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Therapeutic advances in inflammatory bowel disease: Current therapies and future directions

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Therapeutic advances in inflammatory bowel disease: Current therapies and future directions

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Butanol Purified Food Allergy Herbal Formula-2 Has an Immunomodulating Effect *ex-vivo* in Pediatric Crohn's Disease Subjects

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Background: TNF-α has a major role in the pathogenesis of Crohn's disease (CD). In contrast, GM-CSF may be beneficial for its anti-inflammatory role in a subset of patients with CD with antibodies against GM-CSF as seen in prior trials of GM-CSF which resulted in clinical improvement in CD. We developed butanol purified Food Allergy Herbal Formula-2 (B-FAHF-2) by refining FAHF-2. FAHF-2 suppressed TNF-α production by human peripheral blood mononuclear cells (PBMCs) and colonic mucosa, and abrogated colitis in a murine model. We sought to examine the effect of B-FAHF-2 and the herbs that comprise it on TNF-α and GM-CSF production as a potential herbal therapy for the treatment of CD.

Methods: B-FAHF-2 was examined using high pressure liquid chromatography (HPLC) and compared to the original formulation, FAHF-2. PBMCs from pediatric patients with CD were cultured with lipopolysaccharide and B-FAHF-2, individual herbs or medium alone. Colonic biopsy specimens were cultured with or without B-FAHF-2. TNF-α and GM-CSF were measured by enzyme-linked immunosorbent assay (ELISA). B-FAHF-2 efficacy was tested *in vivo* in the $CD45Rb^{hi}$ transfer model.

Results: B-FAHF-2 had a similar HPLC fingerprint as FAHF-2 but decreased TNF-α production by PBMCs and colonic mucosa from pediatric CD subjects at 20% of the FAHF-2 dose. B-FAHF-2 increased GM-CSF production by PBMCs and colonic mucosa from pediatric CD subjects including those with antibodies to GM-CSF. Of B-FAHF-2's herbal constituents, only Huang Bai suppressed TNF-α and increased GM-CSF production. In the murine model, B-FAHF-2 treatment alleviated colitis.

Conclusions: B-FAHF-2 decreased TNF-α production by PBMCs and colonic mucosa from pediatric subjects at a lower dose than FAHF-2. B-FAHF-2 also increased GM-CSF production by PBMCs independent of antibodies. B-FAHF-2 may have a benefit in CD patients.

Keywords: Crohn's disease, herbal therapy, pre-clinical drug testing, immunomodulatory, drug development

INTRODUCTION

Crohn's disease (CD), a form of inflammatory bowel disease (IBD), is a life-long disease characterized by chronic and relapsing inflammation of the gastrointestinal tract. The prevalence of CD varies based on region, race, age, and environment. The annual incidence of CD ranges from 16.7 to 318.5 per 100,000 in North American, 0.6 to 322 per 100,000 in Europe, and 0.88 to 67.9 per 100,000 in Asia and the Middle East (1). Among CD patients, 20–25% are diagnosed before 17 years old. The incidence of pediatric CD is steadily increasing (2). CD has multiple clinical phenotypes and disease severities that determine which therapy is utilized. Currently, there are numerous treatment options for children and adults with moderate-to-severe CD, but few that are approved to treat those with mild-to-moderate disease. The only FDA approved medication for treatment of mild-to-moderate CD in children 8 years and older is Entocort EC (budesonide). The indications allow for up to 10 weeks of use in active disease but not for use as a maintenance therapy in children. Budesonide is a steroid compound and as such may have the same side effects as corticosteroids. Other medications including immunomodulators and biologics are used off-label for mild-to-moderate CD in children and have risks for significant adverse effects. Thus, there is a need for the development of new therapies.

Herbal therapies may fill this therapeutic void and there is a growing interest in complementary treatments (3). Food Allergy Herbal Formula-2 (FAHF-2) is composed of nine Chinese herbal medications that are originally part of the traditional Chinese herbal formula, Wu Mei Wan, that has been used safely to treat colitis in China and Japan for thousands of years (4). FAHF-2 has been shown in both murine and human studies of food allergy to inhibit both adaptive and innate immune pro-inflammatory cytokine responses in peripheral blood mononuclear cells (PBMCs) and *in vivo* (5–10). Interestingly, in food allergic subjects, FAHF-2 induced potent suppression of TNF-α, one of the major inflammatory cytokines involved in the pathogenesis of IBD. Anti-TNF antibody-based biologics are one of the most efficacious groups of medications currently in use to treat IBD. That led to our investigation on the effects of FAHF-2 in IBD. We showed that FAHF-2 suppresses TNF-α, IFN-γ, IL-1β, IL-6, IL-12 and IL-17 production from both PBMCs and inflamed colonic mucosa from pediatric subjects with CD, and abrogates murine colitis in the $CD45RB^{hi}$ transfer model (5). FAHF-2 affects immune responses by disrupting the NF-kB pathway (5).

FAHF-2's effects are immunomodulatory and not globally immunosuppressive as evidenced by the effect FAHF-2 has on the production of IFN-γ in different inflammatory diseases. In food allergy, FAHF-2 stimulates increased production of IFN-γ (8, 11), whereas in CD it decreases IFN-γ production (5). In addition, FAHF-2's effects on chemokines and growth factors shown here for the first time, demonstrate that FAHF-2 suppresses production of numerous chemokines (IP-10, MIP-1β, eotaxin, RANTES, MIG, MCP-1, and MIP-1α) and stimulates production of granulocyte-macrophage colony-stimulating factor (GM-CSF) by PBMCs from pediatric subjects with CD.

GM-CSF in particular plays an integral role in intestinal innate immunity, immune tolerance and the induction of intestinal regulatory T cells (12, 13). Pediatric and adult CD patients with ileal and stricturing or penetrating disease were shown to have higher levels of GM-CSF autoantibodies (14). A recombinant human form of GM-CSF, sargramostim, had positive effects in a small pilot study of CD patients. A subsequent larger placebo-controlled trial showed that GM-CSF therapy decreased disease severity and induced mucosal healing, but failed to demonstrate a clinical response (15). The larger study failed to target subjects with deficiencies or antibodies against GM-CSF and this may have been the reason for its failure. Heterogeneity in pathogenic mechanisms exists. Thus, an increase in GM-CSF may be therapeutic for a subgroup of CD patients, and we therefore continued to investigate the ability of FAHF-2 and its derivatives to alter GM-CSF.

FAHF-2 shows promise as a possible therapy for CD. Clinical trials of FAHF-2 in food allergy however, revealed that the high burden of pills needed in clinical trials contributed to non-compliance (16). Our goal was to lower the pill burden needed to use FAHF-2 in treatment protocols. We therefore developed butanol purified FAHF-2 (B-FAHF-2) which could be used at a fraction of the dose. We also tested the individual herbs that comprise FAHF-2 and B-FAHF-2 for their effects on TNF-α and GM-CSF as representative of their ability to modulate the immune response in CD. We found that the purified form, B-FAHF-2, significantly suppressed the production of TNF-α by PBMCs and intestinal mucosal from pediatric subjects with CD and abrogated colitis in a murine model at a much lower dose than FAHF-2. B-FAHF-2 also increased production of GM-CSF by PBMCs and intestinal mucosa and this was also true in a small subset of our CD subjects with antibodies against GM-CSF. Consistent with traditional Chinese medicine practices, the individual herbs within FAHF-2 and B-FAHF-2 were

Abbreviations: FAHF-2, Food Allergy Herbal Formula-2; B-FAHF-2, butanol purified FAHF-2; IBD, inflammatory bowel disease; CD, Crohn's disease; HPLC, high pressure liquid chromatography; PBMCs, Peripheral blood mononuclear cells.

TABLE 1 | The composition of FAHF-2.

Latin name	Chinese name	Plant part	Occupied percent
Prunus mume	Wu-Mei	Fruit	20%
Zanthoxylum schinifolium	Chuan-Jiao	Pericarp	2%
Angelica sinensis	Dang-Gui	Root	6%
Zingiber officinalis	Gan-Jiang	Root	6%
Cinnamomum cassiae	Gui-Zhi	Burgeon	4%
Phellodendron chinense	Huang-Bai	Cortex	4%
Coptis chinensis	Huang-Lian	Root	6%
Panax ginseng	Hong-Shen	Root	6%
Ganoderma lucidum	Ling-Zhi	Mushroom	46%

not as effective as the whole in suppressing TNF-α and increasing GM-CSF.

MATERIALS AND METHODS

FAHF-2 and B-FAHF-2 Production, Quality Control, and Dose Derivation

FAHF-2 and B-FAHF-2 were obtained from Xiyuan Chinese Medicine Research and Pharmaceutical Manufacturer, Chinese Academy of Chinese Medicine Sciences, Beijing, China, a good manufacturing practice (GMP) certified facility. Herbal components of FAHF-2 are listed in **Table 1**. All herbs were inspected for identity and quality by licensed pharmacists. All plant names have been checked with http://www.theplantlist.org except for *Ganoderma lucidum* which is a fungus and not listed there. Voucher specimens of the raw herbs were archived in the laboratory of Dr. Xiu-Min Li. The manufacturing process of FAHF2 is as follows: *Ganoderma lucidum* powder was generated by decoctions by boiling small pieces *Ganoderma lucidum* twice (2 h each time). *Panax ginseng* was extracted twice with 80% aqueous ethanol, then filtered, combined, and evaporated until there was no residual ethanol. The other 7 herbs were combined and boiled twice in water (1.5 h each time). The decoction was collected, combined, and purified by ethanol precipitation. *Panax ginseng* was mixed with the 7 herbs extract, dried into a powder, and then combined with the *Ganoderma lucidum* powder to form FAHF-2 (17, 18).

Extensive quality control, analytical chemistry data and batch consistency pertaining to the formula have been published previously (11). We identified chemical markers by high-pressure liquid chromatography (HPLC) and liquid chromatography (LC) mass spectrometry. Endotoxin levels were measured using the Pyrogent Plus assay kit (Lonza, MA) and were below 0.03 EU/ml, the limit of sensitivity for this kit.

Given the generally favorable safety profile, the dose ranges of most Chinese herbal formulations are wide. Initially, the dose used in our FAHF-2 studies was converted from the human daily dose of the Wu Mei Wan formula and Ling Zhi extract at a medium to high dose range using a conversion table of equivalent effective dose ratios from humans to animals based on body surface area (19). This dose was consistently effective in protecting peanut allergic mice from anaphylaxis, and at abrogating colitis in a murine model (5). B-FAHF-2 is 20% of FAHF-2 by weight because of the elimination of non-medicinal components and thus the dose used in our experiments is derived by using 20% of the dose of FAHF-2 (250 μg/ml). The effective dose (60 μg/ml) of B-FAHF-2 was found to be 20% that of FAHF-2 based upon cell culture assays and *in vivo* models of peanut allergy (6). For the individual herbs within FAHF-2 and B-FAHF-2, the doses used correspond to the equivalent dose found in the full compound. Equivalent doses were as follows: Wu Mei (WM) 50 μg/ml, Chuan Jiao (HJ) 125 μg/ml, Dang Gui (DJ) 125 μg/ml, Gan Jiang (GJ) 125 μg/ml, Gui Zhi (GZ) 125 μg/ml, Huang Bai (HB) 125 μg/ml, Huang Lian (HL) 125 μg/ml, Hong Shen (HS) 125 μg/ml, and Ling Zhi (LZ) 125 μg/ml.

HPLC of FAHF-2, and B-FAHF-2

The instruments used were a Waters 2690 HPLC system coupled to a 2996 PDA detector (Waters, Milford, MA). B-FAHF-2 tablets were ground, and a 22.5 mg/mL solution was prepared using 1:1 ratio of mobile phase mixture. The B-FAHF-2 solution was centrifuged at 10,000 rpm for 10 minutes. 10μL of the supernatant was injected into the HPLC system and separated on a ZORBAX SB-C18 (4.6× 150 mm, 5 μm) column (Agilent, Santa Clara, CA). The mobile phase A was made of 0.1% of formic acid aqueous solution. Mobile phase B was acetonitrile. The separation was performed at 1 min/mL flow rate following a linear gradient elution of 2–25% mobile phase B in 45 min, 25–35% B in the following 25 min, 35–55% in the next 15 min, 55–75% in another 10 min. This mobile phase composition was maintained for 5 min and then rapidly switched to 2% mobile phase B. An equivalent amount of FAHF-2 formula, 99 mg/mL, was also prepared following the same procedure as for B-FAHF-2. 10μL of FAHF-2 supernatant was analyzed on the HPLC system. Data was collected and processed with Waters' Empower software.

Subjects

Human studies were approved by the Institutional Review Board of the Icahn School of Medicine at Mount Sinai (No. 11-00808). All participants provided written informed consent for participation in the study. Blood samples (n = 29) and inflamed colonic biopsy specimens (n = 20) were collected from newly diagnosed pediatric CD patients (8–19 years old) naïve to medications that could alter immune responses including: immune-modulators, biologics or steroids. CD patients were diagnosed based on standard clinical, radiographic and endoscopic criteria. Blood and colonic biopsy specimens were also collected from non-IBD control subjects (n = 12). Patient characteristics are outlined in **Table 2**.

PBMC Separation, Cell Culture and Cytokine, Chemokine, and Growth Factor Measurements

PBMCs were isolated by Ficoll Hypaque (Pharmacia, Piscataway, NJ) with density gradient centrifugation. Purified PBMCs were cultured in medium containing 5% FBS with or without FAHF-2 (250 μg/ml), B-FAHF-2 (60 μg/ml), individual herbs

TABLE 2 | Subject characteristics based upon the Montreal classification for CD.

	CD ($n = 29$)	Non-IBD ($n = 12$)
Gender (male/female)	21/8	6/6
Age, mean ± SD	13 ± 3.2	15.8 ± 2.5
Location (L2/L3)	6/23	N/A
Behavior (B1/B2/B3)	26/0/3	N/A
Perianal (N/Y)	26/3	N/A

L2, colonic; L3, ileocolonic; B1, non-stricturing, non-penetrating; B2, stricturing; B3, penetrating.

TABLE 3 | Real-time PCR primer sequences.

Target	Forward sequences (5′-3′)	Reverse sequences (5′-3′)
TGF-β1	CCCGAAGCGGACTACTATGC	CGAATGTCTGACGTATTGAAGAACA
TGF-β2	CACCCAGCGCTACATCGATAG	CAGCGTCTGTCACGTCGAA
IL-10	TTTGAATTCCCTGGGTGAGAA	GCTCCACTGCCTTGCTCTTATT
Foxp3	ACTGGGGTCTTCTCCCTCAA	CGTGGGAAGGTGCAGAGTAG
Gapdh	GGTGGTCTCCTCTGACTTCAACA	GTTGCTGTAGCCAAATTCGTTGT

in B-FAHF-2 (doses noted above) or dexamethasone (10^{-3} μM/ml, positive control) for 24 h followed by LPS (2 μg/ml) stimulation or no stimulation for an additional 24 h (8). Cytokine, chemokine and growth factor levels in the culture supernatants were determined by ELISA per the manufacturer's instructions (BD Biosciences), by multiplex immunoassay or by cytometric bead array. Multiplex assays were performed per the manufacturer's instructions (Luminex Human assay, Invitrogen, Grand Island, NY). Cytometric bead array was performed per the manufacturer's instructions (BD Biosciences).

Biopsy Preparation and Culture

Colonic biopsies were washed with PBS and then cultured with or without B-FAHF-2 (60 μg/ml) in complete RPMI (10% FBS and GPS) with phosphatase and protease inhibitors overnight. The volume of culture medium was based upon the weight of each sample so that the size of the biopsies could be standardized. Supernatants were filtered and TNF-α and GM-CSF were assessed by ELISA (BD Biosciences) per the manufacturer's instructions.

GM-CSF Antibody Measurement

Serum was tested by ELISA. Briefly, samples were diluted serially in 4-fold increments from 1/100 to 1/25,000 and assayed for the presence of IgG and IgA against GM-CSF (Sargramostim, Genzyme), as well as irrelevant control recombinant proteins. Each plate included positive control sera from patients with pulmonary alveolar proteinosis as well as a negative control healthy donor serum pool, expected, respectively, to react or not with GM-CSF.

CD45RB^{hi} T Cell Transfer Model of Colitis

Animal studies were approved by the Institutional Animal Care and Use Committee at Mount Sinai. Cells were obtained from C57BL/6 wild type mice and enriched using an EasySep Cell Isolation Kit (STEMCELL Technologies Inc., Vancouver, CA) that depletes $CD8^+$, $CD11b^+$, $CD11c^+$, $CD19^+$, and $B220^+$ cells by negative selection. The resulting $CD4^+$ enriched population was labeled with FITC-conjugated anti-CD4 Ab, APC-conjugated anti-CD62L Ab and PE-conjugated anti-CD45RB Ab (eBiosciences). Subpopulations of $CD4^+$ cells were sorted by flow cytometry. $CD4^+$CD45RBhi T cells (3.5×10^5) were adoptively transferred by intraperitoneal injection into recipient $RAG1^{-/-}$ mice. Transfer was confirmed by flow cytometry of peripheral blood in all mice after 2–4 weeks. To prevent the progression to colitis, the recipient mice were fed B-FAHF-2 (50 mg/day) daily by gavage 24 h after $CD4^+$CD45RBhi T cells transfer. Control mice received water by gavage. Weights were recorded semi-weekly. All mice were sacrificed once any mouse lost 20% of their initial weight. Colonic histology was scored for inflammatory infiltrates and epithelial damage by a pathologist blinded to the treatment group (20, 21). A total score with a maximum of 20 was determined by the summation of the following sub-scores: mucosal involvement: 0 = normal, 1 = 3–10 mucosal neutrophils/hpf, 2 = more than 10 mucosal neutrophils or rare crypt abscesses, 3 = multiple crypt abscesses or erosion; submucosal involvement: 0 = normal,1 = focal aggregates of neutrophils, 2 = neutrophil infiltration with expansion of submucosa, 3 = diffuse neutrophil infiltration; muscularis: 0 = normal, 1 = scattered neutrophils within the muscularis, 2 = neutrophil infiltration with focal effacement of the muscularis, 3 = extensive neutrophil infiltration with transmural effacement of the muscularis; crypt damage: 0 = normal, 1 = loss of the basal one third, 2 = loss of the basal two thirds, 3 = entire crypt loss, 4 = crypt loss with surface erosion, 5 = confluent, extensive erosion; ulcerations: 0 = absence of ulceration, 1 = one to two ulcer foci, 2 = three to four ulcer foci, 3 = confluent extensive ulceration. Colonic samples were cultured overnight in medium containing phosphatase and protease inhibitors. Cytokine secretion was measured in the supernatants (IL-4, IL-6, IL-10, IL-12, TNF-β, IFN-γ, IL-17A) by cytometric bead array (BD Biosciences) and ELISA for GM-CSF (BD Biosciences) per the manufacturer's instructions. RT-PCR was performed to look at regulatory elements within the colonic mucosa including TGFβ1, TGFβ2, Foxp3, and IL-10. Colon samples' RNA extraction and complementary DNA (cDNA) transcription were performed as previously described. (22) SYBR™ Green Master Mix (Thermo Fisher Scientific, Fair lawn, NJ) was used to perform RT-PCR. The target gene mRNA expression was normalized to the untreated group and calculated with the $_{\Delta\Delta}$CT method. The primer sequences are listed in **Table 3**. Immunofluorescence (IF) staining of mouse colon sections was performed according to the previously described protocol with slight modifications (23). Paraffin embedded slides were dewaxed by serial xylene, xylene/ethanol (1:1), 100% ethanol, 95% ethanol, 70% ethanol, and H_2O. Then slides were unmasked in heat antigen retrieval solution in the microwave. After permeabilization for 10 min at room temperature (RT), the slides were blocked with 20% goat serum in PBS for 1 h at room temperature, and then incubated with CD4-FITC and F4/80-APC (eBiosciences,

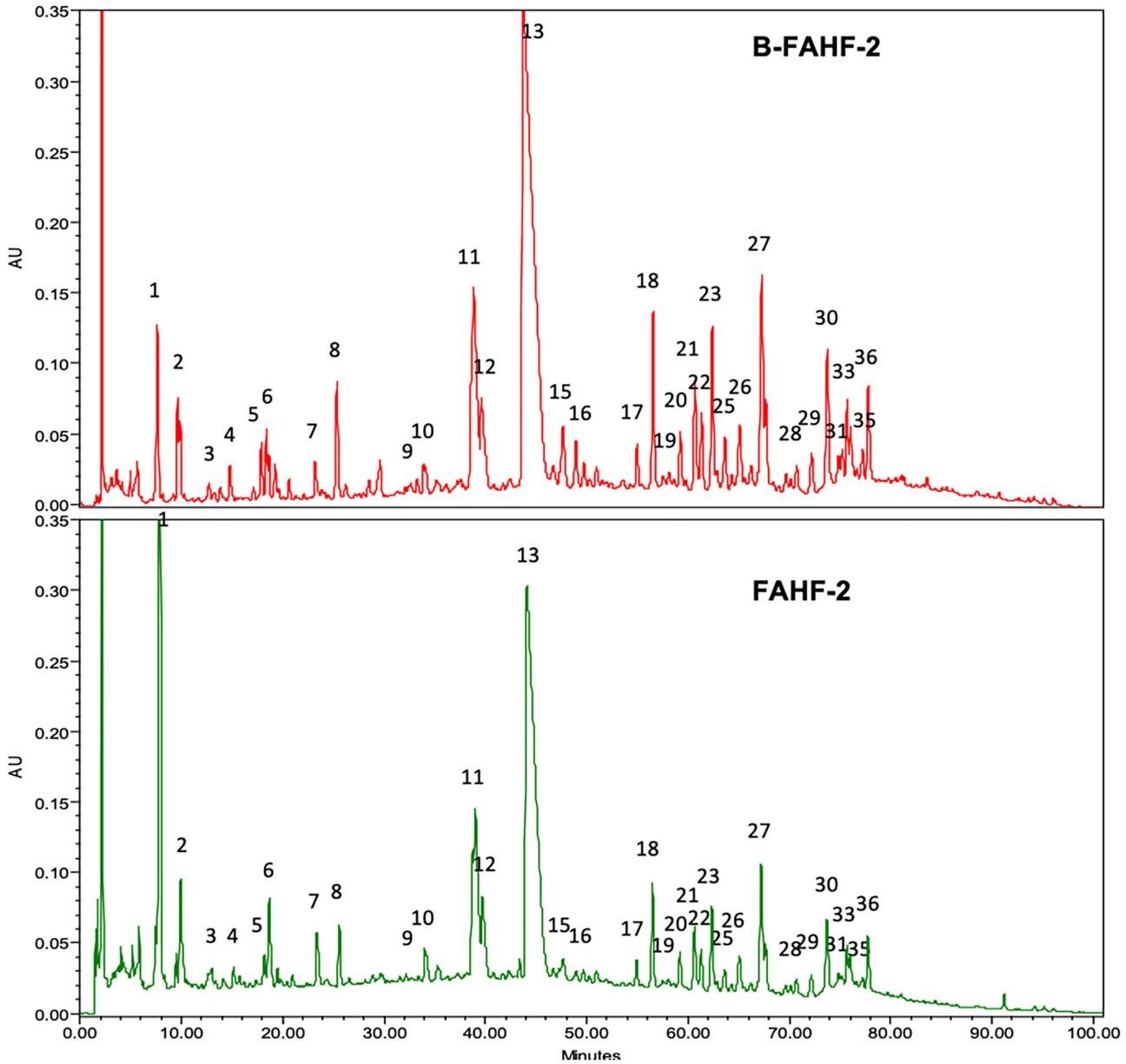

FIGURE 1 | HPLC fingerprints of B-FAHF-2 (top) and FAHF-2 (bottom) demonstrating the presence of the same compounds. The HPLC major peaks in three key herbs of FAHF-2 and B-FAHF-2, Huang-Bai, Ling-Zhi and Wu-Mei overlap with FAHF-2 and B-FAHF-2 in on-line ultraviolet (UV) spectra and retention time (t_R). Huang-Bai contributes to peaks 1, 5–8, 12–14. Ling-Zhi contributes to peaks 1–4, 7, 13, 16–19, 31, 32, 35, 36. Wu-Mei contributes to peak 1–6.

San Diego, CA) overnight at 4°C. DAPI was used to stain cell DNA.

