bidirectional relationship, Sexton et al. assessed symptom activity, intestinal inflammation, and perceived stress using the Manitoba IBD Index, fecal calprotectin in the stool, and Cohen's Perceived Stress Scale at months 0, 3, and 6. Perceived stress at month 0 was found to be positively correlated with disease activity at months 3 and 6 in both UC and CD. Nevertheless, no correlation between intestinal inflammation, evaluated by fecal calprotectin and perceived stress, was found (62).

A total of 403,665 patients with depression and 5323,986 people without a history of depression were followed up for an average of 6.7 years. A total of 0.05% of the depression cohort developed CD, while 0.03% of individuals in the non-depression cohort developed CD. Furthermore, 0.13% of patients in the depression cohort developed UC, and only 0.09% of individuals in the non-depression cohort developed UC. Compared with the non-depression cohort, the unadjusted hazards of CD and UC in the depression cohort increased by 67 and 41%, respectively. After adjusting for various confounding factors, the risk of developing IBD remained significantly increased in the depression cohort (63).

Psychological Comorbidities in Childhood IBD

Despite the limitation of inclusion age criteria for adolescent subjects, the phenomenon that adolescents with IBD have a higher prevalence of anxiety and depression symptoms can still be concluded (64, 65). According to parental reports, emotional problems, including anxious/depressed mood and withdrawn/depressed mood, appear to be more common in adolescents with IBD than National Health and Nutrition Examination in population-based controls. Both parental and self-reported psychosocial symptoms are related to the increased severity of self-perceived IBD symptoms (66). The incidence of mental illnesses, especially depression, among young people with IBD is increasing (67). In a prospective study of 121 patients with IBD aged 16–21 years, 55% reported increased anxiety/depression symptoms and 83% had a reduced quality of life compared with the baseline (68). A study including 374 IBD patients from the Netherlands found elevated symptoms of psychological comorbidities in both adolescents (10–17 years) and young adults (18–25 years), but there was no difference (64). A Swedish study found that IBD children who were identified with younger than 18 years had a three-fold increased hazard ratio for death in adulthood compared to children in the general population. The highest estimated risk of overall mortality was higher in UC patients than in CD patients (69).

Animal Models to Assess Psychological Impact of IBD

Similar phenomena have been observed in animal experiments. Depressive- and anxiety-like behaviors were found in mice with dinitrobenzene sulfonic acid (DNBS)-induced colitis. Upregulated expression of inflammatory genes and mitochondrial dysfunction in the hippocampus might be responsible for the abnormal mouse behaviors (70). Recent studies have shown that mice with chronic colitis exhibit increased anxiety-related behaviors in open-field and acoustic stress tests, accompanied by visceral hypersensitivity and low levels of intestinal inflammation (71).

Overall, IBD patients are more prone to developing emotional disorders than the general population. In addition, depression and anxiety have adverse effects on the course of the disease. Psychological comorbidity and IBD seem to fall into a vicious circle.

The Impact of Stress on IBD

Life always includes stresses which change over time. In adulthood, stress mainly originates from family, work, economic status, and major life-threatening events. Early life and childhood exposure to antibiotics, vaccination, diet, smoke, and psychosocial stress seems to lead to a long-term adverse influence throughout life. The stressors of the above different periods may increase adulthood susceptibility to diabetes, cardiovascular disease, autoimmune disease, stroke, and certain cancers (72–74).

Stress and the Risk of IBD Onset

A Manitoba IBD cohort study in Canada ascertained the first onset of psychotic symptoms via a structured diagnostic interview. The report showed that approximately two-thirds of patients who had both anxiety disorder and IBD actually developed psychiatric symptoms predating the IBD diagnosis by over 2 years. This more than 2-year time interval for diagnosis was also present in more than half of IBD patients with mood disorders. Moreover, IBD patients with lifelong anxiety or mood disorders displayed an earlier onset of IBD symptoms than those without the above disorders, and there was a tendency for an early diagnosis of IBD (75). These results reminded us of the potential interactions between IBD and psychiatric diseases. It is possible that the existence of these psychiatric illnesses may increase the susceptibility of individuals to IBD.