Statistics

Paired *T*-tests with Wilcoxon signed-rank test were used when comparing the same samples without and with treatment. Comparisons between multiple groups were done using one-way ANOVA. This was followed by either non-parametric Mann-Whitney U test or Bonferroni analysis when appropriate. Statistical differences between groups for the colitis model were determined by Mann-Whitney *T*-test assuming non-normally distributed data. Data analysis was done using Prism software (GraphPad, San Diego, CA). A value of $p < 0.05$ was considered statistically significant. *P*-values are indicated by $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

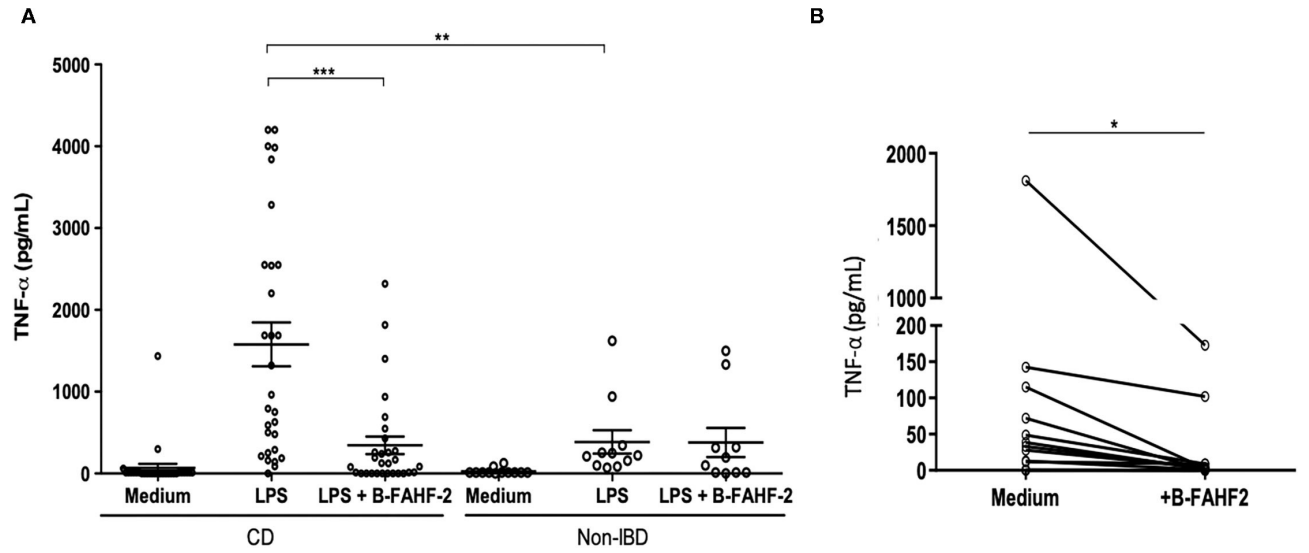

FIGURE 2 | B-FAHF-2 suppressed production of TNF-α by PBMCs and by colonic mucosa from pediatric subjects with CD. **(A)** TNF-α levels from PBMCs after stimulation with LPS in CD and non-IBD controls and then after treatment with B-FAHF-2 (CD $n = 29$, Non-IBD $n = 12$). **(B)** TNF-α levels from colonic biopsies from pediatric subjects with CD cultured in medium or with the addition of B-FAHF-2 ($n = 20$) ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$).

RESULTS

B-FAHF-2 Contained the Same Compounds as FAHF-2 and Suppressed TNF-α Production by PBMCs and Colonic Mucosa From Pediatric Subjects With CD at 20% of the Dose of FAHF-2

After butanol extraction of FAHF-2 was completed, B-FAHF-2 and FAHF-2 were examined by HPLC to ensure that the compounds were similar. HPLC fingerprints (**Figure 1**) demonstrate similar patterns with peaks at similar absorbance units and retention times, indicating that they contain the same compounds.

FAHF-2 suppressed production of TNF-α IFN-γ, and IL-12 from PBMCs from pediatric subjects with CD (5) and therefore we examined B-FAHF-2's effects on TNF-α production as a representative cytokine. We first tested the effect of B-FAHF-2 (60 μg/ml) on TNF-α secretion by PBMCs from 29 CD subjects and 12 non-IBD controls (**Table 2**). PBMCs from CD subjects secreted significantly more TNF-α upon LPS stimulation as compared to non-IBD controls ($p < 0.01$) (**Figure 2A**). TNF-α levels were significantly reduced when CD PBMCs were cultured with B-FAHF-2 ($p < 0.001$) (**Figure 2A**). No cytotoxicity was detected (Data not shown).

To be effective at treating CD, B-FAHF-2 should have effects on intestinal mucosa and inflammatory cytokine secretion. FAHF-2 suppressed production of TNF-α, IL-1β, IL-6, and IL-17 from inflamed colonic mucosa from pediatric subjects with CD (5) and therefore we examined B-FAHF-2's effects on TNF-α production as a representative cytokine. We incubated inflamed colonic mucosa from subjects with CD with and without B-FAHF-2 and quantified TNF-α from the supernatant. B-FAHF-2 significantly suppressed the production of TNF-α from inflamed biopsies from subjects with CD ($p < 0.05$) (**Figure 2B**).

Thus, like FAHF-2, B-FAHF-2 significantly suppressed TNF-α production by PBMCs and inflamed colonic mucosa from pediatric subjects with CD.

B-FAHF-2 Stimulated Increased Production of GM-CSF by PBMCs and Colonic Mucosa From Pediatric Subjects With CD

FAHF-2 had an immunomodulatory effect that is best demonstrated by its stimulation of increased production of GM-CSF while at the same time suppressing production of multiple inflammatory chemokines (IP-10, MIP-1β, eotaxin, RANTES, MIG, MCP-1, and MIP-1α) and HGF from PBMCs from pediatric subjects with CD (**Figure 3**).

Therefore, using GM-CSF as representative of the immunomodulatory capacity FAHF-2, we tested the effect of B-FAHF-2 on GM-CSF secretion by unstimulated PBMCs from all 29 pediatric subjects with CD. PBMCs cultured with B-FAHF-2 produced more GM-CSF than when cultured in medium alone (**Figure 4A**) ($p < 0.05$). B-FAHF-2 also stimulated significantly increased production of GM-CSF from biopsies from pediatric subjects with CD (**Figure 4B**) ($p < 0.05$).

B-FAHF-2 Increased GM-CSF Production by PBMCs From Pediatric Subjects With CD Who Have GM-CSF Antibodies

Given the effect of B-FAHF-2 on GM-CSF and the possibility that it might be beneficial in a subset of patients with GM-CSF antibodies, we tested the serum from all subjects. We found that four of the 29 subjects with CD had GM-CSF antibodies. B-FAHF-2 significantly increased GM-CSF production by PBMCs

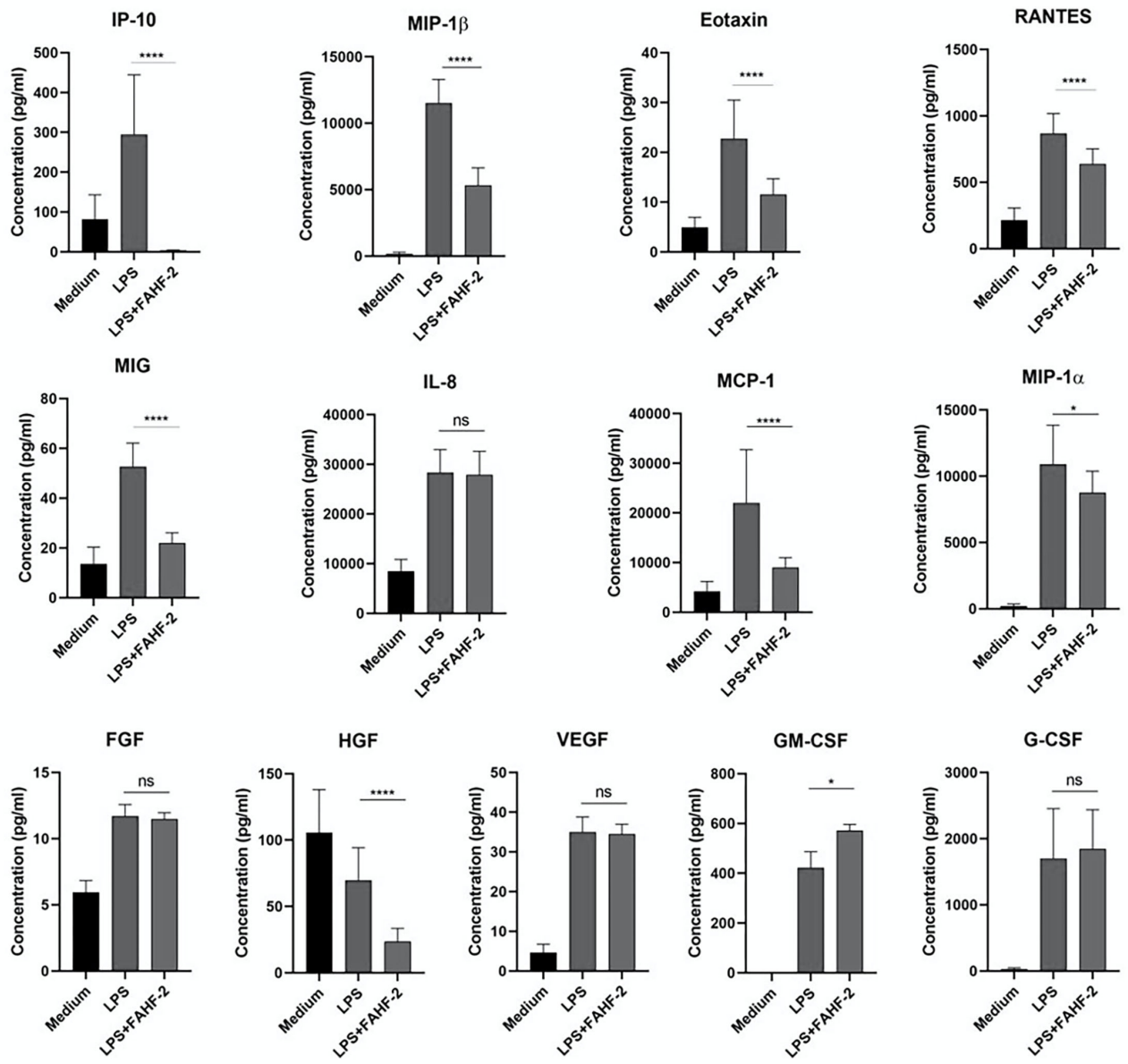

FIGURE 3 | FAHF-2 modulated chemokine and growth factor production by PBMCs from pediatric CD subjects. Production of IP-10, MIP-1β, Eotaxin, RANTES, MIG, MCP-1, MIP-1α, HGF and GM-CSF by LPS- stimulated PBMCs from pediatric CD subjects was significantly altered when cultured with FAHF-2 (LPS+FAHF-2) versus without it (LPS) ($n = 14$, ns, not significant, $^{*}p < 0.05$, $^{****}p < 0.0001$).

from these 4 subjects ($p < 0.05$) (**Figure 5**) despite them having antibodies to GM-CSF.

Only B-FAHF-2 and Huang Bai Were Effective at Both Suppressing TNF-α Production and Inducing GM-CSF Production by PBMCs From Pediatric Subjects With CD

Given the potent suppressive effect of FAHF-2 and B-FAHF-2 on TNF-α production, (5) each herb that comprises them [*Prunus mume (Wu Mei), Zanthoxylum bungeanum* (Chuan Jiao), *Angelica sinensis* (Dang Gui), *Zingiber officinalis* (Gan Jiang), *Cinnamomum cassia* (Gui Zhi), *Phellodendron chinense* (Huang Bai), *Coptis chinensis* (Huang Lian), *Panax ginseng* (Hong Shen) and *Ganoderma lucidum* (Ling Zhi)] was tested for the ability to suppress production of TNF-α from LPS stimulated PBMCs from pediatric subjects with CD. LPS caused a marked increase in production of TNF-α. B-FAHF-2, Dexamethasone (Dexa), Huang Bai (HB), Huang Lian (HL) and Dang Gui (DG) significantly reduced production of TNF-α ($p < 0.0001$, $p < 0.001$, $p < 0.01$, $p < 0.05$, and $p < 0.05$ respectively) (**Figure 6A**).

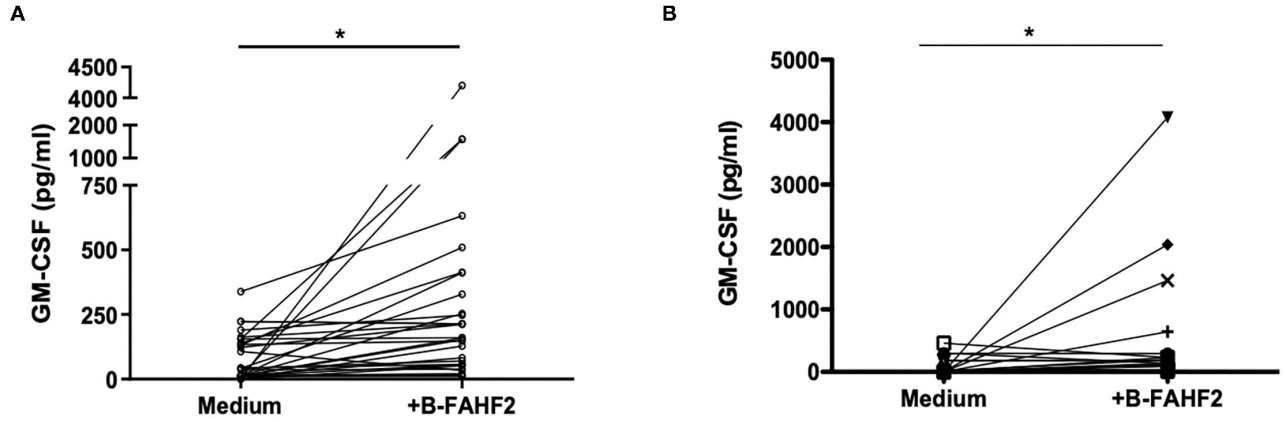

FIGURE 4 | B-FAHF-2 stimulated increased production of GM-CSF by PBMCs and colonic mucosa from pediatric subjects with CD. **(A)** GM-CSF levels from PBMCs from pediatric subjects with CD cultured in medium or with the addition of B-FAHF-2 ($n = 29$). **(B)** GM-CSF levels from colonic biopsies from pediatric subjects with CD cultured in medium or with the addition of B-FAHF-2 ($n = 20$) (*$p < 0.05$).

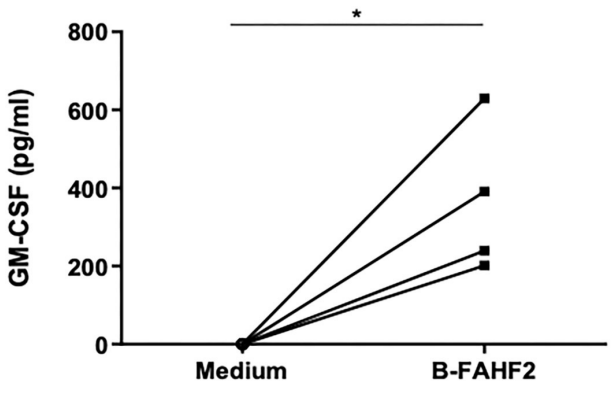

FIGURE 5 | B-FAHF-2 caused increased GM-CSF production by PBMCs from pediatric subjects with CD who have GM-CSF antibodies. GM-CSF levels from PBMCs from four pediatric subjects with CD and GM-CSF antibodies cultured in medium alone or with the addition of B-FAHF-2 ($n = 4$, *$p < 0.05$).

We then tested the effect of B-FAHF-2 and each herb that comprises it on the production of GM-CSF. B-FAHF-2, Ling Zhi (LZ) and Huang Bai (HB) induced significantly increased production of GM-CSF by unstimulated PBMCs from pediatric subjects with CD ($p < 0.05$, $p < 0.001$, and $p < 0.05$, respectively) (**Figure 6B**).

Overall, only B-FAHF-2 and Huang Bai (HB) could both decrease TNF-α and increase GM-CSF production by PBMCs unlike any of the other individual herbs.

B-FAHF-2 Alleviated Colitis in a Murine Model

In the CD45RB^{hi} T cell transfer model of colitis, treatment with FAHF-2 decreased colitis progression as evidenced by decreased weight loss, histological inflammation and production of TNF-α, IFN-γ, IL-6, and IL-17 from the colon (5). Therefore, we assessed the effect of B-FAHF-2 *in vivo* using the CD45RB^{hi} T cell transfer model of colitis, which exhibits features like those found in CD including transmural colitis and elevated levels of TNF-α. B-FAHF-2 treated mice trended toward less weight loss (**Figures 7A,B**) and had significantly less colon shortening than untreated controls ($p < 0.05$) (**Figure 7C**). The B-FAHF-2 treated mice had significantly less histological inflammation with less muscularis inflammatory infiltrates, crypt damage, and ulceration ($p < 0.05$, $p < 0.05$, $p = 0.0548$, and $p < 0.01$, respectively) (**Figures 7D–F**). The scoring system used takes into account changes in neutrophil infiltrates. Thus, we also looked by IF at CD4$^+$ T cell and macrophage infiltrates and found that both were decreased in the B-FAHF-2 treated group (**Figure 7F**). Inflammatory cytokine production by colonic tissue in B-FAHF-2 treated mice was reduced: TNF-α, IFN-γ, and IL-6 ($p < 0.05$, $p < 0.05$, $p < 0.01$, respectively) (**Figure 7G**). Levels of IL-17 and IL-10 were not detectable in both groups (data not shown). Finally, to examine if B-FAHF-2 affects regulatory T cells, we examined TGF- β1, TGF-β2, Foxp3, and IL-10 by RT-PCR and found no significant differences between the groups (data not shown).

Thus, B-FAHF-2 alleviates colitis and inhibits the inflammatory milieu associated with colitis.

DISCUSSION

In this continuation of our studies on FAHF-2 as a potential treatment for CD, we first sought to lessen the pill burden of FAHF-2 required for human trials by purifying the active compounds and then by examining the individual herbs. Our data demonstrates that the purified product, B-FAHF-2, was effective at suppression of TNF-α production by both PBMCs and mucosal biopsies from pediatric subjects with CD at 20% of the dose of FAHF-2. In a murine model, B-FAHF-2 was effective at alleviating colitis at this same dose. B-FAHF-2 also caused an increase in GM-CSF production even in those subjects with antibodies against it. Of the individual herbs within FAHF-2 and

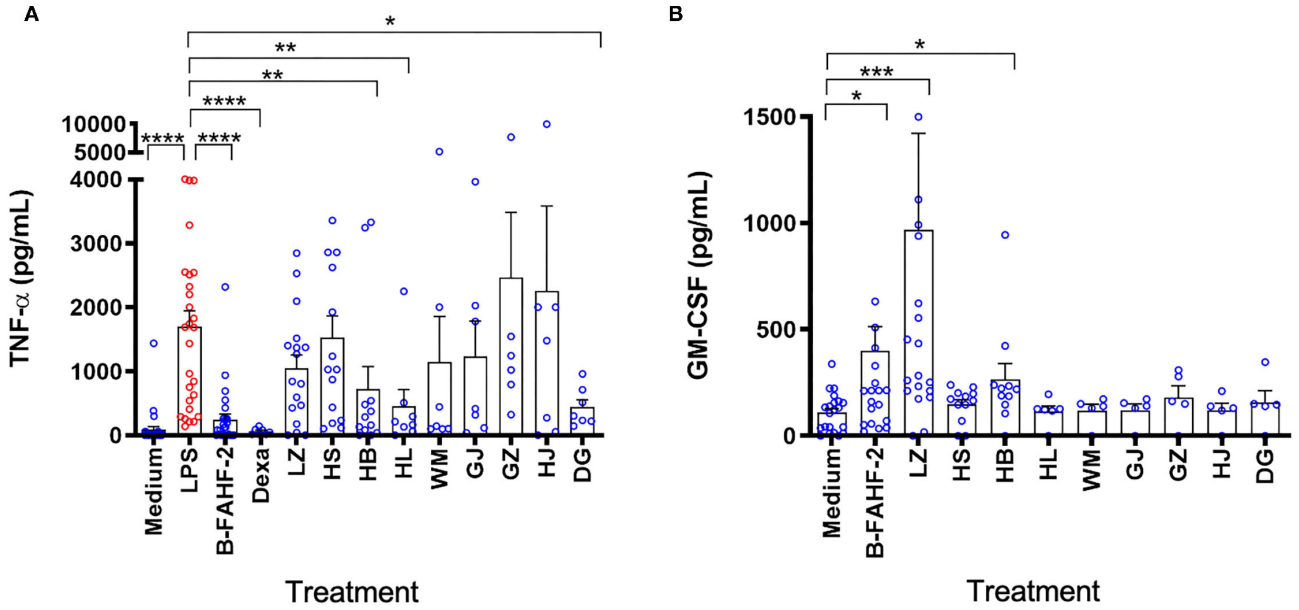

FIGURE 6 | Only B-FAHF-2 and Huang Bai suppressed TNF-α and induced GM-CSF production by PBMCs from pediatric subjects with CD. **(A)** TNF-α levels from PBMCs from pediatric subjects with CD stimulated with LPS and treated with B-FAHF-2, dexamethasone (Dexa), *Ganoderma lucidum*/Ling Zhi (LZ), *Panax ginseng*/Hong Shen (HS), *Phellodendron chinense*/Huang Bai (HB), *Coptis chinensis*/Huang Lian (HL), *Prunus mume/Wu Mei (WM), Zingiber officinalis*/Gan Jiang (GJ), *Cinnamomum cassia*/Gui Zhi (GZ), *Zanthoxylum bungeanum*/Chuan Jiao (HJ), or *Angelica sinensis*/Dang Gui (DG). **(B)** GM-CSF levels from PBMCs from pediatric subjects with CD treated with B-FAHF-2, dexamethasone (Dexa), *Ganoderma lucidum*/ Ling Zhi (LZ), *Panax ginseng*/ Hong Shen (HS), *Phellodendron chinense*/Huang Bai (HB), *Coptis chinensis*/Huang Lian (HL), *Prunus mume/Wu Mei (WM), Zingiber officinalis*/Gan Jiang (GJ), *Cinnamomum cassia*/Gui Zhi (GZ), *Zanthoxylum bungeanum*/Chuan Jiao (HJ), or *Angelica sinensis*/Dang Gui (DG) (n 7–28) (****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ as compared with LPS stimulated PBMCs for TNF-α or as compared to medium alone for GM-CSF).

B-FAHF-2, only Huang Bai was effective at both suppressing TNF-α and stimulating GM-CSF production.

Purification of FAHF-2 using butanol extraction did not affect the ability of B-FAHF-2 to suppress TNF-α production *ex vivo* from human PBMCs and colonic mucosa nor its ability to abrogate colitis in a murine model. The components removed by butanol purification were non-medicinal components including fiber, wax, proteins and starch. Given that B-FAHF-2 has a similar HPLC fingerprint as FAHF-2, and similar effects on TNF-α suppression (5), B-FAHF-2 likely has similar immunomodulatory effects beyond what we have shown here. Importantly, the *in-vitro* dose of FAHF-2 that inhibited 60–70% of TNF-α production was 700 μg/mL whereas that of B-FAHF-2 is only 60 μg/mL. In human trials this equates to a pill burden reduction from 36 pills daily for FAHF-2 to 10 pills maximum per day for B-FAHF-2 (6). This reduction would allow clinical trials to proceed with better participant compliance. An even more purified version of B-FAHF-2 is currently in a phase II trial as an adjunct therapy for children and adults with multiple food allergies (24), and a clinical trial in patients with mild-to-moderate CD, where there is a lack of FDA approved medications, is about to begin (25).

Our results on the effect of FAHF-2 on chemokines and growth factors showed that the formulation is immunomodulatory and not broadly immunosuppressant. Studies have shown that alterations of GM-CSF function may be involved in a subset of IBD cases (14). A recombinant human form of GM-CSF, sargramostim, had positive effects in a pilot study of patients with CD but larger trials did not show improved clinical outcomes (26, 27). This is likely due to heterogeneity in pathogenic mechanisms. Therefore, GM-CSF may be an appropriate therapy for a subgroup of CD patients which the larger clinical trial did not target specifically. We demonstrate for the first time, that B-FAHF-2 increases production of GM-CSF from PBMCs and from colonic mucosa from children with CD. The most likely therapeutic target for this increase would be a subset of CD patients who have been shown to have antibodies against GM-CSF and thus low levels of GM-CSF. In our study population, only 4 children had significant antibodies against GM-CSF and, independent of the presence of antibodies, B-FAHF-2 significantly increased production of GM-CSF by PBMCs from these subjects.

The complexity of Traditional Chinese Medicine makes these therapies particularly difficult to study. Each herbal formulation including B-FAHF-2, incorporates multiple herbs, which in turn are made up of numerous chemicals. The active components of these complex mixtures work synergistically and removal of some components, even if inactive, can destabilize the active components (28). In addition, many of the components may be transformed *in vivo* into more complex active metabolites that could be eliminated unintentionally. Thus, we were not surprised that only one of the individual herbs, Huang Bai,

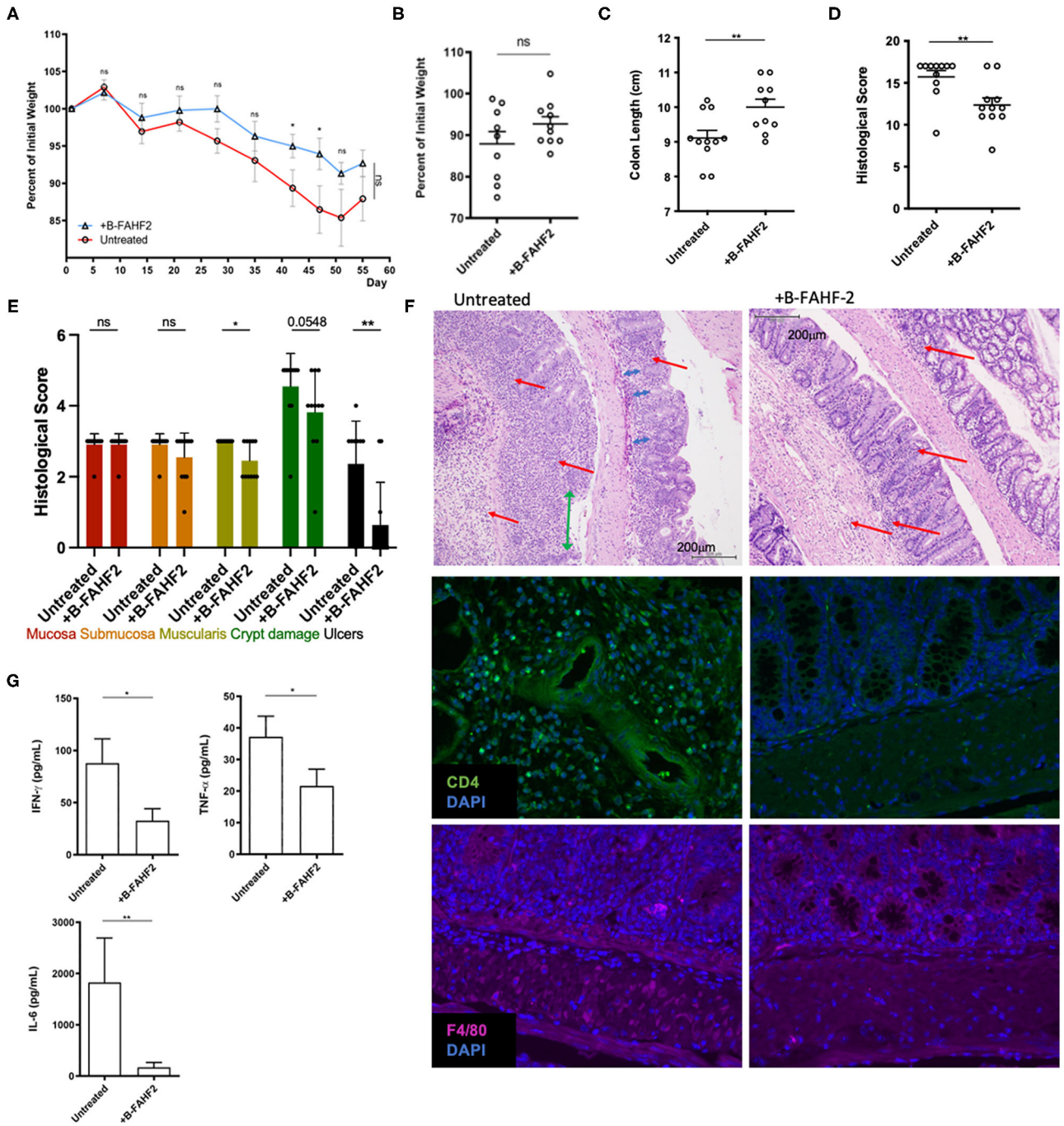

FIGURE 7 | B-FAHF-2 alleviated colitis in the CD45RB^{hi} transfer model. **(A)** Weight curves of mice treated (+B-FAHF-2) or untreated with B-FAHF-2. **(B)** The final percentage of initial body weight as measured at the time the mice were sacrificed. **(C)** Colon length of mice treated (+B-FAHF-2) or untreated with B-FAHF-2. **(D)** The total histological scores of inflammation of the colon of mice treated (+B-FAHF-2) or untreated. **(E)** The histological sub-scores for inflammation in the mucosa, submucosa, and muscularis as well as for crypt damage and the extent of ulceration in mice treated (+B-FAHF-2) or untreated. **(F)** A representative H & E stained histological figure demonstrating the untreated specimen with marked inflammatory infiltrate including neutrophils (red arrow), loss of crypts (blue arrow), crypt distortion, and ulceration (green arrow) of the mucosa vs. the B-FAHF-2 treated specimen which has significantly less inflammatory infiltrate and disordered crypts but no ulceration and minimal loss of crypts. Immunofluorescence staining for CD4$^+$ T cells and macrophages (F4/80) in untreated and +B-FAHF-2 groups. **(G)** Cytokine production by colonic mucosa from mice treated (+B-FAHF-2) or untreated with B-FAHF-2 ($n = 10$ per group, ns, not significant, $^*p < 0.05$, $^{**}p < 0.01$).

could both suppress TNF-α and stimulate GM-CSF production although not with the same effect as B-FAHF-2. Other individual herbs were able to either suppress TNF-α or increase GM-CSF production but none did both. Interestingly, our group has attempted to isolate active compounds within this complex formulation but has not shown that individual compounds or smaller combinations of them have the same effects as the entire mixture together. In addition to the complexity of the formulation which makes it difficult to study, further challenges to performing clinical trials include obtaining reliable sources for the raw herbal components, ensuring no pesticide or heavy metal contamination, and confirming consistency of the chemical makeup of each batch of herbal medicine. Finally, herbal formulas have often been found to have hepatotoxicity. FAHF-2 and B-FAHF-2 were made with the removal of potentially hepatoxic herbs and data in prior murine and human studies have shown no hepatoxicity as evidenced by this formulation receiving an Investigational New Drug status from the FDA (8, 29).

Our study is the next step toward the development of this formulation into an herbal therapy for CD but additional refinement of the formulation and further larger studies must be performed. This study has several other limitations. We used TNF-α and GM-CSF as surrogate markers for the immunomodulatory capabilities of B-FAHF-2 but examination of a multitude of cytokines, chemokines and growth factors should be examined as was done with FAHF-2. In prior studies we examined the mechanism of how FAHF-2 suppressed inflammatory cytokine production and found that the NFκB pathway was inhibited. This is unlikely to explain the mechanism causing increases in GM-CSF production and will need to be investigated in future studies. In addition, we had only a small sample of subjects with antibodies against GM-CSF. The effects of B-FAHF-2 in this sub-group should be confirmed in larger patient populations.

CONCLUSIONS

In conclusion, B-FAHF-2 suppresses production of TNF-α, a major inflammatory cytokine involved in the pathogenesis of CD, to a similar extent as FAHF-2 but at a lower dose. B-FAHF-2 warrants further clinical investigation to determine its efficacy in CD. B-FAHF-2 also increased production of GM-CSF and thus warrants further investigation in a sub-group of CD patients who have low levels of or antibodies against GM-CSF.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Icahn School of Medicine at Mount Sinai Institutional Review Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. The animal study was reviewed and approved by Icahn School of Medicine at Mount Sinai Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

XC and JL: validation, formal analysis, writing—original draft, and visualization. YS and SG: methodology, formal analysis, and writing—review and editing. NY: resources, writing—review, and editing. VG, WH, and EC: formal analysis and writing—review and editing. NP, JJ, and KB: specimen collection and writing—review and editing. MD: conceptualization, writing—review and editing. X-ML: conceptualization, writing—review and editing, supervision, project administration, and funding acquisition. DD: conceptualization, formal analysis, resources, writing—review and editing, visualization, supervision, project administration, and funding acquisition. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology*. (2012) 142:46–54.e42. doi: 10.1053/j.gastro.2011.10.001
2. Ouahed J, Spencer E, Kotlarz D, Shouval DS, Kowalik M, Peng K, et al. Very early onset inflammatory bowel disease: a clinical approach with a focus on the role of genetics and underlying immune deficiencies. *Inflamm Bowel Dis*. (2019) 26:820–42. doi: 10.1093/ibd/izz259
3. Ceballos C, Bao R, Dunkin D, Song Y, Li X-M, Benkov K. Complementary and alternative medicine use at a single pediatric inflammatory bowel disease center. *Gastroenterol Nurs*. (2014) 37:265–71. doi: 10.1097/SGA.0000000000000050
4. China, T.S.P.C.o.T.P.s.R.o. *Pharmacopoeia of the People's Republic of China*. Version 6. Beijing: People's Medical Publishing House (2005).

5. Song Y, Dunkin D, Dahan S, Luga A, Ceballos C, Hoffstadter-Thal K, et al. Anti-inflammatory effects of the Chinese herbal formula FAHF-2 in experimental and human IBD. *Inflamm Bowel Dis*. (2014) 20:144–53. doi: 10.1097/01.MIB.0000436467.37566.48
6. Srivastava K, Nang N, Chen Y, Lopez-Exposito I, Song Y, Lopez-Exposito I et al. Efficacy, safety and immunological actions of butanol-extracted Food Allergy Herbal Formula-2 on peanut anaphylaxis. *Clin Exp Allergy*. (2011) 41:582–91. doi: 10.1111/j.1365-2222.2010.03643.x
7. Srivastava KD, Bardina L, Sampson HA, Li X-M. Efficacy and immunological actions of FAHF-2 in a murine model of multiple food allergies. *Ann Allergy Asthma Immunol*. (2012) 108:351–8.e1. doi: 10.1016/j.anai.2012.03.008
8. Srivastava KD, Kattan JD, Zou ZM, Li JH, Zhang L, Wallenstein S, et al. The Chinese herbal medicine formula FAHF-2 completely blocks anaphylactic reactions in a murine model of peanut allergy. *J Allergy Clin Immunol*. (2005) 115:171–8. doi: 10.1016/j.jaci.2004.10.003
9. Srivastava KD, Qu C, Zhang T, Goldfarb J, Sampson HA, Li X-M. Food Allergy Herbal Formula-2 silences peanut-induced anaphylaxis for a prolonged posttreatment period via IFN-gamma-producing CD8+ T cells. *J Allergy Clin Immunol*. (2009) 123:443–51. doi: 10.1016/j.jaci.2008.12.1107
10. Srivastava KD, Song Y, Yang N, Liu C, Goldberg IE, Nowak-Wegrzyn A, et al. B-FAHF-2 plus oral immunotherapy (OIT) is safer and more effective than OIT alone in a murine model of concurrent peanut/tree nut allergy. *Clin Exp Allergy*. (2017) 47:1038–49. doi: 10.1111/cea.12936
11. Kattan JD, Srivastava KD, Zou ZM, Goldfarb J, Sampson HA, Li X-M. Pharmacological and immunological effects of individual herbs in the Food Allergy Herbal Formula-2 (FAHF-2) on peanut allergy. *Phytother Res*. (2008) 22:651–9. doi: 10.1002/ptr.2357
12. Dabritz J, Weinhage T, Varga G, Wirth T, Walscheid K, Brockhausen A, et al. Reprogramming of monocytes by GM-CSF contributes to regulatory immune functions during intestinal inflammation. *J Immunol*. (2015) 194:2424–38. doi: 10.4049/jimmunol.1401482
13. Weinhage T, Däbritz J, Brockhausen A, Wirth T, Brückner M, Belz M, et al. Granulocyte macrophage colony-stimulating factor-activated CD39+/CD73+ murine monocytes modulate intestinal inflammation via induction of regulatory T cells. *Cell Mol Gastroenterol Hepatol*. (2015) 1:433–49.e1. doi: 10.1016/j.jcmgh.2015.04.005
14. Han X, Uchida K, Jurickova I, Koch D, Willson T, Samson C, et al. Granulocyte-macrophage colony-stimulating factor autoantibodies in murine ileitis and progressive ileal Crohn's disease. *Gastroenterology*. (2009) 136:1261–71.e1–3. doi: 10.1053/j.gastro.2008.12.046
15. Korzenik JR, Dieckgraefe BK, Valentine JF, Hausman DF, Gilbert MJ, Sargramostim in Crohn's Disease Study Group. Sargramostim for active Crohn's disease. *N Engl J Med*. (2005) 352:2193–201. doi: 10.1056/NEJMoa041109
16. Wang J, Jones SM, Pongracic JA, Song Y, Yang N, Sicherer SH, et al. Safety, clinical, and immunologic efficacy of a Chinese herbal medicine (Food Allergy Herbal Formula-2) for food allergy. *J Allergy Clin Immunol*. (2015) 136:962–70.e1. doi: 10.1016/j.jaci.2015.04.029
17. Patil SP, Wang J, Song Y, Noone S, Yang N, Wallenstein S, et al. Clinical safety of Food Allergy Herbal Formula-2 (FAHF-2) and inhibitory effect on basophils from patients with food allergy: extended phase I study. *J Allergy Clin Immunol*. (2011) 128:1259–602. doi: 10.1016/j.jaci.2011.06.015
18. Wang JL, Patil SP, Yang N, Ko J, Lee J, Noone S, et al. Safety, tolerability, and immunologic effects of a food allergy herbal formula in food allergic individuals: a randomized, double-blinded, placebo-controlled, dose escalation, phase 1 study. *Ann Allergy Asthma Immunol*. (2010) 105:75–7. doi: 10.1016/j.anai.2010.05.005
19. Xiu S. *The Experimental Method of Pharmacology*. Beijing: The People's Public Health Publisher (1986).
20. Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest*. (1993) 69:238–49.
21. Ostanin DV, Bao J, Koboziev I, Gray L, Robinson-Jackson SA, Kosloski-Davidson M, et al. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. *Am J Physiol Gastrointest Liver Physiol*. (2009) 296:G135–46. doi: 10.1152/ajpgi.90462.2008
22. Chen X, Berin MC, Gillespie VL, Sampson HA, David Dunkin. Treatment of intestinal inflammation with epicutaneous immunotherapy requires TGF-beta and IL-10 but not Foxp3(+) Tregs. *Front Immunol*. (2021) 12:637630. doi: 10.3389/fimmu.2021.637630
23. Zaqout S, Becker LL, Kaindl AM. Immunofluorescence staining of paraffin sections step by step. *Front Neuroanat*. (2020) 14:582218. doi: 10.3389/fnana.2020.582218
24. *E-B-FAHF-2, Multi OIT and Xolair (Omalizumab) for Food Allergy*. Bethesda, MD: National Library of Medicine. ClinicalTrials.gov [Internet].
25. *Study to Evaluate Safety, Tolerability and Efficacy of Oral E-B-FAHF-2 in Mild-to-Moderate Crohn's Disease*. Bethesda, MD: National Library of Medicine. ClinicalTrials.gov [Internet].
26. Roth L, Macdonald JK, McDonald JW, Chande N. Sargramostim (GM-CSF) for induction of remission in Crohn's disease. *Cochrane Database Syst Rev*. (2011) 2011:CD008538. doi: 10.1002/14651858.CD008538.pub2
27. Dieckgraefe BK, Korzenik JR. Treatment of active Crohn's disease with recombinant human granulocyte-macrophage colony-stimulating factor. *Lancet*. (2002) 360:1478–80. doi: 10.1016/S0140-6736(02)11437-1
28. Liu EH, Qi L-W, Li K, Chu C, Li P. Recent advances in quality control of traditional Chinese medicines. *Comb Chem High Throughput Screen*. (2010) 13:869–84. doi: 10.2174/138620710793360301
29. Wang J. Treatment of food anaphylaxis with traditional Chinese herbal remedies: from mouse model to human clinical trials. *Curr Opin Allergy Clin Immunol*. (2013) 13:386–91. doi: 10.1097/ACI.0b013e3283615bc4

Conflict of Interest: X-ML formerly had patents with Herbal Springs for FAHF-2 for treating food allergy. SG is a named inventor of a patent application related to GM-CSF autoantibodies in immune-related colitis. This is filed through the Icahn School of Medicine at Mount Sinai and is currently unlicensed. SG reports consultancy and/or advisory roles for Merck and OncoMed, and research funding from Bristol-Myers Squibb, Celgene, Genentech, Immune Design, Janssen R&D, Pfizer, Regeneron, and Takeda.

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frontiers in Medicine

REVIEW
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Treatment of Inflammatory Bowel Disease: A Comprehensive Review

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Inflammatory bowel disease (IBD), as a global disease, has attracted much research interest. Constant research has led to a better understanding of the disease condition and further promoted its management. We here reviewed the conventional and the novel drugs and therapies, as well as the potential ones, which have shown promise in preclinical studies and are likely to be effective future therapies. The conventional treatments aim at controlling symptoms through pharmacotherapy, including aminosalicylates, corticosteroids, immunomodulators, and biologics, with other general measures and/or surgical resection if necessary. However, a considerable fraction of patients do not respond to available treatments or lose response, which calls for new therapeutic strategies. Diverse therapeutic options are emerging, involving small molecules, apheresis therapy, improved intestinal microecology, cell therapy, and exosome therapy. In addition, patient education partly upgrades the efficacy of IBD treatment. Recent advances in the management of IBD have led to a paradigm shift in the treatment goals, from targeting symptom-free daily life to shooting for mucosal healing. In this review, the latest progress in IBD treatment is summarized to understand the advantages, pitfalls, and research prospects of different drugs and therapies and to provide a basis for the clinical decision and further research of IBD.

Keywords: inflammatory bowel disease, Crohn disease, ulcerative colitis, therapeutics, recent advance

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INTRODUCTION

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is a chronic and recurrent inflammatory disease that mainly relates to the intestinal tract. Over recent decades, the epidemiology of IBD has changed considerably. The early twenty-first century has witnessed a rapidly rising incidence in newly industrialized countries (1). The morbidity of IBD was about 1.74 per 100,000 person-years in China (2). Although the morbidity turns to be stable in western countries, burden remains high as prevalence exceeds 0.3% (1). As a global disease, IBD not only seriously endangers human health but also brings heavy financial burdens to individuals, families, and society.

The exact cause of IBD remains indistinct, but it is generally accepted that its etiopathology is multifactorial, involving genetic predisposition, mucosal barrier dysfunction, disturbances in the gastrointestinal microbiota, dysregulated immune responses, environmental, and lifestyle factors (3, 4).

The past few years have seen an expansion in IBD therapeutic options. Conventional treatments control symptoms through pharmacotherapy, including aminosalicylates, corticosteroids (CSs),

immunomodulators, and biologics, with other general measures and/or surgical resection if necessary. The introduction of specific inhibitors of tumor necrosis factor (TNF) is a groundbreaking achievement, enabling long-standing remission, and modification of the IBD course in a significant fraction of patients (5). However, primary non-response to TNF inhibitors was observed in up to 40% of patients in clinical trials and 10–20% patients in clinical series; secondary loss of response occurred in ∼23–46% of patients after 1 year of treatment (6), which calls for new therapeutic strategies. New therapeutic strategies are emerging, involving small molecules, apheresis therapy, improved intestinal microecology, cell therapy, and exosome therapy. In addition, patient education on diet and psychology appears to benefit IBD treatment.

Recent progress in therapeutic approaches, especially the emergence of biologics, has not only promoted the transformation of the treatment mode in IBD, but also changed the perspective of IBD therapy. Traditionally, the therapeutic effects are mainly evaluated through clinical symptom score. Nowadays, the disease activity can also be assessed by objective indicators such as endoscopic findings and biomarkers (7). The goals are not only to induce and maintain remission in symptom, to prevent and treat complications but also to achieve mucosal healing. Mucosal healing refers to the elimination of local mucosal inflammation and the restoration of the normal mucosal structure. Although there is still no unified criteria for the determination of mucosal healing, it is usually characterized by the disappearance of endoscopic ulcer (8). Multiple studies are emerging to show that mucosal healing may be associated with reduced rates of clinical recurrence, hospitalization, surgery and disability, and a good long-term prognosis (9–11).

In this review, we not only focus on drugs and therapies that have been approved, but also focus on the potential methods for the treatment of IBD, providing a comprehensive overview for clinicians of available therapies and drugs for IBD treatment.

From the database's inception until October 2021, we conducted a comprehensive search in PubMed and Web of Science. The retrieval strategy is based on Medical Subject Headings (MeSH) and corresponding free words. The major search terms are as follows: "Inflammatory Bowel Disease," "Bowel Diseases, Inflammatory," "IBD," "Crohn's Disease," "Crohn's Enteritis," "Ulcerative Colitis," "Colitis Gravis," "Aminosalicylates," "Mesalazine," "5-Aminosalicylic Acid," "Corticosteroids," "Thiopurines," "Methotrexate," "Calcineurin Inhibitors," "Biologics," "Janus Kinase inhibitors," "Ozanimod," "Etrasimod," "Surgical Procedure," "Apheresis," "Blood Component Removals," "Antibiotics," "Antibacterial Agents," "Probiotics," "Prebiotics," "Synbiotics," "Postbiotics," "Fecal Microbiota Transplantation," "Stem Cell Transplantations," "Exosomes," "Diet." The above search terms were connected by the logical operators "OR" or "And." The research focused on the treatment of IBD. A total of 9,885 references were retrieved. Studies, which are old, repetitive and non-English and those without clear information were excluded. We selected some representative scientific papers and 257 references were finally quoted.

PHARMACOLOGICAL INTERVENTION

At present, pharmacological intervention is important for IBD treatment. The medications mainly include aminosalicylates, CSs, immunomodulators, biologics, and oral small molecules. We mainly introduced their mechanism of action, efficacy, and safety in UC or CD.

Aminosalicylates

Aminosalicylates for IBD mainly include traditional sulfasalazine (SASP) and other types of 5-aminosalicylic acid (5-ASA) drugs. SASP is composed of 5-ASA and sulphapyridine (SP) by diazo bonding and has been used to treat IBD for 80 years. In the treatment of IBD, SASP is the prodrug, SP is the carrier, and 5-ASA is the active part. The mechanisms of action (MOA) of 5-ASA and SASP include interference with the metabolism of arachidonic acid (conversion to prostaglandin and leukin-triene), scavenging of reactive oxygen species, and effects on the function of white blood cells and the production of cytokines (12). Oh-oka et al. have proposed a novel anti-inflammatory mechanism in the colitis treatment: 5-ASA could induce regulatory T cells (Tregs) in the colon through the aryl hydrocarbon receptor pathway, followed by the activation of transforming growth factor (TGF)-β (13).

Studies on the efficacy and safety of 5-ASA preparations in the treatment of IBD are summarized in **Table 1**. Recent studies have reported that oral 5-ASA has better efficacy in UC treatment than placebo and shows similar effects (clinical remission rates) between once-daily dosing and conventional (twice or three times daily) dosing (14). The efficacy of SASP in UC treatment is similar to that of other 5-ASA preparations. However, taking costs into account, SASP may be the preferred option in clinical application, because other 5-ASA preparations are more expensive (14). A case-control study has reported that 5-ASA maintenance therapy can reduce the risk of colorectal cancer by 75% in UC patients (15). A meta-analysis has proved the efficacy of topical 5-ASA in preventing relapse of UC (16).

The therapeutic efficacy of aminosalicylic acid preparations for CD remains controversial. A review has suggested that oral 5-ASA preparations have no significant advantage in maintaining remission in patients with CD (17). However, a retrospective study in the UK found that 5-ASA was widely used as a long-term treatment for CD as about a quarter of patients continued to use 5-ASA for more than 10 years (18). 5-ASA therapy for more than a year could reduce the consumption of related medical resources (including referrals, hospitalization, and surgery) (18). Gjuladin-Hellon et al. have reported the benefit of 5-ASA in preventing relapse of CD in remission after surgery (19). Coward et al. in their Bayesian network meta-analysis found that high-dose mesalamine is an option for inducing remission among mild-to-moderate CD patients preferring to avoid steroids (20). Other studies have also reported the treatment effectiveness of aminosalicylates in CD (21, 22).

Side effects associated with 5-ASA, including flatulence, nausea, abdominal pain, diarrhea, and headache, are generally mild. In contrast, the side effects of SASP, such as infertility, hemolytic anemia, photosensitization, and granulocytosis, are

TABLE 1 | Aminosalicylates.

Type of study	Patients	Treatment	Therapy period	Results/Conclusion	References
A review	UC patients	Oral 5-ASA	NA	5-ASA was more effective than placebo. There was no difference in clinical remission rates between once-daily dosing and conventional (twice or three times daily) dosing. Other 5-ASA formulations appeared to be as efficacious as SASP	(14)
A case-control study	UC patients	5-ASA	NA	Regular 5-ASA therapy reduced colorectal cancer risk by 75%	(15)
A meta-analysis	Patients with quiescent UC	5-ASA	6–24 months	Topical 5-ASA was effective in preventing relapse of UC in remission	(16)
A systematic review	CD patients	Oral 5-ASA	NA	No significant advantage was found in oral 5-ASA for the maintenance of medically-induced remission	(17)
A retrospective study	Adults with CD	5-ASA	NA	5-ASA was widely used as a long-term treatment for CD. The use of CD-related healthcare resources decreased significantly in the year following 5-ASA initiation	(18)
An updated cochrane review	CD patients in remission after surgery	Oral 5-ASA	NA	5-ASA drugs were superior to placebo for maintaining surgically-induced remission of CD. 5-ASA formulations appeared to be safe when compared with placebo, no treatment or biologics	(19)
A bayesian network meta-analysis	Mild-to-Moderate CD patients	Mesalamine, SASP, CSs, and budesonide	8–17 weeks	CSs and high-dose budesonide were effective treatments for inducing remission in mild-to-moderate CD. CSs were more effective than high-dose mesalamine, but high-dose mesalamine was an option among patients preferring to avoid steroids	(20)
A systematic review and meta-analysis	Adults with luminal CD in remission after a surgical resection	5-ASA	NA	5-ASA was of modest benefit in preventing relapse of quiescent CD after a surgical resection	(21)
A systematic review	Patients with mildly to moderately active CD	Aminosalicylates	NA	For induction therapy of mild to moderate CD, SASP had modest efficacy and high dose mesalamine (3–4.5 g/day) was not superior to placebo.	(22)

UC, ulcerative colitis; 5-ASA, 5-aminosalicylic acid; NA, not applicable; SASP, sulfasalazine; CD, Crohn's disease; CSs, corticosteroids.

much more than those of 5-ASA (12). However, a few patients may develop nephrotoxicity within 1 year of 5-ASA administration (23).

CSs

Oral CSs have been used for IBD treatment since the 1950s (24, 25), and can effectively induce remission when a flare occurs. CSs combine with CSs receptors in the cytoplasm, and then CSs receptors are activated. The activated CSs receptors could get into the nucleus and interact with specific proinflammatory transcription factors (such as nuclear factor-kappaB and activator protein-1), which will recruit co-activator complexes (for e.g., histone deacetylation enzymes) to inhibit the transcription of some inflammatory genes (26). Moreover, activated CSs receptors can also bind to specific response elements in the promoter region of anti-inflammatory genes in the nucleus to regulate the expression of anti-inflammatory genes. In addition, the anti-inflammatory effect of CSs may be mediated by different membrane receptors (27).