Recent studies have focused on the long-term effects of early life adversity on the immune system, including impaired cellular immunity, increased inflammation, and accelerated immunosenescence (76, 77). An animal experiment showed that early- life stress results in an altered microbiota and increased visceral sensation and psychiatric illnesses (78). A recent study found that nerve growth factor (NGF)-mediated tropomyosin receptor kinase A (TrkA) signaling mediates bowel dysfunctions that resemble IBS induced by neonatal maternal separation (79). Moreover, there are sex differences in the effects of early life adversity on gut microbiota and emotional behaviors (80). Parental separation in childhood can lead to psychological distress in adulthood to varying degrees. The adverse impact caused by this abnormal family pattern contributes to the development of IBD in adulthood (81). Researchers analyzed the relationship between the annual rhythm of IBD symptom onset and academic semesters in children. The results showed that academic stress may facilitate disease onset in pediatric IBD (82).

In animal models, there seem to be different views as to whether early stress increases the incidence of IBD, which may be related to different patterns and periods of stress (83–85).

Animals exposed to WAS developed acute small intestinal inflammation as evaluated by histological scores in an experimental study. Leukocytic infiltration, intestinal hyperpermeability, increased serum TNF-α, and upregulated IL-17 and IL-6 expression in mucosa have also been discovered during stress (45). In addition, acoustic stress has been found to cause severe enteritis in the healthy intestinal tract (86). Chronic stress can cause excessive growth of pro-inflammatory bacteria and thus induce increased susceptibility to colitis in subjects after fecal microbiota transplant. Stress is known to cause low-grade intestinal inflammation via increased bacterial translocation and the production of poisons (87).

Effect of Stress on IBD Course

Stress causes wide-ranging effects on patients with IBD, especially the recurrence and aggravation of the disease. Some studies have shown that high perceived stress has a bearing on the frequency of symptomatic flares (88). A systematic review of 15 high-quality studies arrived at the conclusion that emotions are associated with abdominal pain symptoms in IBD patients. Among IBD patients, depression, anxiety, and perceived stress are common emotional disorders (89). Although the symptoms become distinctly intensified, IBD activity evaluated by the fecal calprotectin level may not be apparent during perceived stress (90). In contrast, a German cross-sectional study including 1,032 IBD patients, revealed that relevant reported depressive symptoms correlate with increased rates of disease activity (91). In addition to exacerbating symptoms, stress can also lead to relapse in IBD patients (92). Moreover, in a prospective longitudinal study, 60 patients with quiescent IBD were followed-up for up to 18 months. The baseline depression score was found to be connected with the first recrudescence time. In particular, patients with anxiety appeared to have an increased recurrence frequency (93). A multicenter cohort study in Germany found that patients with CD were more likely to be affected by psychological disorders than those with UC or controls. Compared with the healthy controls, both UC and CD patients scored higher on psychological disorders and maladaptive stress coping tests during the active phase. Interestingly, UC patients in remission were minimally affected by psychological disorders, while CD patients in remission showed insecurity and paranoid ideation. The neuroticism score of CD patients was found to be higher than that of the healthy controls, while that of UC patients was not (94). Another study also showed that CD patients with depression were more likely to deteriorate than UC patients with depression (95). In addition, a prospective study found that depression increased the risk of CD, rather than UC, in women (96). A study in Switzerland recruited 468 adults with CD, who were followed-up for 18 months. The results of the study showed that, among those who were under perceived stress, patients with anxiety and depression were more likely to develop worsening of the disease, indicating the significance of emotional elements (97).

Several recent studies have identified factors beyond disease activity associated with pain burden at birth. Family stressors, such as divorce and loss of family members, were found to increase pain-related distress in children by influencing coping and depression symptoms (98). Thirteen percent of children with CD still suffer from abdominal pain despite clinical remission (99).