CSs may be a kind of treatment selection for patients with UC who have not responded to mesalazine within 2–4 weeks, and those with mild-to-moderate CD, especially with extensive lesions (28). CSs have no proven efficacy in maintaining remission in IBD and should not be used for this purpose. Systemic oral CSs may result in numerous side effects, such as opportunistic infections, diabetes mellitus, hypertension, ocular effects, venous thromboembolism (VTE), osteoporosis, etc (29, 30). Steroid dependency or excess was found in ∼15–40% of IBD patients (31, 32). Further investigation should define appropriate corticosteroid use and find measures for the improvement in CSs prescription management.

Studies related to CSs' efficacy in IBD are presented in **Table 2**. Systematic reviews and metanalyses have proved the benefits of CSs in inducing remission of IBD (33). A recent study demonstrated that CSs were more effective than 5-ASA in the treatment of CD (20). Other studies have also reported the efficacy and safety of CSs in IBD treatment (34–40).

Second-generation oral CSs, such as budesonide, are becoming available and may have better safety and tolerability

TABLE 2 | Corticosteroids.

Type of study	Patients	Treatment	Results/Conclusion	Adverse events	References
A review	IBD patients	CSs and aminosalicylates	There were numerous adverse events of CSs, particularly at high doses and prolonged treatment. Therapy with budesonide may result in a better safety profile. 5-ASA treatment is usually well-tolerated, but with regard to the rare nephrotoxic events	CSs: opportunistic infections, diabetes mellitus, hypertension, ocular effects (glaucoma and cataracts), psychiatric complications, hypothalamic-pituitary-adrenal axis suppression and increased fracture risk	(29)
A systematic review	UC patients	Second-Generation oral CSs	Beclomethasone dipropionate and budesonide MMX have better efficacy in the induction of remission in UC than placebo or mesalazine. Second-generation CSs have a more favorable safety and tolerability than systemic CSs	Altered glucose concentration, constipation, menorrhagia, UC exacerbation, headache, nausea	(30)
A multi-center audit	IBD patients	CSs	14.9% of British patients with IBD experienced steroid dependency or excess	NA	(31)
A systematic review and meta-analysis	IBD patients	CSs	CSs were beneficial for inducing remission in UC, and might be effective in CD. Standard CSs were more effective than budesonide	NA	(33)
A prospective observational study	Adult outpatients with UC or CD	Oral prednisone (40 mg/day for 2 weeks, followed by a tapering course of 5 mg/day reduction every week)	CSs was associated with high rate of mood change in IBD patients when disease flares	Frequentmood changes	(34)
A systematic review and meta-analysis	IBD patients with CMV	CSs, TPs, TNF antagonists	Exposure to CSs or TPs, but not anti-TNF drugs, was associated with an increased risk of CMV reactivation in IBD patients	CMV reactivation	(35)
A retrospective review	IBD patients	CSs	Prolonged use of CSs was associated with significant harm to IBD patients	VTE, fragility fracture, infections	(36)
A retrospective survey	UC patients	Oral or intravenous CSs	The majority of UC patients primarily responded to CSs. But after 1 year of treatment, nearly half of patients were assessed as CS dependence	NA	(37)
A retrospective study	Adults with IBD	CSs	The use of CSs significantly increased the risk of VTE	VTE	(38)
A population-based cohort study with a nested case-control analysis	Incident IBD patients aged ≥66 years	Systemic oral CSs	Oral CSs were associated the increase risk of serious infections in elderly-onset IBD patients	Diabetes, chronic respiratory diseases, chronic kidney diseases, cancer	(39)
A retrospective cohort study	UC patients	CSs	About half of newly-diagnosed patients with UC required CSs. Among CS users, one third of the patients had a sustained response after the initial CSs course while two-thirds required further CSs therapy	NA	(40)
Two randomized, double-blind, placebo-controlled, phase 3 studies	Patients with mild-to-moderate active UC	Budesonide MMX (9 or 6 mg once daily)	Budesonide MMX 9 mg resulted in significantly higher combined clinical and colonoscopic remission rates ($P = 0.0002$)	Headache, nausea, abdominal pain, nasopharyngitis	(41)
A phase III, randomized, double-blind, double-dummy, placebo-controlled, parallel-group trial	Patients with active, mild-to-moderate UC	Budesonide MMX (9 mg/day)	Budesonide MMX 9 mg appeared to be safe and more effective than placebo at inducing combined clinical and endoscopic remission in patients with active, mild-to-moderate UC	Headache, flatulence, nausea, blood cortisol decrease	(42)

IBD, inflammatory bowel disease; CSs, corticosteroids; 5-ASA, 5-aminosalicylic acid; UC, ulcerative colitis; MMX, Multi Matrix; NA, not applicable; CD, Crohn's disease; CMV, cytomegalovirus; TNF, tumor necrosis factor; VTE, venous thromboembolism.

profile than conventional CSs. Target delivery of steroids to the site of inflammation potentially reduces systemic side effects (30). Budesonide is a synthetic CS with a high affinity for CSs receptors. While pH-dependent budesonide capsules restrict the release of budesonide to the distal ileum and ascending colon, budesonide Multi Matrix (MMX) is released throughout the entire colon. The tolerability of budesonide MMX at 8 weeks was similar to that of placebo, mesalazine (41), and pH-dependent budesonide (42), which may prompt the use of budesonide in patients who are not suitable for conventional CSs. For the mild-to-moderate CD of the ileum and/or ascending colon, 9 mg budesonide once daily for 8 weeks is recommended with a 2 week taper (28).

Immunomodulators

Immunomodulators are important for patients with IBD and mainly include thiopurines (TPs), methotrexate (MTX), calcineurin inhibitors, and Janus Kinase (JAK) inhibitors. The studies on the efficacy and safety of immunomodulators in IBD are summarized in **Supplementary Table 1**.

TPs

During the progression of IBD, activated T lymphocytes infiltrate the inflammatory site of the intestinal mucosa and produce a variety of cytokines, further aggravating intestinal inflammation. TPs, including azathioprine (AZA), 6-mercaptopurine (MP), and 6-thioguanine (TG) could control intestinal inflammation by inhibiting T lymphocyte proliferation and activation. These inactive 6-TP prodrugs are metabolized into pharmacologically active deoxy-6-thioguanosine phosphate (deoxy-6-TGNP). Deoxy-6-TGNP can interfere with DNA synthesis and inhibit lymphocyte proliferation. Besides, 6-TGNP can bind to Rac1 to form the 6-TGNP-Rac1 complex, thus blocking the activation of Rac1 in T lymphocytes and inhibiting the survival and function of T lymphocytes (43).

It has been proved that AZA has a favorable and similar therapeutic effect on CD and UC, which helps reduce hospitalization and surgery rates of IBD patients (44–46). A meta-analysis has indicated that AZA/6-MP is more effective in preventing UC recurrence than placebo (OR = 2.59, 95% CI: 1.26–5.3) (47). A retrospective cohort study has shown TP's long-term efficacy on UC patients, with a 7-year maintenance remission rate of 43.9% and a colectomy-free survival rate of 88% (48). A prospective, observational study has reported that 70% of steroid-dependent CD patients treated with AZA achieved a 60 month steroid-free remission (49).

However, TPs have many adverse side effects, such as bone marrow suppression (50), liver injury (51), and gastrointestinal intolerance (44), etc. It has been reported that up to 39% of patients with IBD discontinue using TPs due to adverse reactions, most of which occur within 3 months of treatment (44). The use of TPs in IBD treatment has declined due to concerns about adverse drug reactions.

MTX

Low doses of MTX can inhibit the function of several enzymes related to DNA synthesis, and downregulate a variety of inflammatory cytokines [such as interleukin (IL)-1, IL-2, IL-6, IL-8, etc.], thus inhibiting the proliferation of T lymphocytes and inflammatory response (52). A study has found that 72% of patients with active CD achieved clinical remission after 3 months of MTX treatment (53). A randomized controlled trial has shown that in patients with CD who have clinical remission after intramuscular treatment with 25 mg MTX per week, the rate of maintenance remission at 40 weeks of intramuscular treatment with 15 mg MTX per week is 65%, higher than that of the control group (39%, $P = 0.04$) (54). MTX has not been proven to have efficacy in inducing remission in UC (55). In addition, MTX can cause fatigue, nausea, vomiting, diarrhea, peritoneal abscess, hypoalbuminemia, atypical pneumonia, severe rash, etc. (27, 55).

Calcineurin Inhibitors

Calcineurin, activated by calmodulin, can induce dephosphorylation and activation of the nuclear factor of activated T cells (NFAT). Then the activated NFAT will move from cytoplasm to nucleus and combine with the gene regulatory regions to regulate gene transcription of a variety of inflammatory cytokines [TNF-α, interferon (IFN)-c, IL-2, etc.] (56). Calcineurin inhibitors, including Cyclosporine A (CsA) and Tacrolimus (TAC) interfere with the signaling pathway, and therefore inhibit the inflammatory response. CsA and TAC bind to intracellular Cyclophilin A and FK binding protein 12, respectively, forming complexes that inhibit NFAT dephosphorylation (56). In addition, it has been reported that TAC not only has an immunosuppressive effect on T cells, but also inhibits the activation and promotes the apoptosis of macrophages, thereby inhibiting the production of pro-inflammatory cytokines (IL-12/IL-23 and TNF-α) (57).

A randomized controlled trial demonstrated that more than 80% of patients with severe acute refractory UC responded to CsA (58). It has been reported that the 8-day clinical remission rate (84.2 vs. 85.7%) of patients with severe UC treated with intravenous 4 mg/kg CsA is similar to that of patients treated with intravenous 2 mg/kg CsA (59). Stange et al. performed a randomized controlled trial to delineate the long-term effect of CsA on chronic active CD and found that CsA combined with low-dose steroids had no advantage over the sole use of low-dose steroids (60).

Although the MOA of TAC is similar to that of CsA, the immunosuppressive effect of TAC is much higher than that of CsA and is 10–20 times *in vivo* and 30–100 times *in vitro*, respectively (61). Additionally, TAC is well-absorbable through the intestine, and it is similarly effective for refractory UC whether administered intravenously or orally (62). Multiple studies have confirmed the effectiveness of TAC in patients with refractory UC (63–67). A randomized controlled trial showed that 68.4% of patients with refractory UC taking TAC orally after 2 weeks had an improved disease activity index (DAI) score (>4 points, all categories improved), but in the control group, only 10% of patients' score was improved ($P < 0.001$) (63). Yamamoto et al. have revealed that 77.8% of patients with refractory UC respond to TAC and 70.4% of patients have clinical remission within 30 days (64). Komaki et al. performed a systematic review and meta-analysis to exam the efficacy of TAC as rescue therapy

for active UC and found that the 2-week clinical response rate of TAC was significantly higher than that of placebo (RR = 4.61, 95% CI: 2.09–10.17) (67). The clinical response rates at 1 and 3 months were 73% (95% CI: 64–81%) and 76% (95% CI: 59–87%), and the colectomy-free rates at 1, 3, 6, and 12 months were 86, 84, 78, and 69%, respectively. However, the efficacy of TAC in CD treatment remains controversial. McSharry et al. have systematically reviewed the studies assessing the potency of TAC in the treatment of luminal CD and found that the roughly computed remission rate was 44.3% (7–69%) and the partial response rate was 37.1% (14–57%) (68). Iida et al. have probed into the studies from 1950 to December 2017 to show the efficacy of TAC in CD treatment and found that the clinical remission rates for luminal CD patients systemically treated with TAC, perianal CD patients with systemic TAC treatment, and localized CD patients with topical administration of TAC were 37.1, 32.0, and 22.7%, respectively (69).

TAC has a high incidence of adverse side effects, including tremor, renal function damage, infectious diseases, hot flashes, hyperkalemia, headache, etc (63, 64), which should be taken into consideration during clinical practice. Besides, the blood concentration and the patient's general status should be closely monitored when using TAC. The optimal blood trough concentration appeared to be 10–15 ng/ml during remission-induction therapy for refractory UC (63). Recently, a study has reported that the use of calcineurin inhibitors makes 1-year colectomy rates of UC patients who are previously exposed to biologics significantly higher than those of patients who are biologic-naïve (70). Clinical studies exploring the efficacy and safety of CsA and TAC in the treatment of IBD are rare, and more randomized controlled trials are needed.

Biologics

Biologics mainly include pro-inflammatory cytokine inhibitors and integrin antagonists. The pro-inflammatory cytokines, TNF-α and IL-12/23, play an important role in the pathogenesis of IBD. Studies on the efficacy and safety of biologics in IBD treatment are summarized in **Supplementary Table 2**.

Anti-TNF Therapy

TNF-α is a prototypic member of a large family of cytokines that play important roles in inflammation, apoptosis, proliferation, invasion, etc. (71) Overexpression of TNF-α can cause chronic inflammation and lead to autoimmune diseases and tissue damage. Anti-TNF-α monoclonal antibodies, such as Infliximab (IFX) and Adalimumab (ADA), exert therapeutic effects by inhibiting TNF-α-associated inflammatory responses and tissue damage.

IFX therapy may be applied for the treatment of patients who are intolerant or do not respond well to CSs and immunomodulators and are steroid-dependent. A randomized controlled trial evaluating the efficacy of IFX in patients with moderate to severe UC demonstrated that colorectomy rates decreased by 7% after 54 weeks of IFX treatment. UC-related hospitalization and surgery rates saw a decrease as well (72). Present et al. found that the clinical response rate of patients with CD was 68% after intravenous injection of 5 mg/kg IFX, and the complete healing rate of the fistula was 55% (73). The efficacy of IFX was shown in about 2 weeks, and the median time of fistula closure was 3 months. Golimumab is a fully human IgG1 monoclonal antibody against TNF-α with good efficacy and safety. It is approved for use in patients with moderate to severe UC (74, 75) and CD (76, 77), who fail to respond to conventional therapy.

Anti-TNF treatment is not all-encompassing despite its vital role in IBD treatment. Up to 40% of patients do not respond to TNF inhibitors, and nearly 23–46% of patients experience secondary loss-of-response 1 year after anti-TNF-α treatment (6). It may be possible to achieve long-term remission through dose escalation, shorter intervals between infusions (78) or combination therapy (79). Due to anti-TNF agents' dose-related therapeutic benefit, measurement of serum trough level and anti-drug antibody is advocated (80, 81).

Anti-IL-12/23 Therapy

IL-12 and IL-23 are important pro-inflammatory cytokines in intestinal inflammation, mainly produced by antigen-presenting cells. IL-12 is composed of the p35 and p40 subunits, and IL-23 is composed of the p40 and p19 subunits. Preclinical studies have suggested that IL-12 and IL-23 are involved in the pathophysiological process of IBD and play a role in the induction and maintenance of intestinal inflammation (82). In addition, genomic studies have shown an association between the IL-12/IL-23 pathway and CD (83).

Ustekinumab is a fully humanized IgG1 monoclonal antibody that binds to the common p40 subunit of IL-12 and IL-23 to inhibit the binding of IL-12 and IL-23 to the IL-12 receptor on the cell membrane surface of T cells and NK cells, thereby inhibiting intestinal inflammation. Ustekinumab has been approved be effective for the treatment of moderate to severe CD and UC (84). Rutgeerts et al. conducted three-phase randomized controlled clinical trials and found that the 8-week simplified endoscopic activity score for CD decreased by 2.8 in the ustekinumab group while the decrease in the control group was only 0.7 ($P = 0.012$), showing that ustekinumab had a better treatment efficacy than placebo in patients with CD (85). Feagan et al. reported that for patients with moderate to severe CD, the clinical response rates at week 6 in the intravenous ustekinumab group were significantly higher than those in the control group, and the clinical remission rates at week 44 of patients receiving intravenous ustekinumab were also higher than those of patients receiving placebo (86).

Mirikizumab is a humanized monoclonal antibody that targets the unique p19 subunit of IL-23. In a phase 2 trial, patients with moderate to severe UC were randomly divided into 4 groups and were given intravenous placebo, 50 mg mirikizumab, 200 mg mirikizumab, and 600 mg mirikizumab, respectively (87). It was reported that clinical remission rates of patients given 200 mg mirikizumab at week 12 were significantly higher than those of patients given placebo. The clinical remission rates of the other two mirikizumab groups were not significantly different from those of the placebo group. No serious adverse events or unexpected safety problems occurred in any groups during the induction and maintenance treatment.

Risankizumab, a humanized IgG monoclonal antibody against the p19 subunit of IL-23, undergoes phase 2 and phase 3 clinical evaluation. A randomized, double-blind, placebo-controlled phase 2 study demonstrated that risankizumab was superior to placebo in inducing clinical remission in patients with moderate to severe CD (88). Feagan et al. conducted an open-label extension study in patients with moderate to severe CD. It was proved that extended intravenous induction with risankizumab effectively increased clinical response and remission rates at week 26 and subcutaneous maintenance therapy with risankizumab achieved sustained remission until week 52 in ∼70% of patients were in clinical remission at week 26 (89).

Multiple studies have suggested that IL-12/23 and IL-23 antagonists are potential therapeutic options for IBD treatment. Experts recommended IL-12/23 and IL-23 antagonists as a first- or second-line therapy because of their efficacy in biologic-naïve and experienced patients (90).

Anti-integrin Therapy

Integrin, a cell surface glycoprotein receptor, mediates the homing of leucocytes into surrounding tissues by binding to tissue-specific cell adhesion molecules (CAMs). α4β7 integrin plays a key role in the homing of leucocytes to the intestinal mucosa and related lymphoid tissues. The homing of intestinal selective leucocytes is mediated by the binding of α4β7 integrin and mucosal addressin cell adhesion molecule (MAdCAM)-1 (91). The degree of α4β7+ cell infiltration and MAdCAM-1 expression are increased in the intestinal tracts of IBD patients.

Additionally, the specific binding of αEβ7 integrin on leukocytes to E-cadherin on epithelial cells (especially intestinal epithelial mucosal cells) is thought to mediate cell retention (92). The accumulation of leukocytes in the intestinal tract aggravates intestinal inflammatory response. Under this condition, T lymphocytes produce more pro-inflammatory cytokines (IFN-γ, TNF-α, and IL-17A) and IL-9, a cytokine that inhibits epithelial cell repair. These produced cytokines are considered to be important in the pathogenesis of IBD (93). Anti-integrin therapy blocks the effect of integrin on the surface of leucocytes and endothelial CAMs, thereby inhibiting leukocytes from interacting with the intestinal mucosa.

Vedolizumab, a recombinant human IgG1 monoclonal antibody, specifically inhibits the binding of α4β7 integrin to MAdCAM-1, preventing lymphocyte migration to the intestinal tissue, and thereby alleviating local intestinal inflammation. The three-phase randomized controlled clinical trials have proved the effectiveness of vedolizumab in inducing and maintaining remission in patients with IBD (94, 95). In a comprehensive study, vedolizumab has shown great tolerability and safety (96), which may be due to that the intestinal selectivity help to avoid adverse effects of systemic immunosuppression. Based on these findings, vedolizumab has been approved in the United States and Europe to be applied for adult patients with moderate to severe UC and CD showing no response or tolerance to conventional therapy or anti-TNF-α monoclonal antibodies (97). Vedolizumab can only block lymphocyte migration to the intestinal tract and does not directly control the mucosal inflammatory response. Vedolizumab in combination therapy with calcineurin or TNF-α inhibitors is another choice for patients with refractory IBD (98, 99).

Etrolizumab is a monoclonal antibody that selectively targets the β7 subunit of both α4β7 and αEβ7 integrins. Its gut-selectivity and the dual mechanism of action make it an alternative option for IBD treatment (93). A randomized, double-blind, placebo-controlled, phase 2 study indicated that etrolizumab was more effective than placebo in inducing clinical remission at week 10 for patients with moderate-to-severe UC (100). Phase 3 clinical trials are ongoing and several of them have demonstrated that etrolizumab is more effective than placebo in inducing remission for patients with moderate to severe UC (101). The efficacy of etrolizumab in maintaining remission remains to be confirmed. No major safety issues have been found to date.

Carotegrast Methyl (AJM300) is an orally active small molecule inhibitor that specifically targets the α4 subunit of α4β7 and α4β1. A double-blind, placebo-controlled, phase 2a study demonstrated that AJM300 was more effective than placebo at week 8 for patients with UC and had an acceptable safety profile (102).

Additionally, PF-00547659, a fully human monoclonal antibody targeting MAdCAM-1, has been proved to be well-tolerated and effective in inducing remission in patients with moderate to severe UC (103). PN-943 is another emerging orally administered α4β7 antagonist peptide and it has been confirmed that PN-943 is effective for the induction of remission in UC (104). A phase 2 study is ongoing to evaluate the effects of PN-943 (150 and 450 mg twice daily) on moderate to severe UC patients (NCT04504383).

Biological agents are expensive despite the advantages of high selectivity, high efficiency and low toxicity. Besides, primary no-response, secondary loss-of-response, and therapeutic intolerance in IBD treatment with biologics urge researchers to actively explore other therapies.

Small Molecules

Orally absorbed small molecules have attracted great interest of researchers because of the convenience of oral administration. Studies on the efficacy and safety of small molecules for IBD are listed in **Table 3**.

JAK Inhibitors

As novel therapeutic drugs, JAK inhibitors can block multiple signaling pathways. JAK family kinases JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK 2) target a variety of cytokine pathways through cytokine receptors. JAK1, JAK2 and TYK2 are widely expressed in all kinds of cells, but the expression of JAK3 is limited within hematopoietic cells. They interact with the common gamma chain subunit of six cytokine receptors (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) that have a crucial role in lymphopoiesis and homeostasis (112).

Tofacitinib is an oral small-molecule JAK inhibitor that can inhibit all JAKs, preferentially JAK1 and JAK3 and the efficacy of tofacitinib for the treatment of moderate to severe active UC has been approved (113). Sandborn et al. completed three-phase, randomized, and double-blind placebo-controlled trials of tofacitinib therapy in adults with UC and found that in

TABLE 3 | Small molecules.

Type of study	Patients	Treatment	Median treatment duration	Median follow-up duration	Results/Conclusion	Adverse events	References
Three phase 3, randomized, double-blind, placebo-controlled trials	Adults with UC	Tofacitinib (induction therapy:10 mg twice daily for 8 weeks; maintenance therapy: either 5 or 10 mg twice daily for 52 weeks)	8, 8, 52 weeks	8, 8, 52 weeks	Tofacitinib appeared more effective in inducing and maintaining remission in patients with active CD compared with placebo	Increased lipid levels, infections, cardiovascular events	(105)
A phase 2, double-blind, randomized, placebo-controlled trial	Patients with moderate-to-severe CD	Filgotinib (GLPG0634, GS-6034) (200 mg once daily)	10 weeks	20 weeks	Filgotinib was more effective for inducing remission than placebo, and it had an acceptable safety profile	Infections	(106)
A multicenter, double-blind, phase 2b study	Adults with moderately to severely active UC and an inadequate response, loss of response, or intolerance to CSs, immunosuppressors, and/or biologics	Upadacitinib (7.5, 15, 30, or 45 mg once daily)	8 weeks	8 weeks	Upadacitinib (45 mg) was more efficacious as induction therapy than placebo	Increased serum lipid levels and creatine phosphokinase, herpes zoster, pulmonary embolism, deep venous thrombosis	(107)
A double-blind, placebo-controlled phase 2 trial	Adults with moderate-to-severe UC	Ozanimod (RPC1063) (0.5 or 1 mg daily)	32 weeks	32 weeks	Ozanimod at a daily dose of 1 mg resulted in a slightly higher rate of clinical remission of UC than placebo	Pyrexia, arthralgia, alanine aminotransferase increased, rash, vomiting, orthostatic hypotension, aspartate aminotransferase increased, hyperbilirubinemia, insomnia, nasopharyngitis, proctalgia	(108)
A phase 3, multicenter, randomized, double-blind, placebo-controlled trial	Patients with moderately to severely active	Oral ozanimod hydrochlorid (1 mg once daily) for induction therapy	10, 10, 52 weeks	10, 10, 52 weeks	Ozanimod resulted in significantly increased incidences of clinical response and clinical remission for both induction and maintenance period	Elevated liver aminotransferase levels, nasopharyngitis, headache, arthralgia	(109)
A single-arm, phase 2, prospective observer-blinded endpoint study	Adults with moderately to severely active CD	Ozanimod (0.25 mg daily for 4 days, followed by 3 days at 0.5 mg daily, then 1.0 mg daily for a further 11 weeks, followed by a 100-week extension)	12 weeks	112 weeks	Endoscopic, histological, and clinical improvements were seen within 12 weeks of initiating ozanimod therapy in patients with moderately to severely active CD	CD(flare), abdominal pain, lymphopenia, arthralgia, nausea	(110)
A phase 2, proof of concept, double-blind, parallel-group study	Patients with moderately to severely active UC	Etrasimod (APD334) (1 or 2 mg once daily)	12 weeks	12 weeks	Erasimod 2 mg was more effective than placebo in producing clinical and endoscopic improvements	Anemia, urinary tract infection, headache, blood creatine phosphokinase increased, gamma-glutamyltransferase increased, sinusitis, fever, hyperlipasemia	(111)

UC, ulcerative colitis; CD, Crohn's disease; CSs, corticosteroids.

the OCTAVE Induction 1 trial, the 8-week remission rate of tofacitinib induction therapy was 18.5%, higher than that of the control group (8.2%, $P = 0.007$) (105). In the OCTAVE Induction 2 trial, the 8-week remission rate was 16.6% in the tofacitinib treatment group vs. 6.6% in the control group ($P < 0.001$). In the OCTAVE Sustain trial, for UC patients with clinical responses to tofacitinib induction therapy, the 52-week remission rates of the 5 mg tofacitinib group, 10 mg tofacitinib group, and the control group were 34.3, 40.6, and 11.1%, respectively ($P < 0.001$, compared with the control group) (105). According to the study published recently, Tofacitinib has fast onset of action and seems to be effective even in cases of acute severe UC or refractory to anti-TNF-α (114, 115). The long-term safety of tofacitinib remains unclear, and the main side effects are herpes zoster virus infection and thrombosis (105). Therefore, clinical trials of other subtypes of selective JAK inhibitors are still ongoing to improve the benefit-to-risk ratio of JAK inhibitors.

Filgotinib is an oral selective JAK1 inhibitor. A phase 2, double-blind clinical trial of CD patients reported that 47% of the patients in the filgotinib group while 23% in the placebo group ($P = 0.0077$) had clinical remission at 10 weeks (106).

Upadacitinib is another selective JAK1 inhibitor. Sandborn et al. accomplished a phase 2b, multicenter and double-blind clinical trial of patients with moderate-to-severe refractory UC. No patients receiving placebo achieved clinical remission at week 8 and the rates of clinical remission in patients receiving 7.5, 15, 30, or 45 mg upadacitinib were 8.5, 14.3, 13.5, and 19.6%, respectively ($P = 0.052$, $P = 0.013$, $P = 0.011$, and $P = 0.002$, compared with placebo). Additionally, 14.9, 30.6, 26.9, and 35.7% of patients receiving 7.5, 15, 30, or 45 mg upadacitinib achieved endoscopic improvement (endoscopic subscore ≤ 1) at week 8, while only 2.2% of patients receiving placebo achieved endoscopic improvement ($P = 0.033$, $P < 0.001$, $P < 0.001$, and $P < 0.001$, compared with placebo, respectively) (107). In the 45 mg upadacitinib group, one patient developed herpes zoster, and another patient had pulmonary embolism and deep vein thrombosis. In addition, increased serum lipid levels and creatine phosphokinase were reported after upadacitinib treatment.

Deucravacitinib is a kind of highly selective TYK2 inhibitor and exerts less or no activity toward JAK3 (116). Deucravacitinib can significantly decrease the levels of IL-12 and IL-23 which may be helpful for IBD treatment (117). However, the clinical trials for deucravacitinib in IBD treatment are still ongoing and the efficacy and safety of deucravacitinib should still be better evaluated.

Sphingosine-1-Phosphate Receptor Modulators and Agonists

S1P is a lipid mediator which is derived from membrane sheath lipid metabolism. S1P is produced intracellularly and can be translocated to extracellular regions, where it plays a regulatory role in the immune system by activating specific receptors. Ozanimod (RPC1063) and Etrasimod (APD334), as S1P receptor (S1PR) modulator and agonist, are already being studied for the treatment of UC.

Ozanimod is an oral and selective S1PR modulator that acts on S1PR-1 and S1PR-5. It induces peripheral blood lymphocytes to isolate in the lymph nodes, thereby reducing the number of activated lymphocytes circulating to the inflammatory sites. Sandborn et al. first studied the effect of ozanimod on UC (108). This double-blind, placebo-controlled phase 2 trial showed that patients receiving ozanimod (1 mg/day) had a higher rate of clinical remission. In a recently published phase 3 multicenter randomized, placebo-controlled study, a total of 1,012 patients were included in the induction period and 457 patients in the maintenance period (109). Results showed a significantly higher clinical remission rate among patients receiving ozanimod than those receiving placebo during induction (18.4 vs. 6.0%, $P < 0.001$) and maintenance (37.0 vs. 18.5% among patients with a response at week 10, $P < 0.001$). The incidence of clinical response was also tremendously higher with ozanimod than with placebo during both the induction (47.8 vs. 25.9%, $P < 0.001$) and maintenance (60.0 vs. 41.0%, $P < 0.001$). Despite the risk of raised liver aminotransferase levels, ozanimod was more effective than placebo in inducing and maintaining the remission of moderately to severely active UC. Feagan et al. conducted a phase 2 prospective study to evaluate the effects of ozanimod in moderate to severe CD. After induction therapy for 12 weeks, 23.2, 39.1, and 56.5% of the patients experienced endoscopic response, clinical remission, and clinical response, respectively (110). Despite the lack of a contemporaneous control group, similar endoscopic and histopathology improvements to those in controlled trials of effective agents verified the therapeutic benefits of ozanimod. Additionally, the observer-blinded design of this study makes the results more persuasive.

Etrasimod, an oral S1PRS agonist, is selective for S1PR-1, S1PR-4, and S1PR-5. A phase 2 randomized, double-blind, placebo-controlled study was performed to assess the therapeutic effects of etrasimod for patients with moderately to severely active UC, which reported that patients receiving etrasimod at a daily dose of 2 mg achieved better clinical ($P = 0.009$) and endoscopic improvements ($P = 0.003$) than patients receiving placebo (111). There is no study reporting the treatment effects of etrasimod in CD. More studies are needed to further evaluate the efficacy and safety of ozanimod and etrasimod in IBD.

SURGICAL TREATMENT

With the development of biologics, significant progress has been made in the drug treatment of IBD, but surgery is still an important means for IBD treatment. Despite the increased number of hospitalized patients in recent years, the rate of surgery for CD has decreased from 10 to 8.8% ($P < 0.001$), and that for UC has decreased from 7.7 to 7.5% ($P < 0.001$) (118). A study in the New York State Database demonstrated that in the era of biologics, the mortality rate of CD patients after non-selective surgery declined, but that of UC patients increased to 15% (119). There is still room for improvement in surgical and perioperative management.

The absolute indications of operation in UC patients principally include complicated massive bleeding, intestinal perforation, carcinogenesis and highly suspected carcinogenesis. Relative indications include: (1) Patients with severe UC fail to respond to active medical treatment, and patients with

toxic megacolon have no response to medical treatment, earlier surgical intervention is suggested. (2) The medical treatment effect is poor and/or adverse drug reactions have seriously affected the quality of life (120).

For localized ileocaecal CD patients who failed to respond or relapse after initial medical therapy or preferred surgery to continued drug therapy, laparoscopic resection is recommended. Due to the poor long-term outcomes, surgical options for perianal Crohn's fistula can only be offered to a selected group of patients after consultation, especially patients with complex diseases and ongoing disease activity (120). One study showed that the symptomatic recurrence rate of CD patients was 20% at 1 year and 34% at 3 years after ileocolectomy, while the endoscopic recurrence rate reached 73 and 85%, respectively (121). Regular post-operative endoscopic examination may help monitor recurrence and develop prevention and treatment plans.

NOVEL THERAPIES

Emerging therapeutic approaches, such as apheresis therapy, improved intestinal microecology, cell therapy, and exosome therapy, were reviewed in this section. More research on these therapies may provide new treatment options for IBD, bringing both opportunities and challenges.

Apheresis Therapy

Apheresis therapy is a novel treatment for IBD developed in Japan, whose main mechanism is to reduce the local inflammatory response by isolating and absorbing one or more specific leukocytes (such as granulocytes, monocytes and activated lymphocytes) in the peripheral blood (122).

Adsorptive granulocyte/monocyte apheresis (GMA) has been shown to be effective in inducing remission in patients with UC and CD. A meta-analysis showed that GMA was more effective in inducing clinical remission in patients with active UC than CSs (OR = 2.23, 95% CI: 1.38–3.60) and that the incidence of adverse events associated with GMA was significantly lower than that with CSs (123). The efficacy (overall efficacy rate of about 70%) and safety of GMA were initially confirmed by a multicenter clinical trial in China that involved 34 patients with active UC (124). Motoya et al. found that the clinical remission rate of UC treated with GMA was 46.4% with no increase of adverse events in older patients with IBD (125). Recently, a retrospective analysis showed that nearly 80% of patients with UC achieved clinical remission after GMA treatment (126). Fukuchi et al. investigated the efficacy of GMA combined with TPs in the treatment of patients with early-diagnosed CD (127). The clinical remission rate and mucosal healing rate during the 52-week long treatment were 81.8 and 50%, respectively, without any serious adverse reactions.

GMA is quite popular among patients with IBD. About half of patients expressed their satisfaction with the effect of GMA after treatment, and 80% showed agreement to be treated with this technique again in the future, regardless of the response to the treatment (128). However, evidence for the effectiveness of GMA maintenance therapy is scarce and further studies are needed.

The Improvement of Intestinal Microecology

Changes in the composition and function of the intestinal microbiota were found in patients with IBD (129). Although the specific mechanism of IBD remains unclear, the occurrence of IBD is found closely related to the imbalance of intestinal microecology. The imbalance between beneficial bacteria and harmful pathogenic bacteria in patients can trigger abnormal immune response in genetically susceptible people (129). Meanwhile, the inflammatory cells and factors can cause the intestinal mucosa injury. In IBD patients, the biodiversity of intestinal microbiota was decreased, with the most pronounced changes in the number of normal anaerobic bacteria such as Bacteroides, Eubacteria and Lactobacilli. Mucosal inflammation in IBD has been proved associated with the loss of these normal anaerobic bacteria (130).

On the theoretical basis of intestinal microbiota disorder, researchers have found potentially effective treatment methods for IBD by improving intestinal microecology with progressive achievements in recent years, including antibiotics, probiotics, prebiotics, postbiotics, synbiotics, and fecal microbiota transplantation (FMT).

Antibiotics

Researchers have made more efforts to explore the role of antibiotics in the treatment of IBD. Antibiotics are expected to be a future treatment choice for IBD, given their potential influence on the intestinal microbiota composition. A systematic review and meta-analysis published in 2011 demonstrated the positive effects of antibiotics for both UC and CD (131). Uchino et al. conducted a randomized controlled trial and proved that combined oral and intravenous antibiotics in CD patients could decrease the incidence of incisional infection (7.4 vs.16.6%, $P = 0.01$) compared with intravenous antimicrobial prophylaxis alone (132). They concluded the absence of oral antibiotics as an independent risk factor for surgical site infections and revealed the importance of preoperative oral antibiotics for CD patients. In the 3rd European evidence-based consensus, the use of antibiotics in CD patients is appropriate for septic complications, symptoms attributable to bacterial overgrowth or perineal disease (133). However, a Cochrane database systematic review in 2019 reported that the efficacy of antibiotics in inducing remission of CD appeared be modest and might not be clinically meaningful (134). The effect on the maintenance of remission and the risk of severe adverse events in CD was unclear. Moreover, studies on the effect of antibiotics in UC are insufficient to recommend antibiotics for induction or maintenance of remission (135).

It is important to note that using antibiotics may be an independent risk factor for IBD. Higher cumulative exposure to systemic antibiotic therapy, particularly treatments with a greater spectrum of microbial coverage, may be associated with a higher risk of new-onset IBD (136). Compared with patients taking no antibiotics, the patients taking antibiotics showed a OR value of 1.88 (95% CI: 1.79–1.98) for the occurrence of IBD, 1.74 (95% CI: 1.64–1.85) for UC, and 2.27 (95% CI: 2.06–2.49) for CD, respectively (136). Additionally, Shaw et al. found that changes in intestinal symbiotic bacteria caused by antibiotics use

in infants and children were associated with the development of IBD (137). Of 36 children with IBD, 58% had received antibiotics within the first year of life while only 39% of the 360 normal children received that. Patients who received antibiotics within the first year of life were 2.9 times more likely to develop IBD than those who did (95% CI: 1.2–7.0). Moreover, Balram *et al.* found that antibiotics use within 30 days of diagnosis appeared to increase the risk of clostridium difficile infection (CDI) among IBD patients (OR = 1.85, 95% CI: 1.36–2.52) (138). Therefore, further emphasis should be attached to the management of antibiotics use.

Probiotics, Prebiotics, Synbiotics, and Postbiotics

Probiotics are live microorganisms that are intended to have health benefits when consumed or applied to the body. They can reduce epithelial cell apoptosis and attenuate intestinal mucosal inflammation (139). Probiotics are usually bacteria that produce lactic acid, which can be obtained by ingesting fermented foods, such as yogurt, fermented dairy products, and fermented byproducts of cured meats (140). Prebiotics can be health-promoting substrates selectively utilized by host microbes and supplemented by the intake of legumes, fruits, and vegetables. Generally, beneficial prebiotics includes polyols (sugar alcohols), oligosaccharides, and soluble fiber (141). Synbiotics are the synergistic combination of probiotics and prebiotics found in foods, drugs, and supplements (142–144). The main microbial-derived metabolites are postbiotics, including bile acids, short-chain fatty acids, and tryptophan metabolites (145).

Probiotics, prebiotics, and synbiotics have been proved beneficial in IBD, especially the combination ones in UC (140, 146). Subgroup analyses showed that synbiotics might be more effective than probiotics or prebiotics in inducing or maintaining IBD remission (140). Additionally, probiotics, prebiotics, or synbiotics in combination with conventional drugs were superior to conventional drugs alone (147). A randomized controlled trial demonstrated that regular consumption of kefir (a fermented probiotic dairy product) containing lactobacilli can modulate intestinal biota so as to improve quality of life in patients with IBD (148).

Multiple studies have shown the potential effects of probiotics in UC but not in CD (146, 149–151). Some studies found a beneficial effect of probiotics on active UC (149, 150), but the effect showed little or no difference in clinical remission compared to 5-ASA (149). Some studies suggested that probiotics were as effective as 5-ASA in preventing the recurrence of inactive UC but showed no benefit in inducing remission of active UC over placebo (151). Bjarnason et al. conducted a single-center, randomized, double-blind, placebo-controlled trial, and found a multi-strain probiotic (Symprove^{TM}, Symprove Ltd, Farnham, United Kingdom) might reduce the intestinal inflammation in patients with UC (152). Many studies found no significant effects of probiotics for the treatment of CD (153, 154).

Probiotics appeared to be safe and well-tolerated. In a systematic review and meta-analysis, there was no significant evidence to prove the risk for the overall side effects (RR = 1.35, 95% CI: 0.93–1.94) and for gastrointestinal symptoms (RR = 1.78, 95% CI: 0.99–3.20) was higher in IBD patients taking probiotics than in those exposed to placebo (155). Probiotic supplements that were based on Lactobacillus and Bifidobacterium or more than one strain were more likely to be effective for IBD remission. It suggested the dose of 10^{10}-10^{12} CFU/day as a reference range for using probiotics to relieve IBD (140).

Postbiotics could act as immunomodulators and motivate anti-inflammatory response (156), suggesting that postbiotics may be a treatment for IBD. A study assessing the potential role of postbiotics in an *ex-vivo* organ culture model showed that potent postbiotics could protect healthy tissue against inflammatory attacks and concluded that postbiotics could be an effective and safe choice for acute IBD (157). More in-depth studies are needed to elucidate their role in the treatment of IBD.

FMT

FMT is a new therapy that transplants the functional micromicrobiota from the feces of healthy donors into the gastrointestinal tract of patients suffering from intestinal microbiome disorders to reconstruct the intestinal microecology and cure disease. FMT has been shown to be effective in the treatment of recurrent and refractory CDI with a high success rate of 90% (158). In recent years, the potential of FMT in the treatment of IBD has been further unleashed. The Australian consensus on the clinical use of FMT acknowledged for the first time the efficacy of FMT in inducing remission in patients with mild to moderate UC (159).

The main advantage of FMT lies in the complete ecosystem it provides from healthy individuals, including the full spectrum of microbial organisms, which may address intestinal microdysbiosis and dysfunction in patients with IBD (160). FMT has gained development and application for its efficacy and safety in the treatment of IBD. FMT was superior to placebo in achieving clinical remission at week 7 in a randomized study on patients with active UC (161). A meta-analysis of the efficacy of FMT in IBD demonstrated that the clinical remission rate in UC and CD patients receiving FMT was 33% (95% CI: 23–43%) and 52% (95% CI: 31–72%), respectively (162). Meanwhile, the association between FMT and clinical remission in patients with UC was discovered in a meta-analysis of 4 randomized controlled trials (OR = 2.89, 95% CI: 1.36–6.13).

Multiple studies confirmed that, the intestinal microbial diversity of the recipient increased after FMT (162–164) and showed a similarity to the microbiota of donor (162), thus giving great importance of the selection of donor because the intestinal microorganism status of the donor can affect the efficacy of FMT. It was found that the higher the microbial richness of the donor, the higher the success rate of transplantation treatment (163). A double-blind, randomized controlled trial reported that 27% of the patients allocated FMT and 8% of those assigned placebo achieved steroid-free clinical remission with endoscopic remission or response (RR = 3.6, 95% CI 1.1–11.9; $p = 0.021$) (165). A multicenter, randomized, double-blind clinical trial was conducted to evaluate the efficacy of FMT protocols in adults with mildly to moderately active UC using an anaerobically prepared stool. The results proved that patients receiving anaerobically prepared pooled donor FMT

had a higher steroid-free remission rate (32%) than those (9%) receiving autologous FMT processed under aerobic conditions at week 8 ($P = 0.03$) (166).

The optimal route and regimen of FMT administration requires further study. A meta-analysis suggested that FMT administration via the lower gastrointestinal tract was more effective than the upper gastrointestinal tract in patients with UC (162). However, no unified standard has been made. Compared with traditional drug therapy, the time-consuming and labor-consuming colonoscopic FMT with the unknown safety of long-term frequent operation cannot be used as a routine choice for IBD treatment. The emergence of encapsulation methods, such as liquefaction, freezing, and freeze-drying, provides new ideas for the application of FMT in the maintenance treatment of IBD. The clinical efficacy of oral capsule FMT in the treatment of refractory CDI has been validated (167–169).

Combing FMT and antibiotics may improve the efficiency of IBD treatment. A meta-analysis demonstrated that patients with UC who received antibiotics before FMT had a higher rate of clinical remission than patients who did not before FMT (54 vs. 25%, $P = 0.03$) (170). However, it remains unclear how to select the appropriate antibiotics or combinations of antibiotics for different patients to achieve the optimal intestinal microecology after antibiotic treatment and FMT treatment. Bacteriological and metabolic analyses of fecal samples before and after FMT revealed that compared to patients who did not achieve remission, patients achieving remission after FMT had higher concentrations of Eubacterium hallii and Roseburia inulivorans and increased levels of short-chain fatty acid biosynthesis and secondary bile acids.

There are challenges before the application of FMT in the treatment of IBD: (1) The long-term efficacy is unknown: most clinical studies' period is short with the longest ones lasting 1 year in the treatment of UC (161, 166). (2) The safety is unknown: the reported side effects of FMT treatment in IBD patients include common gastrointestinal reactions (e.g., bloating, diarrhea, and abdominal pain) (162), complications related to administration route (e.g., aspiration pneumonia and intestinal perforation) (163, 171), as well as IBD related ones (e.g., toxic hypercolon and sepsis) (162). However, few long-term statistics on the safety of FMT in patients with IBD have been collected. (3) The feasibility of universal implementation and management is unknown: the lack of unified standards for the use of FMT in IBD around the world limits its further application (172).

In summary, FMT is expected to be a new option for IBD treatment. Future research should focus on the donor-receptor matching based on microbial characterization (165), selection of administration routes, and determination of optimal intensity of treatment. Meanwhile, additional preclinical studies and clinical trials are necessary to provide data on the long-term efficacy and safety of FMT.

Stem Cell Transplantation

Stem cells can differentiate into more than one type of cells in the body and keep dividing and proliferating (173). Stem cell transplantation can promote the regeneration of injured tissue and help restore specific tissue functions, thus restoring the integrity of the intestinal mucosal barrier in patients with IBD. In recent years, advances in stem cell biology have opened new grounds for the application of stem cells of different types in the treatment of IBD.

The cells involved in the pathophysiological process of IBD include inflammatory cells of the lamina propria, intestinal mesenchymal cells and intestinal epithelial cells (IECs). Therefore, haematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and intestinal stem cells (ISCs) are candidates for IBD cell therapy.

HSCs Transplantation

HSCs have the ability to migrate to injured tissues and facilitate tissue renovation, renewal, and regeneration (174, 175). We usually choose autologous HSCs for HSCs transplantation (HSCT) because of gastrointestinal disorders and even the occurrence of IBD (176, 177) after allogeneic HSCT. Autologous HSCT rebuilds the host's immune system by generating new self-tolerant lymphocytes after chemotherapy-induced elimination of self- or auto-reactive lymphocytes (178). The tissue sources of HSCs for therapeutic use are mainly derived from bone marrow, umbilical cord and peripheral blood, with the most specific marker of the cell surface glycoprotein CD34.

HSCT is the most widely used cell regeneration therapy for its feasibility in clinical practice. Early reports showed that 2 patients with severe CD who had no response to anti-TNF-α therapy achieved clinical remission and maintained for more than 1 year after large dose of immunosuppression and autologous HSCT (179). A European retrospective study of 82 patients with severe refractory CD showed 68% of patients had complete remission or significant improvement in symptoms after treatment with autologous HSCT, with a median follow-up of 41 months (6–174 months) (180). Lindsay et al. found that within 1 year after autologous HSCT, 38% of patients with CD achieved a 3-month steroid-free remission, and about half of these patients achieved mucosal healing (181). However, the safety and long-term efficacy of HSCT in the treatment of IBD call for further discussion. A retrospective study reported the unpromising long-term remission of autologous HSCT because most patients required salvage or maintenance treatment within 1 year after autologous HSCT (182). A cohort study showed that although the majority of CD patients relapse within 5 years after autologous HSCT, 80% of relapsed patients returned to clinical remission after re-treatment with HSCT (183). In addition, infectious adverse events [viral infection (180), sepsis, and pneumonia] were common within 100 days after autologous HSCT (184). Some researchers believe that autologous HSCT with low intensity is safe and effective for refractory CD (185). In a study with 14 refractory CD patients, autologous HSCT with low-dose cyclophosphamide was used (186). Compared with previous studies using high-dose cyclophosphamide, the duration of anemia and neutropenia was shorter. The lower intensity cyclophosphamide still achieved an effective treatment for refractory CD, with 13 patients achieving disease remission at 30 days.

In conclusion, although many studies have confirmed the efficacy of HSCT in the treatment of some patients with

refractory CD, caution should be exercised due to the high risk of adverse events after HSCT. In addition, the number of HSCs is limited, accounting for only 1/100,000 (187). Meanwhile, patients' unique conditions must be fully considered before treated with autologous HSCT.

MSCs Transplantation

MSCs are widely distributed in various tissues (bone marrow, peripheral blood, fat, skeletal muscle, etc.). They have strong ability to proliferate and can differentiate into various mesodermal cell types (adipocytes, osteoblasts, or chondrocytes) under specific induction conditions *in vitro* (188). Due to the low immunogenicity of MSCs, allogeneic MSCs transplantation can be used safely without immunosuppression (178). The superiority of MSCs also lies in their tissue-specific differentiation, strong immunomodulation, and plentiful trophic factor production (178).

The main tissue sources of MSCs used for treatment are bone marrow, umbilical cord and adipose tissue, and other sources like amniotic membrane and fetal membrane. Both adipose-derived stem cells (ADSCs) and bone marrow mesenchymal stem cells (BMSCs) showed the morphological and immunophenotypic characteristics of MSCs, with positive expression of MSCs markers and negative expression of hematopoietic markers (189). Compared to BMSCs, ADSCs can secrete higher levels of anti-inflammatory cytokines (IL-6 and TGF-β) involved in immune regulation. Therefore, ADSCs may have stronger immunomodulatory ability than BMSCs (190).

A phase 1 clinical study demonstrated the safety of autologous BMSCs transplantation in patients with refractory CD (191). A phase 2 clinical study showed that after intravenous administration of allogeneic BMSCs for 4 weeks, 80% of patients with refractory CD had a clinical response, and over half of the patients achieved clinical remission. Additionally, 47% of the patients had endoscopic improvement, with a low incidence of adverse events (192). Dietz et al. explored the efficacy of autologous MSCs on perianal fistula of patients with CD through a phase 1 trial and recorded a clinical cure rate of over 80% at 6 months (193). A phase 3 clinical trial demonstrated that the healing rate of complex anal fistulas was ∼40% after 6 months of allogeneic ADSCs transplantation (194). A meta-analysis and systematic review reported that patients receiving MSCs transplantation had a higher rate of fistula healing than patients receiving placebo (61.75 vs. 40.46%, $P < 0.05$). MSCs transplantation, especially ADSCs, was well-tolerated with a lower incidence of adverse events than placebo (195).

MSCs regulate immune response by down-regulating the levels of pro-inflammatory cytokines (TNF-α, IFN-γ, 1L-12, IL-17, etc.) and up-regulating the level of anti-inflammatory cytokines (IL-10). MSCs directly target the Th-17 cells and increase their expression of FoxP3 mRNA, thus switching them into Tregs and inhibiting their production of inflammatory cytokines. MSCs can also inhibit the Th1-driven autoimmune response (196–198). The effect of donor MSCs on the recipient is mainly through paracrine release of various cytokines (199), which not only participate in the regulation of immune response but also promote tissue repair (178). Intraperitoneal BMSCs in mouse model of colitis localize to the peritoneum and produce sufficient immunoregulatory molecules there (200). Therefore, targeted transplantation is not necessary for MSCs transplantation to work.

MSCs transplantation with immunomodulatory effects can promote intestinal epithelial remodeling, which is expected to be an effective method for the treatment of IBD. Available clinical data have shown the potential therapeutic effect of MSCs transplantation on IBD and its complications, but MSCs have not been approved for clinical use. Therefore, more randomized controlled studies are needed to provide data support for the application of MSCs transplantation.

ISCs Transplantation

Intestinal epithelium consists of villi and crypt which is renewed every 2–6 days in healthy individuals due to the continuous proliferation of ISCs at the base of the intestinal crypt. ISCs have the ability to regenerate and differentiate into different types of IECs, such as goblet cells, endocrine cells, tuft cells, and absorptive cells. IBD damages the intestinal epithelium. Therefore, researchers hope to regenerate and repair the damaged intestinal epithelium through the transplantation of ISCs cultured *in vitro*, thus promoting the mucosal healing of patients with IBD. However, culturing ISCs *in vitro* remains a challenge in ISCs transplantation.