Constraint stress has been reported to aggravate spontaneous colitis in *IL-10*$^{-/-}$ mice compared to those without stress (100). Another study demonstrated that neonatal maternal separation induces disruption of the colon barrier and exacerbates colitis symptoms in adult *IL-10*$^{-/-}$ mice (83). Moreover, a recent study revealed that chronic stress damages the gut microbiota and increases susceptibility to DSS-induced colitis in mice. The downregulated expression of mucin-2 and lysozyme caused by stress is implicated in the disturbance of the microbiota (47). Additionally, 12 weeks of WAS significantly increased the relative abundance of the *Clostridium* genus, which produces the toxin phospholipase C in C57BL/6 mice. WAS has also been proven to change the concentration of luminal secreted immunoglobulin A, which is probably connected to gut microbiota alterations and colitis-associated deterioration (51).

Effect of Stress on IBD Prognosis

Psychosocial dysfunction has a negative effect on the treatment of IBD in adults. Psychosocial dysfunction causes adverse effects on the quality of life in IBD patients. IBD patients with psychological comorbidities seem to have more hospitalizations than those without (101). The Inflammatory Bowel Disease Questionnaire (IBDQ), which involves bowel symptoms (bowel movements and abdominal pain), systemic symptoms, and emotional and social factors, was applied to assess the health-related quality of life (HRQOL) of IBD patients. The data showed that an increased level of perceived stress, as judged by a 10-item Perceived Stress Scale, is one of the most predictive factors of reduced HRQOL (102). It has been confirmed that psychological symptoms, perceived stress, and disease severity can have a deleterious effect on HRQOL (103, 104). An IBD cohort study in Boston, including 5,405 CD and 5,429 UC patients, found that CD patients with emotional or anxiety disorders had a 28% increase in the risk of surgery compared to those without psychosocial disorders (105).

A recent study found that stress can inhibit endogenous opioids and can switch their signaling in dorsal root ganglion neurons from inhibition to excitation during chronic colitis, causing exacerbated pain and requiring increased doses of opioid analgesics in IBD patients (106). For adolescent patients with IBD, medication non-adherence is regarded as a major health care problem. A systematic review suggested that psychosocial factors, including poor child-coping strategies, family dysfunction, anxiety, and depressive symptoms, are relevant to medication non-adherence, which may lead to an unnecessary escalation in treatment and can jeopardize IBD therapy outcomes (107).

Due to the bidirectional effect between stress and IBD, patients may fall into a vicious cycle, leading to a poor prognosis. Therefore, attention should be paid to the role of stress therapy in the management of IBD.

MANAGEMENT OF IBD: TARGETING STRESS

According to the present clinical practice guidelines for IBD, therapeutic interventions mainly involve 5-aminosalicylic acid (ASA), corticosteroids, immunomodulators, antibiotics, probiotics, and anti-TNF agents. These traditional treatments can effectively relieve symptoms and promote mucosal healing (108). However, with the in-depth study of the adverse effects of stress on IBD, mental healing seems to be the ultimate treatment goal of IBD and is expected to surpass mucosal healing. Relieving psychological stress is of great benefit to improving symptoms and increasing quality of life. The emerging field of psychogastroenterology focuses on the application of brain-gut psychotherapies, which are considered to be an integral part of the management of digestive diseases (109). Although psychotherapy has limitations, it should be considered to be one of the therapeutic strategies for IBD.

Pharmacological Treatments

Pain symptoms in IBD patients appear to be associated with inflammation of enteric neurons (110). Enteric neurological abnormalities, including glial cell hyperplasia, and hypertrophy, were more pronounced in CD patients than UC patients (111), and appear to be associated with worsening symptoms and prognosis in CD patients. Intestinal inflammation may induce visceral hypersensitivity through the peripheral and central systems (112). Antidepressants are beneficial for relieving chronic pain, especially in patients with emotional disorders (113). Studies have demonstrated that a high percentage of IBD patients are treated with psychotropic drugs (114), with ∼30% of patients taking antidepressants (115). Selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) are the most common drugs used for the treatment of IBD patients with emotional complications, especially anxiety and depression (116). TCAs has been proven to have anti-inflammatory effects in animal intestines. Meanwhile, TCAs seem to relieve severe pain in IBD patients, even at low doses. However, TCAs can also cause side effects, such as dry mouth, blurred vision, and constipation, especially at high doses. These side effects usually recede after a few weeks. Tetracyclic antidepressants are beneficial for patients affected by sleep disorders and pain, but they have not been trialed in IBD patients (117). Moreover, propranolol, a β1-adrenoreceptor/β2-adrenoreceptor inhibitor, has been proven to suppress neutrophil infiltration in the colon and to attenuate tissue injuries caused by chronic stress, suggesting a potential therapeutic value of neuroprotectants, which guard against the recurrence of IBD by inhibiting immune activation (29).

Psychological Interventions

Nondrug psychological interventions for IBD include cognitive behavioral therapy (CBT), medical hypnosis, and mindfulness meditation. Some studies have shown that these methods reduce gastrointestinal symptoms in IBD patients (117, 118).

IBD-specific CBT is helpful in promoting a higher quality of life and reducing anxiety and depression in IBD patients who have a low level of HRQOL (119). In addition, a benchmark study found that CBT may help IBD patients with moderate to severe mood disorders (120). *Moser G* used gut-directed hypnotherapy (GHT) in the treatment of IBD. The results showed that GHT may prolong the remission duration of patients with inactive UC (121). Clinical hypnosis has been used to guide adolescents to cope with various diseases. Hypnosis can effectively relieve chronic abdominal pain in adolescents with IBD (122). Current mindfulness therapies include mindfulness stress reduction and mindfulness behavioral cognitive therapy, most of which are used in adults and show effectiveness in patients suffering from IBD. Furthermore, the physical and mental intervention of the Breath-Body-Mind Workshop (BBMW) were found to be beneficial for IBD patients for the alleviation of symptoms and emotional disorders (123). As an accepted decompression method, yoga appears to be a safe and efficacious method for the treatment of UC patients (124). In a study of adolescent IBD patients, yoga was found to be an effective complementary therapy. Unfortunately, this was a short survey with a small sample size (31).

A randomized controlled trial showed that psychotherapy (psychoeducation, problem-solving, and relaxation) for patients with IBD did not inhibit disease progression or relapse but enhanced the quality of life (125). A parallel group, randomized and controlled trial evaluated the effectiveness of a disease-specific CBT protocol on anxiety, depressive symptoms and HRQOL in adolescents and young adults with IBD. The preliminary results showed that IBD-specific CBT added to standard medical care did not perform better than standard medical care alone in improving psychological symptoms or HRQOL in youths with IBD (126).

Brain-Gut-Microbiota Axis Interventions

With the clear evidence of gut dysbiosis in IBD, novel treatments will doubtless require a microbiota-modulating approach (127). This has been an active field of research, with mixed results.

Although exposure to antibiotics is considered to be a potential risk factor for IBD (11, 128, 129), several meta-analyses have revealed that antibiotics are effective in inducing remission and treating flares in patients with IBD (130, 131). Antibiotic therapy remains controversial, especially considering the current mixed results and the potential risks of systemic adverse events and bacterial antibiotic resistance (132, 133). Rifaximin, a non-systemic bactericidal antibiotic, may be therapeutically beneficial for IBD (134). A study found that *Lactobacillus* species were significantly enriched after oral administration of rifaximin. Moreover, rifaximin treatment protected against the intestinal inflammation, barrier damage, and visceral hypersensitivity caused by chronic water avoidance and repeat restraint stressors in Wistar rats (135).