Sato et al. developed a technique for long-term culture of ISCs *in vitro* by culturing ISCs in a 3D structure named organoid that mimics the ISCs niche environment (201). The ecology of the ISCs *in vivo* was simulated by providing appropriate growth factors and the required extracellular matrix *in vitro* (202). The source of ISCs specimens for *in vitro* culture were the biopsy tissue of patients during endoscopic examination (203). A study reported that the position-specific function of adult ISCs was inherently programmed, and their differentiation fate was independent of position-specific extracellular signaling (204). These findings provided an important basis for the use of ISCs as donor cells for cell transplantation therapy. Another major breakthrough supporting the clinical application of ISCs is that organoids grown *in vitro* can be transplanted and integrated into the recipient intestinal epithelium. Yui et al. transplanted the donor organoid cells into the colon of the dextran sulfate sodium (DSS)-colitis mouse model using the intraluminal transplantation method. Those cells engrafted and covered the lesions in recipient mice and constituted a single-layered epithelium, which formed self-renewing crypts that were functionally and histologically normal (205).

However, several difficulties need to be overcome when treating IBD with ISCs. First of all, research on ISCs transplantation for IBD is still in its infancy with no clinical trials and limited repeated animal experiments. Secondly, many issues need to be addressed before establishing an accurate *in vitro* culture scheme of human ISCs. Thirdly, it is not clear at the moment whether autologous or allogeneic transplantation of ISCs is favorable to treat IBD patients. Moreover, methods to deliver the donor ISCs efficiently to the desired site, e.g., endoscopic technology, need to be established. Finally, the

conditions under which patients can receive transplantation and the contraindications to the procedure need to be specified.

Development in stem cell biology has improved the feasibility of the culturing ISCs *in vitro*. However, there are many issues to address before establishing a safe and effective transplantation scheme for ISCs cultured *in vitro* to patients with IBD.

Exosome Therapy

Exosomes are nanoscale microvesicles released from various types of cells and are widely distributed in biological fluids, which contain important regulatory factors that act on adjacent or distal cells through the systemic system and function in a variety of biological signaling pathways. It has been demonstrated that exosome-mediated immune responses play an important role in the pathogenesis of IBD (206).

The lipid bilayer of exosomes encases various functional components but no organelles. Their functions mainly depend on their internal functional components, like proteins, nucleic acids, and other substances. The structure of exosomes themselves is also important. Studies have shown that exosome structure can increase the stability of internal biological components (207, 208). Exosomes are involved in numerous physiological processes, such as immune regulation, tissue repair, and regeneration (209). Therefore, exosomes have great clinical potential in the treatment of IBD.

The main sources of exosomes are stem cells, immune cells, IECs, body fluids, food and parasites. Stem cell-derived exosomes contribute to stem cell self-renewal, injury repair, and immune regulation. Mao et al. found that exosomes released by MSCs in human umbilical cord blood could reduce inflammatory response in mouse model of IBD and contribute to the recovery of damaged tissues and organs (210). Liu et al. further confirmed the therapeutic effect of MSCs-derived exosomes on IBD and deemed that the therapeutic effects depended on macrophages (211). Exosomes derived from immune cells (such as macrophages, monocytes, and dendritic cells) can evade clearance by the immune system, thereby prolonging their cycle and duration of action. It was reported that exosomes produced by IL-10-treated dendritic cells inhibited colitis of mouse model (212). Another study suggested that TGF-β1 gene-modified exosomes alleviated colitis in mouse model (207). Exosomes produced by IECs are crucial to IECs-induced immune tolerance (206). Though exosomes can be isolated from blood (213), amniotic fluid (214), urine (215), and breast milk (216), their relevance to IBD treatment drawn inadequate academic attentions in the past. However, exosomes derived from food have gained recent popularity. Xiao et al. isolated exosome-like nanoparticles from 11 vegetables and fruits (217) and Zhao et al. isolated exosomes from coconut water (218). A study proved that milk could be the carrier of chemical drugs or other biological components with a targeted effect similar to exosomes (219). Recently, it has been found that extracellular vesicles (EVs) secreted by whipworm can interact with host cells and participate in the regulation of inflammation and immunity (220). EVs produced by hookworm can inhibit the production of inflammatory cytokines (IL-6, IL-1β, IFNγ, and IL-17), thereby alleviating colitis in mouse model (221).

Epithelial restitution is essential for barrier function repairment at injured mucosal surfaces. Prolonged breaches in epithelial barrier function result in inflammation and further damage. Endogenous annexin A1 (ANXA1) in exosomes can heal damaged intestinal epithelium through transducing the formyl peptide receptor signaling pathway (222). Patients with active IBD have higher levels of serum EVs containing ANXA1 than healthy controls (222). Polymeric nanomaterials containing exogenous ANXA1 mimetic peptides can target the impaired intestine and accelerate the intestinal healing and the recovery of intestinal epithelial barrier function in mouse model of UC (222). Heat shock proteins (HSPs) are important elements in the body's defense against various damaging factors. It has been shown that HSPs in exosomes, such as HSP20, HSP27, HSP70 family, and HSP90, are involved in the pathogenesis of IBD (223, 224). Cellular prion protein (PrPc) is a glycosylphosphatidylinositol-anchored glycoprotein ubiquitous in the cellular junctions of many tissues. PrPc has also been found in platelet-released exosomes (225). A study found reduced PrPc levels at the epithelial cell-cell junction of the colon in patients with IBD (226). PrPc regulates IEC-cell junction and plays an important role in maintaining intestinal barrier function (226). Exosomes isolated from vegetables and fruits have anti-inflammatory properties, and their internal miRNAs (small non-coding RNAs) can regulate human mRNA (217).

Design of new drug formulations using exosomal structures may provide new insights for the treatment of IBD. Both animal and clinical studies are required to verify the efficacy of exosomes on IBD before clinical use.

Others

There are also many emerging therapeutic methods for IBD treatment. ABX464, as a novel drug candidate for human immunodeficiency virus (HIV), has shown strong antiviral properties (227). Recent studies have discovered that ABX464 can upregulate the expression of miR-124, thus inhibiting the inflammatory response for IBD treatment (228, 229). A phase 2 study in moderate to severe UC patients treated with oral administration of ABX464 or placebo was conducted (230). After treating 8 weeks, the difference of the endoscopic improvement was significant between ABX 464 (50 mg daily) group and placebo group ($P < 0.05$). In addition, the overall safety of ABX464 was satisfactory with no obvious side effects. Mongerson, as an oral anti-sense small oligonucleotide, can decrease the translation of SMAD7, which will result in the anti-inflammatory response of TGF-b on mucosa (231). A clinical trial indicated that the administration of Mongerson (40 and 160 mg daily) for 2 weeks was better than placebo at inducing the remission of CD (232). However, the longer efficacy and safety of Mongerson for IBD treatment still need further investigation. IL-10 is a kind of proinflammatory cytokine that can decrease a number of proinflammatory signals associated with IBD (233). However, the application of IL-10 has been limited by some side effects (*e.g.*, anemia and thrombocytopenia) and gut-restricted distribution. AMT-101 is a chimera produced by genetically fusing non-toxic fragment of cholix to human IL-10 (233). AMT-101 can efficiently cross the epithelial barrier and selectively

activate human IL-10 receptors in the intestinal lamina propria (233), which allows IL-10 to play a targeted anti-inflammatory role without causing systemic side effects. Further clinical studies are needed to evaluate the efficacy and safety of AMT-101 for IBD treatment.

GENERAL MEASURES AND EDUCATION

There are several general measures for prevention and treatment of the complications in patients with IBD. Many factors within the patient may influence the outcome of IBD treatment, including diet, mood, and other lifestyle factors. In addition, education is necessary for patients and could help patients manage these factors scientifically.

General Measures

Patients with IBD are vulnerable to water and electrolyte balance disorders and malnutrition and there are severe cases such as chronic anemia, and high homocysteinemia that threaten the patients' life. Therefore, appropriate symptomatic treatment measures are necessary. Disordered water and electrolyte balance and acid-base balance should be corrected. Anemic patients should be transfused. Patients with hypoproteinemia should be injected with human albumin. Body mass index (BMI), iron, calcium, and vitamins (especially vitamin D and B12) should be monitored and adjusted accordingly. Nutritional support treatment should be given to patients with severe illnesses. Enteral nutrition is the first choice, and parenteral nutrition can be supplemented if enteral nutrition is insufficient (234). Patients with abdominal pain and diarrhea should take anticholinergic drugs or antidiarrheal drugs when necessary. Patients with severe poisoning symptoms should be given broad-spectrum antibiotics by the intravenous route.

IBD has been proved an independent risk factor for recurrence VTE (RR = 2.5; 95% CI: 1.4–4.2; $P = 0.001$) (235). Because of the high morbidity and mortality of VTE, thromboprophylaxis is essential, which is mainly achieved by correcting risk factors and using drugs. Correcting risk factors refers to controlling disease activity and avoiding long-term bed rest. Low molecular weight heparin is recommended for drug prophylaxis. However, thromboprophylaxis in IBD patients has not been well-implemented due to the lack of awareness or safety concerns.

Education

Diet

Diet alters the composition of the gut microbiome and the production of absorbable metabolites (236), which are important messengers in the interactions among diet, the gut microbiome, and the host (237). As a result, diet may affect the disease activity, symptoms and prognosis of IBD.

Certain components of the diet have anti-inflammatory or pro-inflammatory properties and will affect the course of IBD. Dietary inflammatory index (DII) is an index to quantify the potential inflammatory effect in the diet, which reflects a large literature and population base, and is associated with international standard (238, 239). A recent analysis found that dietary patterns with a high DII could increase the risk of CD (240). It has been shown that DII is positively correlated with disease activity in CD patients and there is no correlation between DII and disease activity of UC (241). However, a study of Iranian patients with IBD indicated that there was no association between DII and disease activity, which may be due to the small sample size ($n = 143$) or influence of other variables (242).

Exploring the influence of dietary interventions on IBD disease activity helps provide dietary guidelines for patients. An review reported that more than half of IBD patients were deficient in micronutrients, such as iron, vitamin B12, vitamin D, vitamin K, and folic acid (243). It has been proved that the supplementation of Vitamin D, which can modulate the immune response and reduce inflammation, may improve outcomes of the treatment of patients with IBD as the active component of vitamin D [1,25-(OH)D3] can interact with T cells and regulate immune response mediated by T cells (244). Vitamin D can also inhibit the inflammatory activity of dendritic cells, induce antimicrobial activity and regulate the production of cytokines to enhance the anti-inflammatory effect (244). In addition, vitamin D supplementation can help increase bone density and reduce the risk of fracture in IBD patients. However, vitamin D is a fat-soluble vitamin and should not be overused. Further research is needed to determine the optimal serum vitamin D levels for optimal therapeutic effects (244).

One study found that CD patients on a high-fiber diet were 40% less likely to have disease recurrence within 6 months than those on a low-fiber diet (245). However, a Cochrane review in 2019 analyzed 18 studies and indicated that the effects of dietary interventions, including high fiber, low refined carbohydrates, low microparticle, low calcium, symptoms-guided diet, highly restricted organic and low red processed meat diets, on CD and UC were uncertain (246). Albenberg et al. implemented a randomized controlled trial and confirmed that low red processed meat diet couldn't reduce the relapse rate in patients with quiescent CD (247). One of the most frequently used elimination diets in CD patients is the diet of low-FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols). Recently, a single-blind, randomized and controlled trial performed by Cox et al. initially demonstrated that the low-FODMAP diet made significant differences among patients with CD in remission, in symptom relief and improvement of life quality, but not in irritable bowel syndrome severity scores (248). Studies revealed that in stool samples collected at the end of the study period, patients on the low-FODMAP diet had a significantly lower abundance of Bifidobacterium adolescentis, Bifidobacterium longum, and Faecalibacterium prausnitzii than patients on the control diet. Bifidobacteria and Faecalibacterium prausnitzii, which have immune-regulatory effects, can increase peripheral blood mononuclear cell IL-10 production *in vitro* (249, 250). Faecalibacterium prausnitzii showed anti-inflammatory effects and was associated with lower post-operative ileal CD recurrence (250). Despite this, there were no detrimental effects of a low-FODMAP diet on patients with quiescent IBD (248).

Mediterranean-style diet (DINE-CD) is another kind of dietary intervention for IBD treatment. DINE-CD requires a diet

high in omega-3′s and low in omega-6′s, which may reduce intestinal inflammation (251). A study found that too much intake of meat, omega-6′s fatty acids, and total fats resulted in high risks of IBD (252). In this study, the benefits of fruits and vegetables and the disadvantages of high meat and fats intake indicate the promise of DINE-CD for IBD treatment. However, some researchers found that adherence to DINE-CD was very low for IBD patients (253). Significantly, these patients would like to extend their nutritional knowledge for a better remission effect.

There is a deficiency of well-designed randomized controlled trials in this area and further research is needed to investigate the effects of various dietary interventions on IBD patients.

Mood and Psychology

IBD patients may have abdominal pain, diarrhea, mucinous blood, and other symptoms. The recurring symptoms and long-term medication will bring a heavy economic burden to the family, so IBD patients are prone to anxiety, depression and other adverse emotions. Patients and their families should be positively guided to have a full understanding of the disease. They are expected to be psychologically prepared and learn to correctly deal with the symptoms. Patients in the active phase of disease should have adequate rest, vent their negative emotions properly, and avoid excessive psychological pressure. They are encouraged to communicate with others and cooperate with treatment to reduce the recurrence of the disease.

Others

A systematic review has demonstrated that for patients with CD, smoking cessation can reduce recurrence risk by 65% compared with continued smokers. Smoking can reduce the drug efficacy and increase surgical and post-operative recurrence rate (254). In addition, patients should take medication as prescribed and participate in regular follow-ups.

DISCUSSION

At present, the treatment of IBD is primarily pharmacological, consisting mainly of aminosalicylates, CSs, immunomodulators, and biologics. However, a considerable number of patients fail to achieve clinical remission after treatment, or lose response over time. Additionally, more clinical data on the long-term safety of drugs are required. With the deepening of research, new therapies for IBD treatment are coming into view, mainly including apheresis therapy, improvement of intestinal microecology, stem cell transplantation, and exosome therapy. These non-approved novel therapies are often applied in investigational protocols, but are limited by their unclear impact on IBD. We still confront with many unresolved challenges before applying these emerging treatment options into clinical management. More research including long-term data are required to minimize the risk and optimize the treatment outcomes.

With the application of biologics, the therapeutic objectives of IBD have changed, and a new concept named mucosal healing has become well-known. It should be noted that histological mucosal healing is different from endoscopic mucosal healing. In cases of endoscopic remission, histological inflammation may persist and be associated with adverse outcomes. Endoscopy maintains a key role in monitoring mucosal healing nowadays. Histological assessment has not been widely used because of the lack of a valid valuation system and complex heterogeneity of disease (255). Nevertheless, histological mucosal healing has the potential to become a higher therapeutic target, considering recent developments in histologic assessment tools in UC (256).

With growing appreciation for mucosal healing, a treat-to-target strategy has gained wide acceptance. In order to verify the progress realized in the therapeutic path, an objective evaluation of mucosal inflammatory response is crucial. In the past, clinical symptom scores (such as CD activity index) were used to evaluate the treatment efficacy. Nowadays, endoscopy, histology, radiology, immunobiochemical monitoring biomarkers, quality of life assessment, and other methods have been introduced to provide more valuable references for the assessment of disease activity. There is no doubt that a multidisciplinary team, in which strong coordination between doctors, other health professionals (technicians, radiologists, biologists) and the patients is needed. Studies have shown that compared with focusing on clinical symptoms alone, targeting mucosal healing or inflammation control appears to be more cost-effective (257).

In the progress of achieving personalized and precise therapy, there are both opportunities and challenges. Doctors should fully grasp the indications, contraindications, as well as evidence-based medicine of various drugs and treatments, so as to develop individualized treatment plans based on the comprehensive assessment of the patient. The treatment should be flexible and changed according to the patient's response to the treatment. Additionally, self-management and regular follow-up of patients should not be neglected. Timely communication and close cooperation between doctors and patients are equally essential to effective treatment strategies. All the above play a necessary role in the induction and maintenance of remission in patients with IBD.

AUTHOR CONTRIBUTIONS

ZC performed the literature search and wrote the paper. SW revised the paper. JL designed the review and revised the paper. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.765474/full#supplementary-material

REFERENCES

1. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet.* (2017) 390:2769–78. doi: 10.1016/S0140-6736(17)32448-0
2. Pasquali S, Gronchi A. Neoadjuvant chemotherapy in soft tissue sarcomas: latest evidence and clinical implications. *Ther Adv Med Oncol.* (2017) 9:415–29. doi: 10.1177/1758834017705588
3. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. *Lancet.* (2017) 389:1756–70. doi: 10.1016/S0140-6736(16)32126-2
4. Ocansey DKW, Zhang L, Wang Y, Yan Y, Qian H, Zhang X, et al. Exosome-mediated effects and applications in inflammatory bowel disease. *Biol Rev Camb Philos Soc.* (2020) 95:1287–307. doi: 10.1111/brv.12608
5. Nielsen OH, Ainsworth MA. Tumor necrosis factor inhibitors for inflammatory bowel disease. *N Engl J Med.* (2013) 369:754–62. doi: 10.1056/NEJMct1209614
6. Ben-Horin S, Kopylov U, Chowers Y. Optimizing anti-TNF treatments in inflammatory bowel disease. *Autoimmun Rev.* (2014) 13:24–30. doi: 10.1016/j.autrev.2013.06.002
7. Nakase H, Hirano T, Wagatsuma K, Ichimiya T, Yamakawa T, Yokoyama Y, et al. Artificial intelligence-assisted endoscopy changes the definition of mucosal healing in ulcerative colitis. *Dig Endosc.* (2021) 33:903–11. doi: 10.1111/den.13825
8. Neurath MF. New targets for mucosal healing and therapy in inflammatory bowel diseases. *Mucosal Immunol.* (2014) 7:6–19. doi: 10.1038/mi.2013.73
9. Okamoto R, Watanabe M. Investigating cell therapy for inflammatory bowel disease. *Expert Opin Biol Ther.* (2016) 16:1015–23. doi: 10.1080/14712598.2016.1177019
10. Schnitzler F, Fidder H, Ferrante M, Noman M, Arijs I, Van Assche G, et al. Mucosal healing predicts long-term outcome of maintenance therapy with infliximab in Crohn's disease. *Inflamm Bowel Dis.* (2009) 15:1295–301. doi: 10.1002/ibd.20927
11. Huang S, Li L, Ben-Horin S, Mao R, Lin S, Qiu Y, et al. Mucosal healing is associated with the reduced disabling disease in Crohn's disease. *Clin Transl Gastroenterol.* (2019) 10:e00015. doi: 10.14309/ctg.0000000000000015
12. Punchard NA, Greenfield SM, Thompson RP. Mechanism of action of 5-arninosalicylic acid. *Mediators Inflamm.* (1992) 1:151–65. doi: 10.1155/S0962935192000243
13. Oh-Oka K, Kojima Y, Uchida K, Yoda K, Ishimaru K, Nakajima S, et al. Induction of colonic regulatory t cells by mesalamine by activating the aryl hydrocarbon receptor. *Cell Mol Gastroenterol Hepatol.* (2017) 4:135–51. doi: 10.1016/j.jcmgh.2017.03.010
14. Murray A, Nguyen TM, Parker CE, Feagan BG, MacDonald JK. Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev.* (2020) 8:Cd000543. doi: 10.1002/14651858.CD000543.pub5
15. Eaden J, Abrams K, Ekbom A, Jackson E, Mayberry J. Colorectal cancer prevention in ulcerative colitis: a case-control study. *Aliment Pharmacol Ther.* (2000) 14:145–53. doi: 10.1046/j.1365-2036.2000.00698.x
16. Ford AC, Khan KJ, Sandborn WJ, Hanauer SB, Moayyedi P. Efficacy of topical 5-aminosalicylates in preventing relapse of quiescent ulcerative colitis: a meta-analysis. *Clin Gastroenterol Hepatol.* (2012) 10:513–9. doi: 10.1016/j.cgh.2011.10.043
17. Akobeng AK, Zhang D, Gordon M, MacDonald JK. Oral 5-aminosalicylic acid for maintenance of medically-induced remission in Crohn's disease. *Cochrane Database Syst Rev.* (2016) 9:Cd003715. doi: 10.1002/14651858.CD003715.pub3
18. Hart A, Ng SC, Watkins J, Paridaens K, Edwards JO, Fullarton JR, et al. The use of 5-aminosalicylates in Crohn's disease: a retrospective study using the UK Clinical practice research datalink. *Ann Gastroenterol.* (2020) 33:500–7. doi: 10.20524/aog.2020.0521
19. Gjuladin-Hellon T, Gordon M, Iheozor-Ejiofor Z, Akobeng AK. Oral 5-aminosalicylic acid for maintenance of surgically-induced remission in Crohn's disease. *Cochrane Database Syst Rev.* (2019) 6:Cd008414. doi: 10.1002/14651858.CD008414.pub3
20. Coward S, Kuenzig ME, Hazlewood G, Clement F, McBrien K, Holmes R, et al. Comparative effectiveness of mesalamine, sulfasalazine, corticosteroids, and budesonide for the induction of remission in crohn's disease: a bayesian network meta-analysis. *Inflamm Bowel Dis.* (2017) 23:461–72. doi: 10.1097/MIB.0000000000001023
21. Ford AC, Khan KJ, Talley NJ, Moayyedi P. 5-aminosalicylates prevent relapse of Crohn's disease after surgically induced remission: systematic review and meta-analysis. *Am J Gastroenterol.* (2011) 106:413–20. doi: 10.1038/ajg.2010.317
22. Lim WC, Hanauer S. Aminosalicylates for induction of remission or response in Crohn's disease. *Cochrane Database Syst Rev.* (2010) 2010:Cd008870. doi: 10.1002/14651858.CD008870
23. Gisbert JP, González-Lama Y, Maté J. 5-Aminosalicylates and renal function in inflammatory bowel disease: a systematic review. *Inflamm Bowel Dis.* (2007) 13:629–38. doi: 10.1002/ibd.20099
24. Creed TJ, Probert CS. Review article: steroid resistance in inflammatory bowel disease - mechanisms and therapeutic strategies. *Aliment Pharmacol Ther.* (2007) 25:111–22. doi: 10.1111/j.1365-2036.2006.03156.x
25. Truelove SC, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. *Br Med J.* (1955) 2:1041–8. doi: 10.1136/bmj.2.4947.1041
26. Hayashi R, Wada H, Ito K, Adcock IM. Effects of glucocorticoids on gene transcription. *Eur J Pharmacol.* (2004) 500:51–62. doi: 10.1016/j.ejphar.2004.07.011
27. Lichtenstein GR, Abreu MT, Cohen R, Tremaine W. American gastroenterological association institute technical review on corticosteroids, immunomodulators, and infliximab in inflammatory bowel disease. *Gastroenterology.* (2006) 130:940–87. doi: 10.1053/j.gastro.2006.01.048
28. Barrett K, Saxena S, Pollok R. Using corticosteroids appropriately in inflammatory bowel disease: a guide for primary care. *Br J Gen Pract.* (2018) 68:497–8. doi: 10.3399/bjgp18X699341
29. Curkovic I, Egbring M, Kullak-Ublick GA. Risks of inflammatory bowel disease treatment with glucocorticosteroids and aminosalicylates. *Dig Dis.* (2013) 31:368–73. doi: 10.1159/000354699
30. D'Haens G. Systematic review: second-generation vs. conventional corticosteroids for induction of remission in ulcerative colitis. *Aliment Pharmacol Ther.* (2016) 44:1018–29. doi: 10.1111/apt.13803
31. Selinger CP, Parkes GC, Bassi A, Fogden E, Hayee B, Limdi JK, et al. A multi-centre audit of excess steroid use in 1176 patients with inflammatory bowel disease. *Aliment Pharmacol Ther.* (2017) 46:964–73. doi: 10.1111/apt.14334
32. Chhaya V, Saxena S, Cecil E, Subramanian V, Curcin V, Majeed A, et al. Steroid dependency and trends in prescribing for inflammatory bowel disease - a 20-year national population-based study. *Aliment Pharmacol Ther.* (2016) 44:482–94. doi: 10.1111/apt.13700
33. Ford AC, Bernstein CN, Khan KJ, Abreu MT, Marshall JK, Talley NJ, et al. Glucocorticosteroid therapy in inflammatory bowel disease: systematic review and meta-analysis. *Am J Gastroenterol.* (2011) 106:590–9; quiz 600. doi: 10.1038/ajg.2011.70
34. Ou G, Bressler B, Galorport C, Lam E, Ko HH, Enns R, et al. Rate of corticosteroid-induced mood changes in patients with inflammatory bowel disease: a prospective study. *J Can Assoc Gastroenterol.* (2018) 1:99–106. doi: 10.1093/jcag/gwy023
35. Shukla T, Singh S, Tandon P, McCurdy JD. Corticosteroids and thiopurines, but not tumor necrosis factor antagonists, are associated with cytomegalovirus reactivation in inflammatory bowel disease: a systematic review and meta-analysis. *J Clin Gastroenterol.* (2017) 51:394–401. doi: 10.1097/MCG.0000000000000758
36. Waljee AK, Wiitala WL, Govani S, Stidham R, Saini S, Hou J, et al. Corticosteroid use and complications in a US inflammatory bowel disease cohort. *PLoS ONE.* (2016) 11:e0158017. doi: 10.1371/journal.pone.0158017
37. Li J, Wang F, Zhang HJ, Sheng JQ, Yan WF, Ma MX, et al. Corticosteroid therapy in ulcerative colitis: clinical response and predictors. *World J Gastroenterol.* (2015) 21:3005–15. doi: 10.3748/wjg.v21.i10.3005
38. Higgins PD, Skup M, Mulani PM, Lin J, Chao J. Increased risk of venous thromboembolic events with corticosteroid vs biologic therapy for inflammatory bowel disease. *Clin Gastroenterol Hepatol.* (2015) 13:316–21. doi: 10.1016/j.cgh.2014.07.017
39. Brassard P, Bitton A, Suissa A, Sinyavskaya L, Patenaude V, Suissa S. Oral corticosteroids and the risk of serious infections in patients with elderly-onset inflammatory bowel diseases. *Am J Gastroenterol.* (2014) 109:1795–802; quiz (1803). doi: 10.1038/ajg.2014.313

40. Khan N, Abbas A, Williamson A, Balart L. Prevalence of corticosteroids use and disease course after initial steroid exposure in ulcerative colitis. *Dig Dis Sci.* (2013) 58:2963–9. doi: 10.1007/s10620-013-2748-0
41. Sandborn WJ, Danese S, D'Haens G, Moro L, Jones R, Bagin R, et al. Induction of clinical and colonoscopic remission of mild-to-moderate ulcerative colitis with budesonide MMX 9 mg: pooled analysis of two phase 3 studies. *Aliment Pharmacol Ther.* (2015) 41:409–18. doi: 10.1111/apt.13076
42. Travis SP, Danese S, Kupcinskas L, Alexeeva O, D'Haens G, Gibson PR, et al. Once-daily budesonide MMX in active, mild-to-moderate ulcerative colitis: results from the randomised CORE II study. *Gut.* (2014) 63:433–41. doi: 10.1136/gutjnl-2012-304258
43. Shin JY, Wey M, Umutesi HG, Sun X, Simecka J, Heo J. Thiopurine prodrugs mediate immunosuppressive effects by interfering with rac1 protein function. *J Biol Chem.* (2016) 291:13699–714. doi: 10.1074/jbc.M115.694422
44. Jharap B, Seinen ML, de Boer NK, van Ginkel JR, Linskens RK, Kneppelhout JC, et al. Thiopurine therapy in inflammatory bowel disease patients: analyses of two 8-year intercept cohorts. *Inflamm Bowel Dis.* (2010) 16:1541–9. doi: 10.1002/ibd.21221
45. Gisbert JP, Niño P, Cara C, Rodrigo L. Comparative effectiveness of azathioprine in Crohn's disease and ulcerative colitis: prospective, long-term, follow-up study of 394 patients. *Aliment Pharmacol Ther.* (2008) 28:228–38. doi: 10.1111/j.1365-2036.2008.03732.x
46. Pugliese D, Aratari A, Festa S, Ferraro PM, Monterubbianesi R, Guidi L, et al. Sustained clinical efficacy and mucosal healing of thiopurine maintenance treatment in ulcerative colitis: a real-life study. *Gastroenterol Res Pract.* (2018) 2018:4195968. doi: 10.1155/2018/4195968
47. Gisbert JP, Linares PM, McNicholl AG, Maté J, Gomollón F. Meta-analysis: the efficacy of azathioprine and mercaptopurine in ulcerative colitis. *Aliment Pharmacol Ther.* (2009) 30:126–37. doi: 10.1111/j.1365-2036.2009.04023.x
48. Yamada S, Yoshino T, Matsuura M, Kimura M, Koshikawa Y, Minami N, et al. Efficacy and safety of long-term thiopurine maintenance treatment in japanese patients with ulcerative colitis. *Intest Res.* (2015) 13:250–8. doi: 10.5217/ir.2015.13.3.250
49. Pinto AL, Chebli LA, Ribeiro MS, Pace FH, Moraes JP, do Amaral FJ Jr, et al. Azathioprine therapy in steroid-dependent patients with Crohn disease: results of a 10-year longitudinal follow-up study. *Med Sci Monit.* (2009) 15:Pi19-26.
50. Connell WR, Kamm MA, Ritchie JK, Lennard-Jones JE. Bone marrow toxicity caused by azathioprine in inflammatory bowel disease: 27 years of experience. *Gut.* (1993) 34:1081–5. doi: 10.1136/gut.34.8.1081
51. Walker TR, Leichter AM. 6-Thioguanine can cause serious liver injury in inflammatory bowel disease patients. *J Pediatr Gastroenterol Nutr.* (2004) 38:232–3. doi: 10.1097/00005176-200402000-00028
52. van Dieren JM, Kuipers EJ, Samsom JN, Nieuwenhuis EE, van der Woude CJ. Revisiting the immunomodulators tacrolimus, methotrexate, and mycophenolate mofetil: their mechanisms of action and role in the treatment of IBD. *Inflamm Bowel Dis.* (2006) 12:311–27. doi: 10.1097/01.MIB.0000209787.19952.53
53. Lémann M, Chamiot-Prieur C, Mesnard B, Halphen M, Messing B, Rambaud JC, et al. Methotrexate for the treatment of refractory Crohn's disease. *Aliment Pharmacol Ther.* (1996) 10:309–14. doi: 10.1111/j.0953-0673.1996.00309.x
54. Feagan BG, Fedorak RN, Irvine EJ, Wild G, Sutherland L, Steinhart AH, et al. A comparison of methotrexate with placebo for the maintenance of remission in Crohn's disease. North American crohn's study group investigators. *N Engl J Med.* (2000) 342:1627–32. doi: 10.1056/NEJM200006013422202
55. Chande N, Wang Y, MacDonald JK, McDonald JW. Methotrexate for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev.* (2014) 2014:Cd006618. doi: 10.1002/14651858.CD006618.pub3
56. Naganuma M, Fujii T, Watanabe M. The use of traditional and newer calcineurin inhibitors in inflammatory bowel disease. *J Gastroenterol.* (2011) 46:129–37. doi: 10.1007/s00535-010-0352-z
57. Yoshino T, Nakase H, Honzawa Y, Matsumura K, Yamamoto S, Takeda Y, et al. Immunosuppressive effects of tacrolimus on macrophages ameliorate experimental colitis. *Inflamm Bowel Dis.* (2010) 16:2022–33. doi: 10.1002/ibd.21318
58. Lichtiger S, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, et al. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J Med.* (1994) 330:1841–5. doi: 10.1056/NEJM199406303302601
59. Van Assche G, D'Haens G, Noman M, Vermeire S, Hiele M, Asnong K, et al. Randomized, double-blind comparison of 4 mg/kg versus 2 mg/kg intravenous cyclosporine in severe ulcerative colitis. *Gastroenterology.* (2003) 125:1025–31. doi: 10.1016/S0016-5085(03)01214-9
60. Stange EF, Modigliani R, Peña AS, Wood AJ, Feutren G, Smith PR. European trial of cyclosporine in chronic active Crohn's disease: a 12-month study. The European study group. *Gastroenterology.* (1995) 109:774–82. doi: 10.1016/0016-5085(95)90384-4
61. Kino T, Hatanaka H, Miyata S, Inamura N, Nishiyama M, Yajima T, et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK-506 *in vitro*. *J Antibiot.* (1987) 40:1256–65. doi: 10.7164/antibiotics.40.1256
62. Fellermann K, Tanko Z, Herrlinger KR, Witthoeft T, Homann N, Bruening A, et al. Response of refractory colitis to intravenous or oral tacrolimus (FK506). *Inflamm Bowel Dis.* (2002) 8:317–24. doi: 10.1097/00054725-200209000-00002
63. Ogata H, Matsui T, Nakamura M, Iida M, Takazoe M, Suzuki Y, et al. A randomised dose finding study of oral tacrolimus (FK506) therapy in refractory ulcerative colitis. *Gut.* (2006) 55:1255–62. doi: 10.1136/gut.2005.081794
64. Yamamoto S, Nakase H, Mikami S, Inoue S, Yoshino T, Takeda Y, et al. Long-term effect of tacrolimus therapy in patients with refractory ulcerative colitis. *Aliment Pharmacol Ther.* (2008) 28:589–97. doi: 10.1111/j.1365-2036.2008.03764.x
65. Högenauer C, Wenzl HH, Hinterleitner TA, Petritsch W. Effect of oral tacrolimus (FK 506) on steroid-refractory moderate/severe ulcerative colitis. *Aliment Pharmacol Ther.* (2003) 18:415–23. doi: 10.1046/j.1365-2036.2003.01662.x
66. Baumgart DC, Pintoffl JP, Sturm A, Wiedenmann B, Dignass AU. Tacrolimus is safe and effective in patients with severe steroid-refractory or steroid-dependent inflammatory bowel disease–a long-term follow-up. *Am J Gastroenterol.* (2006) 101:1048–56. doi: 10.1111/j.1572-0241.2006.00524.x
67. Komaki Y, Komaki F, Ido A, Sakuraba A. Efficacy and safety of tacrolimus therapy for active ulcerative colitis; a systematic review and meta-analysis. *J Crohns Colitis.* (2016) 10:484–94. doi: 10.1093/ecco-jcc/jjv221
68. McSharry K, Dalzell AM, Leiper K, El-Matary W. Systematic review: the role of tacrolimus in the management of Crohn's disease. *Aliment Pharmacol Ther.* (2011) 34:1282–94. doi: 10.1111/j.1365-2036.2011.04873.x
69. Iida T, Nojima M, Nakase H. Therapeutic efficacy and adverse events of tacrolimus in patients with crohn's disease: systematic review and meta-analysis. *Dig Dis Sci.* (2019) 64:2945–54. doi: 10.1007/s10620-019-05619-1
70. Dwadasi S, Zafer M, Goens D, Paknikar R, Dalal S, Cohen RD, et al. Inpatient therapy with calcineurin inhibitors in severe ulcerative colitis. *Inflamm Bowel Dis.* (2020) 25:1896–905. doi: 10.1093/ibd/izaa326
71. Aggarwal BB, Gupta SC, Kim JH. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. *Blood.* (2012) 119:651–65. doi: 10.1182/blood-2011-04-325225
72. Sandborn WJ, Rutgeerts P, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Colectomy rate comparison after treatment of ulcerative colitis with placebo or infliximab. *Gastroenterology.* (2009) 137:1250–60; quiz 1520. doi: 10.1053/j.gastro.2009.06.061
73. Present DH, Rutgeerts P, Targan S, Hanauer SB, Mayer L, van Hogezand RA, et al. Infliximab for the treatment of fistulas in patients with Crohn's disease. *N Engl J Med.* (1999) 340:1398–405. doi: 10.1056/NEJM199905063401804
74. Sandborn WJ, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, et al. Subcutaneous golimumab maintains clinical response in patients with moderate-to-severe ulcerative colitis. *Gastroenterology.* (2014) 146:96–109.e1. doi: 10.1053/j.gastro.2013.06.010
75. Limdi JK. Golimumab for ulcerative colitis: adding perspective to the pursuit. *Frontline Gastroenterol.* (2018) 9:232–3. doi: 10.1136/flgastro-2017-100929
76. Martineau C, Flourié B, Wils P, Vaysse T, Altwegg R, Buisson A, et al. Efficacy and safety of golimumab in Crohn's disease: a French national retrospective study. *Aliment Pharmacol Ther.* (2017) 46:1077–84. doi: 10.1111/apt.14371

77. Adegbola SO, Sahnan K, Warusavitarne J, Hart A, Tozer P. Anti-TNF therapy in crohn's disease. *Int J Mol Sci.* (2018) 19:2244. doi: 10.3390/ijms19082244
78. Yamada S, Yoshino T, Matsuura M, Minami N, Toyonaga T, Honzawa Y, et al. Long-term efficacy of infliximab for refractory ulcerative colitis: results from a single center experience. *BMC Gastroenterol.* (2014) 14:80. doi: 10.1186/1471-230X-14-80
79. Panaccione R, Ghosh S, Middleton S, Márquez JR, Scott BB, Flint L, et al. Combination therapy with infliximab and azathioprine is superior to monotherapy with either agent in ulcerative colitis. *Gastroenterology.* (2014) 146:392–400.e3. doi: 10.1053/j.gastro.2013.10.052
80. Yarur AJ, Rubin DT. Therapeutic drug monitoring of anti-tumor necrosis factor agents in patients with inflammatory bowel diseases. *Inflamm Bowel Dis.* (2015) 21:1709–18. doi: 10.1097/MIB.0000000000000380
81. Nakase H, Motoya S, Matsumoto T, Watanabe K, Hisamatsu T, Yoshimura N, et al. Significance of measurement of serum trough level and anti-drug antibody of adalimumab as personalised pharmacokinetics in patients with Crohn's disease: a subanalysis of the DIAMOND trial. *Aliment Pharmacol Ther.* (2017) 46:873–82. doi: 10.1111/apt.14318
82. Neurath MF. IL-23: a master regulator in Crohn disease. *Nat Med.* (2007) 13:26–8. doi: 10.1038/nm0107-26
83. Wang K, Zhang H, Kugathasan S, Annese V, Bradfield JP, Russell RK, et al. Diverse genome-wide association studies associate the IL12/IL23 pathway with Crohn disease. *Am J Hum Genet.* (2009) 84:399–405. doi: 10.1016/j.ajhg.2009.01.026
84. Almradi A, Hanzel J, Sedano R, Parker CE, Feagan BG, Ma C, et al. Clinical Trials of IL-12/IL-23 inhibitors in inflammatory bowel disease. *BioDrugs.* (2020) 34:713–721. doi: 10.1007/s40259-020-00451-w
85. Rutgeerts P, Gasink C, Chan D, Lang Y, Pollack P, Colombel JF, et al. Efficacy of ustekinumab for inducing endoscopic healing in patients with Crohn's disease. *Gastroenterology.* (2018) 155:1045–1058. doi: 10.1053/j.gastro.2018.06.035
86. Feagan BG, Sandborn WJ, Gasink C, Jacobstein D, Lang Y, Friedman JR, et al. Ustekinumab as induction and maintenance therapy for crohn's disease. *N Engl J Med.* (2016) 375:1946–60. doi: 10.1056/NEJMoa1602773
87. Sandborn WJ, Ferrante M, Bhandari BR, Berliba E, Feagan BG, Hibi T, et al. Efficacy and safety of mirikizumab in a randomized phase 2 study of patients with ulcerative colitis. *Gastroenterology.* (2020) 158:537–49.e10. doi: 10.1053/j.gastro.2019.08.043
88. Feagan BG, Sandborn WJ, D'Haens G, Panés J, Kaser A, Ferrante M, et al. Induction therapy with the selective interleukin-23 inhibitor risankizumab in patients with moderate-to-severe Crohn's disease: a randomised, double-blind, placebo-controlled phase 2 study. *Lancet.* (2017) 389:1699–709. doi: 10.1016/S0140-6736(17)30570-6
89. Feagan BG, Panés J, Ferrante M, Kaser A, D'Haens GR, Sandborn WJ, et al. Risankizumab in patients with moderate to severe Crohn's disease: an open-label extension study. *Lancet Gastroenterol Hepatol.* (2018) 3:671–80. doi: 10.1016/S2468-1253(18)30233-4
90. Wong U, Cross RK. Expert opinion on interleukin-12/23 and interleukin-23 antagonists as potential therapeutic options for the treatment of inflammatory bowel disease. *Expert Opin Investig Drugs.* (2019) 28:473–9. doi: 10.1080/13543784.2019.1597053
91. Wyant T, Fedyk E, Abhyankar B. An overview of the mechanism of action of the monoclonal antibody vedolizumab. *J Crohns Colitis.* (2016) 10:1437–44. doi: 10.1093/ecco-jcc/jjw092
92. Fiorino G, Gilardi D, Danese S. The clinical potential of etrolizumab in ulcerative colitis: hypes and hopes. *Therap Adv Gastroenterol.* (2016) 9:503–12. doi: 10.1177/1756283X16647935
93. Tang MT, Keir ME, Erickson R, Stefanich EG, Fuh FK, Ramirez-Montagut T, et al. Review article: nonclinical and clinical pharmacology, pharmacokinetics and pharmacodynamics of etrolizumab, an anti-β7 integrin therapy for inflammatory bowel disease. *Aliment Pharmacol Ther.* (2018) 47:1440–52. doi: 10.1111/apt.14631
94. Feagan BG, Rutgeerts P, Sands BE, Hanauer S, Colombel JF, Sandborn WJ, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* (2013) 369:699–710. doi: 10.1056/NEJMoa1215734
95. Sandborn WJ, Feagan BG, Rutgeerts P, Hanauer S, Colombel JF, Sands BE, et al. Vedolizumab as induction and maintenance therapy for Crohn's disease. *N Engl J Med.* (2013) 369:711–21. doi: 10.1056/NEJMoa1215739
96. Colombel JF, Sands BE, Rutgeerts P, Sandborn W, Danese S, D'Haens G, et al. The safety of vedolizumab for ulcerative colitis and Crohn's disease. *Gut.* (2017) 66:839–51. doi: 10.1136/gutjnl-2015-311079
97. Scribano ML. Vedolizumab for inflammatory bowel disease: from randomized controlled trials to real-life evidence. *World J Gastroenterol.* (2018) 24:2457–67. doi: 10.3748/wjg.v24.i23.2457
98. Christensen B, Gibson PR, Micic D, Colman RJ, Goeppinger SR, Kassim O, et al. Safety and efficacy of combination treatment with calcineurin inhibitors and vedolizumab in patients with refractory inflammatory bowel disease. *Clin Gastroenterol Hepatol.* (2019) 17:486–93. doi: 10.1016/j.cgh.2018.04.060
99. Pellet G, Stefanescu C, Carbonnel F, Peyrin-Biroulet L, Roblin X, Allimant C, et al. Efficacy and safety of induction therapy with calcineurin inhibitors in combination with vedolizumab in patients with refractory ulcerative colitis. *Clin Gastroenterol Hepatol.* (2019) 17:494–501. doi: 10.1016/j.cgh.2018.08.081
100. Vermeire S, O'Byrne S, Keir M, Williams M, Lu TT, Mansfield JC, et al. Etrolizumab as induction therapy for ulcerative colitis: a randomised, controlled, phase 2 trial. *Lancet.* (2014) 384:309–18. doi: 10.1016/S0140-6736(14)60661-9
101. Sandborn WJ, Vermeire S, Tyrrell H, Hassanali A, Lacey S, Tole S, et al. Etrolizumab for the treatment of ulcerative colitis and Crohn's disease: an overview of the phase 3 clinical program. *Adv Ther.* (2020) 37:3417–31. doi: 10.1007/s12325-020-01366-2
102. Yoshimura N, Watanabe M, Motoya S, Tominaga K, Matsuoka K, Iwakiri R, et al. Safety and efficacy of AJM300, an oral antagonist of α4 integrin, in induction therapy for patients with active ulcerative colitis. *Gastroenterology.* (2015) 149:1775–83.e2. doi: 10.1053/j.gastro.2015.08.044
103. Vermeire S, Sandborn WJ, Danese S, Hébuterne X, Salzberg BA, Klopocka M, et al. Anti-MAdCAM antibody (PF-00547659) for ulcerative colitis (TURANDOT): a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet.* (2017) 390:135–44. doi: 10.1016/S0140-6736(17)30930-3
104. Gubatan J, Keyashian K, Rubin SJS, Wang J, Buckman CA, Sinha S. Anti-Integrins for the treatment of inflammatory bowel disease: current evidence and perspectives. *Clin Exp Gastroenterol.* (2021) 14:333–42. doi: 10.2147/CEG.S293272
105. Sandborn WJ, Su C, Sands BE, D'Haens GR, Vermeire S, Schreiber S, et al. Tofacitinib as induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* (2017) 376:1723–36. doi: 10.1056/NEJMoa1606910
106. Vermeire S, Schreiber S, Petryka R, Kuehbacher T, Hebuterne X, Roblin X, et al. Clinical remission in patients with moderate-to-severe Crohn's disease treated with filgotinib (the FITZROY study): results from a phase 2, double-blind, randomised, placebo-controlled trial. *Lancet.* (2017) 389:266–75. doi: 10.1016/S0140-6736(16)32537-5
107. Sandborn WJ, Ghosh S, Panes J, Schreiber S, D'Haens G, Tanida S, et al. Efficacy of upadacitinib in a randomized trial of patients with active ulcerative colitis. *Gastroenterology.* (2020) 158:2139–49.e14. doi: 10.1053/j.gastro.2020.02.030
108. Sandborn WJ, Feagan BG, Wolf DC, D'Haens G, Vermeire S, Hanauer SB, et al. Ozanimod induction and maintenance treatment for ulcerative colitis. *N Engl J Med.* (2016) 374:1754–62. doi: 10.1056/NEJMoa1513248
109. Sandborn WJ, Feagan BG, D'Haens G, Wolf DC, Jovanovic I, Hanauer SB, et al. Ozanimod as induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* (2021) 385:1280–91. doi: 10.1056/NEJMoa2033617
110. Feagan BG, Sandborn WJ, Danese S, Wolf DC, Liu WJ, Hua SY, et al. Ozanimod induction therapy for patients with moderate to severe Crohn's disease: a single-arm, phase 2, prospective observer-blinded endpoint study. *Lancet Gastroenterol Hepatol.* (2020) 5:819–28. doi: 10.1016/S2468-1253(20)30188-6
111. Sandborn WJ, Peyrin-Biroulet L, Zhang J, Chiorean M, Vermeire S, Lee SD, et al. Efficacy and safety of etrasimod in a phase 2 randomized trial of patients with ulcerative colitis. *Gastroenterology.* (2020) 158:550–61. doi: 10.1053/j.gastro.2019.10.035
112. Ghoreschi K, Laurence A, O'Shea JJ. Janus kinases in immune cell signaling. *Immunol Rev.* (2009) 228:273–87. doi: 10.1111/j.1600-065X.2008.00754.x