Supplementation with prebiotics and probiotics is favorable for reducing stress-related behavior and HPA activation. Probiotics such as *Bifidobacterium* and *Lactobacillus* can alleviate anxiety and depression (136). Arase et al. studied microbiota-targeted therapies and found that a probiotic *Lactobacillus strain* can assist in protecting against enteritis aggravated by stress (137). Animal studies have found that *Bifidobacterium P122, Lactobacillus LA804*, and *Lactobacillus Switzerland* are beneficial

in colitis (138). *B. longum 536* alleviates the symptoms of patients with mild to moderately active UC (139). However, a recent study found that the *B. breve* strain in Yakult did not delay relapse time, compared to a matched placebo in patients with inactive UC. This result may be related to a deficiency in the amount of *B. breve* (140). Most studies have suggested that probiotics are beneficial for IBD patients. The effectiveness and safety of probiotics for alleviating intestinal inflammation in patients with IBD needs more exploration (141).

FMT has become a helpful and increasingly available therapy because of stool banks (142). Randomized controlled studies have indicated that FMT appears to be somewhat effective in the treatment of UC (143, 144). In a prospective trial, 21 children with a median age of 12 years, with IBD refractory to medical treatment, were subjected to a single FMT. Clinical responses were observed in 57 and 28% of the patients at 1 and 6 months after FMT, respectively (145). Paramsothy et al. performed a systematic review and meta-analysis to assess the effectiveness and safety of FMT in IBD. A total of 53 studies (41 in UC, 11 in CD, 4 in pouchitis) published before January 2017 were included. The results showed that the rates of clinical remission in UC, CD, and pouchitis were 36, 50.5, and 21.5%, respectively. Sub-analyses suggested that remission in UC improved with lower gastrointestinal tract administration and an increased number of FMT infusions (146). However, some researchers did not find significant differences in the efficacy of FMT, which may be related to the number and cycle of enemas, preparation process, and limited numbers (147, 148). More large-scale studies evaluating the safety and efficacy of FMT for IBD patients would be a further promising direction.

Rooks et al. demonstrated that genetic inactivation of quorum-sensing *Escherichia coli* regulator C (QseC) can reduce the virulence and colonization capacity in a pathogenic, IBD-associated *E. coli* strain. Further results indicated that biochemical inhibition of QseC can reduce intestinal inflammation in a variety of preclinical IBD models, and provides a new approach for the treatment of colitis (149). Additionally, dietary interventions that modulate the interaction between the immune system and microbiota can also be an option for the treatment of IBD (150).

Studies of the efficacies of psychotherapy and psychopharmacological treatments in patients with IBD are controversial and limited. A systematic literature review has shown that 1/3 of the included 43 studies supported the effectiveness of psychotherapy on the quality of life and disease activity (113). More research is needed to validate this result. As an adjuvant therapy, stress management cannot completely replace drugs. In addition, the study of the gut microbiome and dietary therapy may be future directions for the treatment of IBD.

PERSPECTIVES

Sufficient evidence has indicated that the incidence of psychiatric comorbidities in patients with IBD is higher than that in healthy controls. In turn, these comorbidities exacerbate IBD symptoms and promote intestinal inflammation. The underlying mechanisms may involve alterations of the neuroendocrine system and the brain-gut-microbiota axis. However, the exact mechanisms underlying mucosal immune activation remain to be explored. The effects of psychological stress on IBD should be emphasized, especially in children and adolescents, because of the unique psychological problems encountered in the pediatric population. Thus far, some clinical data support the view that stress management, such as through relaxation exercises, is beneficial to IBD patients, particularly those who are refractory. The psychological score may act as a new measure to evaluate the severity and prognosis of IBD. In the course of future IBD treatment, emotional management, stress release, the use of psychotropic drugs, and care from family needs to be emphasized. Additionally, it should be highlighted that the combination of mucosal and psychological healing as the ultimate goal of therapy will improve the prognosis. The management of IBD goes far beyond traditional drugs and surgical treatment. What is more important is the practice of psychogastroenterology, which appears to be promising.

AUTHOR CONTRIBUTIONS

YS and LL wrote the manuscript. HC, RX, KJ, and BW designed the review. YS, LL, and RX participated in the literature search. YS and RX designed and created the figure. HC made critical revisions. All authors read the manuscript and ultimately approved the article.

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