113. Tran V, Shammas RM, Sauk JS, Padua D. Evaluating tofacitinib citrate in the treatment of moderate-to-severe active ulcerative colitis: design, development and positioning of therapy. *Clin Exp Gastroenterol.* (2019) 12:179–91. doi: 10.2147/CEG.S150908
114. Chaparro M, Garre A, Mesonero F, Rodríguez C, Barreiro-de Acosta M, Martínez-Cadilla J, et al. Tofacitinib in ulcerative colitis: real-world evidence from the ENEIDA registry. *J Crohns Colitis.* (2021) 15:35–42. doi: 10.1093/ecco-jcc/jjaa145
115. Honap S, Pavlidis P, Ray S, Sharma E, Anderson S, Sanderson JD, et al. Tofacitinib in acute severe ulcerative colitis-a real-world tertiary center experience. *Inflamm Bowel Dis.* (2020) 26:e147–9. doi: 10.1093/ibd/izaa157
116. Danese S, Peyrin-Biroulet L. Selective tyrosine kinase 2 inhibition for treatment of inflammatory bowel disease: new hope on the rise. *Inflamm Bowel Dis.* (2021) 27:1694–53. doi: 10.1093/ibd/izab135
117. De Vries LCS, Ghiboub M, van Hamersveld PHP, Welting O, Verseijden C, Bell MJ, et al. Tyrosine kinase 2 signalling drives pathogenic T cells in colitis. *J Crohns Colitis.* (2021) 15:617–30. doi: 10.1093/ecco-jcc/jjaa199
118. Lowe SC, Sauk JS, Limketkai BN, Kwaan MR. Declining rates of surgery for inflammatory bowel disease in the era of biologic therapy. *J Gastrointest Surg.* (2021) 25:211–9. doi: 10.1007/s11605-020-04832-y
119. Justiniano CF, Aquina CT, Becerra AZ, Xu Z, Boodry CI, Swanger AA, et al. Postoperative mortality after nonelective surgery for inflammatory bowel disease patients in the era of biologics. *Ann Surg.* (2019) 269:686–91. doi: 10.1097/SLA.0000000000002628
120. Lamb CA, Kennedy NA, Raine T, Hendy PA, Smith PJ, Limdi JK, et al. British society of gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut.* (2019) 68 (Suppl. 3):s1–106. doi: 10.1136/gutjnl-2019-318484
121. Rutgeerts P, Geboes K, Vantrappen G, Beyls J, Kerremans R, Hiele M. Predictability of the postoperative course of Crohn's disease. *Gastroenterology.* (1990) 99:956–63. doi: 10.1016/0016-5085(90)90613-6
122. Naganuma M, Yokoyama Y, Motoya S, Watanabe K, Sawada K, Hirai F, et al. Efficacy of apheresis as maintenance therapy for patients with ulcerative colitis in an open-label prospective multicenter randomised controlled trial. *J Gastroenterol.* (2020) 55:390–400. doi: 10.1007/s00535-019-01651-0
123. Yoshino T, Nakase H, Minami N, Yamada S, Matsuura M, Yazumi S, et al. Efficacy and safety of granulocyte and monocyte adsorption apheresis for ulcerative colitis: a meta-analysis. *Dig Liver Dis.* (2014) 46:219–26. doi: 10.1016/j.dld.2013.10.011
124. Lai YM, Yao WY, He Y, Jiang X, Gu YB, Chen MH, et al. Adsorptive granulocyte and monocyte apheresis in the treatment of ulcerative colitis: the first multicenter study in China. *Gut Liver.* (2017) 11:216–25. doi: 10.5009/gnl15408
125. Motoya S, Tanaka H, Shibuya T, Osada T, Yamamoto T, Hongo H, et al. Safety and effectiveness of granulocyte and monocyte adsorptive apheresis in patients with inflammatory bowel disease in special situations: a multicentre cohort study. *BMC Gastroenterol.* (2019) 19:196. doi: 10.1186/s12876-019-1110-1
126. Li N, Mao J, Tang H, Zhu L, Tan X, Bi J, et al. Efficacy and safety of adsorptive granulomonocytapheresis in Chinese patients with ulcerative colitis: a retrospective analysis of 50 cases with focus on factors impacting clinical efficacy. *J Clin Apher.* (2020) 35:271–80. doi: 10.1002/jca.21787
127. Fukuchi T, Nakase H, Ubukata S, Matsuura M, Yoshino T, Toyonaga T, et al. Therapeutic effect of intensive granulocyte and monocyte adsorption apheresis combined with thiopurines for steroid- and biologics-naïve Japanese patients with early-diagnosed Crohn's disease. *BMC Gastroenterol.* (2014) 13:124. doi: 10.1186/1471-230X-14-124
128. Rodríguez-Lago I, Benítez JM, García-Sánchez V, Gutiérrez A, Sempere L, Ginard D, et al. Granulocyte and monocyte apheresis in inflammatory bowel disease: the patients' point of view. *Gastroenterol Hepatol.* (2018) 41:423–31. doi: 10.1016/j.gastrohep.2018.04.007
129. Ocansey DKW, Wang L, Wang J, Yan Y, Qian H, Zhang X, et al. Mesenchymal stem cell-gut microbiota interaction in the repair of inflammatory bowel disease: an enhanced therapeutic effect. *Clin Transl Med.* (2019) 8:31. doi: 10.1186/s40169-019-0251-8
130. Ott SJ, Musfeldt M, Wenderoth DF, Hampe J, Brant O, Fölsch UR, et al. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut.* (2004) 53:685–93. doi: 10.1136/gut.2003.025403
131. Khan KJ, Ullman TA, Ford AC, Abreu MT, Abadir A, Marshall JK, et al. Antibiotic therapy in inflammatory bowel disease: a systematic review and meta-analysis. *Am J Gastroenterol.* (2011) 106:661–73. doi: 10.1038/ajg.2011.72
132. Uchino M, Ikeuchi H, Bando T, Chohno T, Sasaki H, Horio Y, et al. Efficacy of preoperative oral antibiotic prophylaxis for the prevention of surgical site infections in patients with Crohn disease: a randomized controlled trial. *Ann Surg.* (2019) 269:420–6. doi: 10.1097/SLA.0000000000002567
133. Gomollón F, Dignass A, Annese V, Tilg H, Van Assche G, Lindsay JO III, et al. European evidence-based consensus on the diagnosis and management of Crohn's disease 2016: Part 1: diagnosis and medical management. *J Crohns Colitis.* (2017) 11:3–25. doi: 10.1093/ecco-jcc/jjw168
134. Townsend CM, Parker CE, MacDonald JK, Nguyen TM, Jairath V, Feagan BG, et al. Antibiotics for induction and maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev.* (2019) 2:Cd012730. doi: 10.1002/14651858.CD012730.pub2
135. Harbord M, Eliakim R, Bettenworth D, Karmiris K, Katsanos K, Kopylov U, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 2: current management. *J Crohns Colitis.* (2017) 11:769–84. doi: 10.1093/ecco-jcc/jjx009
136. Nguyen LH, Örtqvist AK, Cao Y, Simon TG, Roelstraete B, Song M, et al. Antibiotic use and the development of inflammatory bowel disease: a national case-control study in Sweden. *Lancet Gastroenterol Hepatol.* (2020) 5:986–95. doi: 10.1016/S2468-1253(20)30267-3
137. Shaw SY, Blanchard JF, Bernstein CN. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. *Am J Gastroenterol.* (2010) 105:2687–92. doi: 10.1038/ajg.2010.398
138. Balram B, Battat R, Al-Khoury A, D'Aoust J, Afif W, Bitton A, et al. Risk factors associated with clostridium difficile infection in inflammatory bowel disease: a systematic review and meta-analysis. *J Crohns Colitis.* (2019) 13:27–38. doi: 10.1093/ecco-jcc/jjy143
139. Yan F, Cao H, Cover TL, Washington MK, Shi Y, Liu L, et al. Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGFR-dependent mechanism. *J Clin Invest.* (2011) 121:2242–53. doi: 10.1172/JCI44031
140. Zhang XF, Guan XX, Tang YJ, Sun JF, Wang XK, Wang WD, et al. Clinical effects and gut microbiota changes of using probiotics, prebiotics or synbiotics in inflammatory bowel disease: a systematic review and meta-analysis. *Eur J Nutr.* (2021) 60:2855–75. doi: 10.1007/s00394-021-02503-5
141. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: the international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol.* (2017) 14:491–502. doi: 10.1038/nrgastro.2017.75
142. Angelino D, Martina A, Rosi A, Veronesi L, Antonini M, Mennella I, et al. Glucose- and lipid-related biomarkers are affected in healthy obese or hyperglycemic adults consuming a whole-grain pasta enriched in prebiotics and probiotics: a 12-week randomized controlled trial. *J Nutr.* (2019) 149:1714–23. doi: 10.1093/jn/nxz071
143. Yoshimatsu Y, Yamada A, Furukawa R, Sono K, Osamura A, Nakamura K, et al. Effectiveness of probiotic therapy for the prevention of relapse in patients with inactive ulcerative colitis. *World J Gastroenterol.* (2015) 21:5985–94. doi: 10.3748/wjg.v21.i19.5985
144. Sheng K, He S, Sun M, Zhang G, Kong X, Wang J, et al. Synbiotic supplementation containing bifidobacterium infantis and xylooligosaccharides alleviates dextran sulfate sodium-induced ulcerative colitis. *Food Funct.* (2020) 11:3964–74. doi: 10.1039/D0FO00518E
145. Banfi D, Moro E, Bosi A, Bistoletti M, Cerantola S, Crema F, et al. Impact of microbial metabolites on microbiota-gut-brain axis in inflammatory bowel disease. *Int J Mol Sci.* (2021) 22. doi: 10.3390/ijms22041623
146. Ganji-Arjenaki M, Rafieian-Kopaei M. Probiotics are a good choice in remission of inflammatory bowel diseases: a meta analysis and systematic review. *J Cell Physiol.* (2018) 233:2091–103. doi: 10.1002/jcp.25911
147. Mardini HE, Grigorian AY. Probiotic mix VSL#3 is effective adjunctive therapy for mild to moderately active ulcerative colitis: a meta-analysis. *Inflamm Bowel Dis.* (2014) 20:1562–7. doi: 10.1097/MIB.0000000000000084

148. Yilmaz I, Dolar ME, Özpinar H. Effect of administering kefir on the changes in fecal microbiota and symptoms of inflammatory bowel disease: a randomized controlled trial. *Turk J Gastroenterol.* (2019) 30:242–53. doi: 10.5152/tjg.2018.18227
149. Kaur L, Gordon M, Baines PA, Iheozor-Ejiofor Z, Sinopoulou V, Akobeng AK. Probiotics for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev.* (2020) 3:Cd005573. doi: 10.1002/14651858.CD005573.pub3
150. Pabón-Carrasco M, Ramirez-Baena L, Vilar-Palomo S, Castro-Méndez A, Martos-García R, Rodríguez-Gallego I. Probiotics as a coadjuvant factor in active or quiescent inflammatory bowel disease of adults-a meta-analytical study. *Nutrients.* (2020) 12:2628. doi: 10.3390/nu12092628
151. Derwa Y, Gracie DJ, Hamlin PJ, Ford AC. Systematic review with meta-analysis: the efficacy of probiotics in inflammatory bowel disease. *Aliment Pharmacol Ther.* (2017) 46:389–400. doi: 10.1111/apt.14203
152. Bjarnason I, Sission G, Hayee B. A randomised, double-blind, placebo-controlled trial of a multi-strain probiotic in patients with asymptomatic ulcerative colitis and Crohn's disease. *Inflammopharmacology.* (2019) 27:465–73. doi: 10.1007/s10787-019-00595-4
153. Limketkai BN, Akobeng AK, Gordon M, Adepoju AA. Probiotics for induction of remission in Crohn's disease. *Cochrane Database Syst Rev.* (2020) 7:Cd006634. doi: 10.1002/14651858.CD006634.pub3
154. Bourreille A, Cadiot G, Le Dreau G, Laharie D, Beaugerie L, Dupas JL, et al. Saccharomyces boulardii does not prevent relapse of Crohn's disease. *Clin Gastroenterol Hepatol.* (2013) 11:982–7. doi: 10.1016/j.cgh.2013.02.021
155. Dore MP, Bibbò S, Fresi G, Bassotti G, Pes GM. Side effects associated with probiotic use in adult patients with inflammatory bowel disease: a systematic review and meta-analysis of randomized controlled trials. *Nutrients.* (2019) 11:2913. doi: 10.3390/nu11122913
156. Aguilar-Toalá JE, Garcia-Varela R, Garcia HS, Mata-Haro V, González-Córdova AF, Vallejo-Cordoba B, et al. Postbiotics: an evolving term within the functional foods field. *Trends Food Sci Technol.* (2018) 75:105–14. doi: 10.1016/j.tifs.2018.03.009
157. Tsilingiri K, Barbosa T, Penna G, Caprioli F, Sonzogni A, Viale G, et al. Probiotic and postbiotic activity in health and disease: comparison on a novel polarised *ex-vivo* organ culture model. *Gut.* (2012) 61:1007–15. doi: 10.1136/gutjnl-2011-300971
158. Quraishi MN, Widlak M, Bhala N, Moore D, Price M, Sharma N, et al. Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory clostridium difficile infection. *Aliment Pharmacol Ther.* (2017) 46:479–93. doi: 10.1111/apt.14201
159. Haifer C, Kelly CR, Paramsothy S, Andresen D, Papanicolas LE, McKew GL, et al. Australian consensus statements for the regulation, production and use of faecal microbiota transplantation in clinical practice. *Gut.* (2020) 69:801–10. doi: 10.1136/gutjnl-2019-320260
160. Kahn SA, Vachon A, Rodriquez D, Goeppinger SR, Surma B, Marks J, et al. Patient perceptions of fecal microbiota transplantation for ulcerative colitis. *Inflamm Bowel Dis.* (2013) 19:1506–13. doi: 10.1097/MIB.0b013e318281f520
161. Moayyedi P, Surette MG, Kim PT, Libertucci J, Wolfe M, Onischi C, et al. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology.* (2015) 149:102–9.e6. doi: 10.1053/j.gastro.2015.04.001
162. Paramsothy S, Paramsothy R, Rubin DT, Kamm MA, Kaakoush NO, Mitchell HM, et al. Faecal microbiota transplantation for inflammatory bowel disease: a systematic review and meta-analysis. *J Crohns Colitis.* (2017) 11:1180–99. doi: 10.1093/ecco-jcc/jjx063
163. Vermeire S, Joossens M, Verbeke K, Wang J, Machiels K, Sabino J, et al. Donor species richness determines faecal microbiota transplantation success in inflammatory bowel disease. *J Crohns Colitis.* (2016) 10:387–94. doi: 10.1093/ecco-jcc/jjv203
164. Paramsothy S, Nielsen S, Kamm MA, Deshpande NP, Faith JJ, Clemente JC, et al. Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. *Gastroenterology.* (2019) 156:1440–54.e2. doi: 10.1053/j.gastro.2018.12.001
165. Paramsothy S, Kamm MA, Kaakoush NO, Walsh AJ, van den Bogaerde J, Samuel D, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet.* (2017) 389:1218–28. doi: 10.1016/S0140-6736(17)30182-4
166. Costello SP, Hughes PA, Waters O, Bryant RV, Vincent AD, Blatchford P, et al. Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: a randomized clinical trial. *JAMA.* (2019) 321:156–64. doi: 10.1001/jama.2018.20046
167. Staley C, Hamilton MJ, Vaughn BP, Graiziger CT, Newman KM, Kabage AJ, et al. Successful resolution of recurrent clostridium difficile infection using freeze-dried, encapsulated fecal microbiota; pragmatic cohort study. *Am J Gastroenterol.* (2017) 112:940–7. doi: 10.1038/ajg.2017.6
168. Youngster I, Russell GH, Pindar C, Ziv-Baran T, Sauk J, Hohmann EL. Oral, capsulized, frozen fecal microbiota transplantation for relapsing clostridium difficile infection. *JAMA.* (2014) 312:1772–8. doi: 10.1001/jama.2014.13875
169. Kao D, Roach B, Silva M, Beck P, Rioux K, Kaplan GG, et al. Effect of oral capsule- vs colonoscopy-delivered fecal microbiota transplantation on recurrent clostridium difficile infection: a randomized clinical trial. *JAMA.* (2017) 318:1985–93. doi: 10.1001/jama.2017.17077
170. Keshteli AH, Millan B, Madsen KL. Pretreatment with antibiotics may enhance the efficacy of fecal microbiota transplantation in ulcerative colitis: a meta-analysis. *Mucosal Immunol.* (2017) 10:565–6. doi: 10.1038/mi.2016.123
171. Rossen NG, Fuentes S, van der Spek MJ, Tijssen JG, Hartman JH, Duflou A, et al. Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. *Gastroenterology.* (2015) 149:110–8.e4. doi: 10.1053/j.gastro.2015.03.045
172. Haifer C, Leong RW, Paramsothy S. The role of faecal microbiota transplantation in the treatment of inflammatory bowel disease. *Curr Opin Pharmacol.* (2020) 55:8–16. doi: 10.1016/j.coph.2020.08.009
173. Jin J. Stem cell treatments. *JAMA.* (2017) 317:330. doi: 10.1001/jama.2016.17822
174. Kavanagh DP, Kalia N. Hematopoietic stem cell homing to injured tissues. *Stem Cell Rev Rep.* (2011) 7:672–82. doi: 10.1007/s12015-011-9240-z
175. Clevers H, Loh KM, Nusse R. Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. *Science.* (2014) 346:1248012. doi: 10.1126/science.1248012
176. Boussen I, Sokol H, Aractingi S, Georges O, Hoyeau-Idrissi N, Hugot JP, et al. Inflammatory bowel disease after allogeneic stem cell transplantation. *Bone Marrow Transplant.* (2015) 50:1365–6. doi: 10.1038/bmt.2015.138
177. Sonwalkar SA, James RM, Ahmad T, Zhang L, Verbeke CS, Barnard DL, et al. Fulminant crohn's colitis after allogeneic stem cell transplantation. *Gut.* (2003) 52:1518–21. doi: 10.1136/gut.52.10.1518
178. Nagaishi K, Arimura Y, Fujimiya M. Stem cell therapy for inflammatory bowel disease. *J Gastroenterol.* (2015) 50:280–6. doi: 10.1007/s00535-015-1040-9
179. Burt RK, Traynor A, Oyama Y, Craig R. High-dose immune suppression and autologous hematopoietic stem cell transplantation in refractory Crohn disease. *Blood.* (2003) 101:2064–6. doi: 10.1182/blood-2002-07-2122
180. Brierley CK, Castilla-Llorente C, Labopin M, Badoglio M, Rovira M, Ricart E, et al. Autologous haematopoietic stem cell transplantation for crohn's disease: a retrospective survey of long-term outcomes from the european society for blood and marrow transplantation. *J Crohns Colitis.* (2018) 12:1097–103. doi: 10.1093/ecco-jcc/jjy069
181. Lindsay JO, Allez M, Clark M, Labopin M, Ricart E, Rogler G, et al. Autologous stem-cell transplantation in treatment-refractory Crohn's disease: an analysis of pooled data from the ASTIC trial. *Lancet Gastroenterol Hepatol.* (2017) 2:399–406. doi: 10.1016/S2468-1253(17)30056-0
182. Snowden JA, Ansari A, Sachchithanantham S, Jackson G, Thompson N, Lobo A, et al. Autologous stem cell transplantation in severe treatment-resistant Crohn's disease: long-term follow-up of UK patients treated on compassionate basis. *QJM.* (2014) 107:871–7. doi: 10.1093/qjmed/hcu095
183. López-García A, Rovira M, Jauregui-Amezaga A, Marín P, Barastegui R, Salas A, et al. Autologous haematopoietic stem cell transplantation for refractory crohn's disease: efficacy in a single-centre cohort. *J Crohns Colitis.* (2017) 11:1161–68. doi: 10.1093/ecco-jcc/jjx054
184. Hawkey CJ, Allez M, Clark MM, Labopin M, Lindsay JO, Ricart E, et al. Autologous hematopoetic stem cell transplantation for refractory crohn disease: a randomized clinical trial. *JAMA.* (2015) 314:2524–34. doi: 10.1001/jama.2015.16700

185. Pockley AG, Lindsay JO, Foulds GA, Rutella S, Gribben JG, Alexander T, et al. Immune reconstitution after autologous hematopoietic stem cell transplantation in crohn's disease: current status and future directions. A review on behalf of the EBMT autoimmune diseases working party and the autologous stem cell transplantation in refractory CD-low intensity therapy evaluation study investigators. *Front Immunol.* (2018) 9:646. doi: 10.3389/fimmu.2018.00646
186. Ruiz MA, Kaiser RL, Jr., de Quadros LG, Piron-Ruiz L, Peña-Arciniegas T, et al. Low toxicity and favorable clinical and quality of life impact after non-myeloablative autologous hematopoietic stem cell transplant in Crohn's disease. *BMC Res Notes.* (2017) 10:495. doi: 10.1186/s13104-017-2824-1
187. Mishra R, Dhawan P, Srivastava AS, Singh AB. Inflammatory bowel disease: therapeutic limitations and prospective of the stem cell therapy. *World J Stem Cells.* (2020) 12:1050–1066. doi: 10.4252/wjsc.v12.i10.1050
188. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. *Cytotherapy.* (2006) 8:315–7. doi: 10.1080/14653240600855905
189. Mohamed-Ahmed S, Fristad I, Lie SA, Suliman S, Mustafa K, Vindenes H, et al. Adipose-derived and bone marrow mesenchymal stem cells: a donor-matched comparison. *Stem Cell Res Ther.* (2018) 9:168. doi: 10.1186/s13287-018-0914-1
190. Nauta AJ, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. *Blood.* (2007) 110:3499–506. doi: 10.1182/blood-2007-02-069716
191. Duijvestein M, Vos AC, Roelofs H, Wildenberg ME, Wendrich BB, Verspaget HW, et al. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. *Gut.* (2010) 59:1662–9. doi: 10.1136/gut.2010.215152
192. Forbes GM, Sturm MJ, Leong RW, Sparrow MP, Segarajasingam D, Cummins AG, et al. A phase 2 study of allogeneic mesenchymal stromal cells for luminal Crohn's disease refractory to biologic therapy. *Clin Gastroenterol Hepatol.* (2014) 12:64–71. doi: 10.1016/j.cgh.2013.06.021
193. Dietz AB, Dozois EJ, Fletcher JG, Butler GW, Radel D, Lightner AL, et al. Autologous mesenchymal stem cells, applied in a bioabsorbable matrix, for treatment of perianal fistulas in patients with Crohn's disease. *Gastroenterology.* (2017) 153:59–62.e2. doi: 10.1053/j.gastro.2017.04.001
194. Herreros MD, Garcia-Arranz M, Guadalajara H, De-La-Quintana P, Garcia-Olmo D. Autologous expanded adipose-derived stem cells for the treatment of complex cryptoglandular perianal fistulas: a phase III randomized clinical trial (FATT 1: fistula advanced therapy trial 1) and long-term evaluation. *Dis Colon Rectum.* (2012) 55:762–72. doi: 10.1097/DCR.0b013e318255364a
195. Cao Y, Su Q, Zhang B, Shen F, Li S. Efficacy of stem cells therapy for Crohn's fistula: a meta-analysis and systematic review. *Stem Cell Res Ther.* (2021) 12:32. doi: 10.1186/s13287-020-02095-7
196. González MA, Gonzalez-Rey E, Rico L, Büscher D, Delgado M. Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. *Gastroenterology.* (2009) 136:978–89. doi: 10.1053/j.gastro.2008.11.041
197. Gonzalez-Rey E, Anderson P, González MA, Rico L, Büscher D, Delgado M. Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. *Gut.* (2009) 58:929–39. doi: 10.1136/gut.2008.168534
198. Planat-Benard V, Varin A, Casteilla L. MSCs and inflammatory cells crosstalk in regenerative medicine: concerted actions for optimized resolution driven by energy metabolism. *Front Immunol.* (2021) 12:626755. doi: 10.3389/fimmu.2021.626755
199. Watanabe S, Arimura Y, Nagaishi K, Isshiki H, Onodera K, Nasuno M, et al. Conditioned mesenchymal stem cells produce pleiotropic gut trophic factors. *J Gastroenterol.* (2014) 49:270–82. doi: 10.1007/s00535-013-0901-3
200. Sala E, Genua M, Petti L, Anselmo A, Arena V, Cibella J, et al. Mesenchymal stem cells reduce colitis in mice via release of TSG6, independently of their localization to the intestine. *Gastroenterology.* (2015) 149:163–76.e20. doi: 10.1053/j.gastro.2015.03.013
201. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology.* (2011) 141:1762–72. doi: 10.1053/j.gastro.2011.07.050
202. Sato T, Clevers H. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. *Science.* (2013) 340:1190–4. doi: 10.1126/science.1234852
203. VanDussen KL, Marinshaw JM, Shaikh N, Miyoshi H, Moon C, Tarr PI, et al. Development of an enhanced human gastrointestinal epithelial culture system to facilitate patient-based assays. *Gut.* (2015) 64:911–20. doi: 10.1136/gutjnl-2013-306651
204. Middendorp S, Schneeberger K, Wiegerinck CL, Mokry M, Akkerman RD, van Wijngaarden S, et al. Adult stem cells in the small intestine are intrinsically programmed with their location-specific function. *Stem Cells.* (2014) 32:1083–91. doi: 10.1002/stem.1655
205. Yui S, Nakamura T, Sato T, Nemoto Y, Mizutani T, Zheng X, et al. Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5? stem cell. *Nat Med.* (2012) 18:618–23. doi: 10.1038/nm.2695
206. Xu AT, Lu JT, Ran ZH, Zheng Q. Exosome in intestinal mucosal immunity. *J Gastroenterol Hepatol.* (2016) 31:1694–99. doi: 10.1111/jgh.13413
207. Cai Z, Zhang W, Yang F, Yu L, Yu Z, Pan J, et al. Immunosuppressive exosomes from TGF-β1 gene-modified dendritic cells attenuate Th17-mediated inflammatory autoimmune disease by inducing regulatory T cells. *Cell Res.* (2012) 22:607–10. doi: 10.1038/cr.2011.196
208. Zhang H, Wang L, Li C, Yu Y, Yi Y, Wang J, et al. Exosome-induced regulation in inflammatory bowel disease. *Front Immunol.* (2019) 10:1464. doi: 10.3389/fimmu.2019.01464
209. Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. *Nat Rev Immunol.* (2014) 14:195–208. doi: 10.1038/nri3622
210. Mao F, Wu Y, Tang X, Kang J, Zhang B, Yan Y, et al. Exosomes derived from human umbilical cord mesenchymal stem cells relieve inflammatory bowel disease in mice. *Biomed Res Int.* (2017) 2017:5356760. doi: 10.1155/2017/5356760
211. Liu H, Liang Z, Wang F, Zhou C, Zheng X, Hu T, et al. Exosomes from mesenchymal stromal cells reduce murine colonic inflammation via a macrophage-dependent mechanism. *JCI Insight.* (2019) 4:e31273. doi: 10.1172/jci.insight.131273
212. Yang X, Meng S, Jiang H, Chen T, Wu W. Exosomes derived from interleukin-10-treated dendritic cells can inhibit trinitrobenzene sulfonic acid-induced rat colitis. *Scand J Gastroenterol.* (2010) 45:1168–77. doi: 10.3109/00365521.2010.490596
213. Baranyai T, Herczeg K, Onódi Z, Voszka I, Módos K, Marton N, et al. Isolation of exosomes from blood plasma: qualitative and quantitative comparison of ultracentrifugation and size exclusion chromatography methods. *PLoS ONE.* (2015) 10:e0145686. doi: 10.1371/journal.pone.0145686
214. Xiao GY, Cheng CC, Chiang YS, Cheng WT, Liu IH, Wu SC. Exosomal miR-10a derived from amniotic fluid stem cells preserves ovarian follicles after chemotherapy. *Sci Rep.* (2016) 6:23120. doi: 10.1038/srep23120
215. Street JM, Koritzinsky EH, Glispie DM, Yuen PST. Urine exosome isolation and characterization. *Methods Mol Biol.* (2017) 1641:413–23. doi: 10.1007/978-1-4939-7172-5_23
216. Hock A, Miyake H, Li B, Lee C, Ermini L, Koike Y, et al. Breast milk-derived exosomes promote intestinal epithelial cell growth. *J Pediatr Surg.* (2017) 52:755–9. doi: 10.1016/j.jpedsurg.2017.01.032
217. Xiao J, Feng S, Wang X, Long K, Luo Y, Wang Y, et al. Identification of exosome-like nanoparticle-derived microRNAs from 11 edible fruits and vegetables. *PeerJ.* (2018) 6:e5186. doi: 10.7717/peerj.5186
218. Zhao Z, Yu S, Li M, Gui X, Li P. Isolation of exosome-like nanoparticles and analysis of MicroRNAs derived from coconut water based on small RNA high-throughput sequencing. *J Agric Food Chem.* (2018) 66:2749–57. doi: 10.1021/acs.jafc.7b05614
219. Munagala R, Aqil F, Jeyabalan J, Gupta RC. Bovine milk-derived exosomes for drug delivery. *Cancer Lett.* (2016) 371:48–61. doi: 10.1016/j.canlet.2015.10.020
220. Eichenberger RM, Talukder MH, Field MA, Wangchuk P, Giacomin P, Loukas A, et al. Characterization of trichuris muris secreted proteins and extracellular vesicles provides new insights into host-parasite communication. *J Extracell Vesicles.* (2018) 7:1428004. doi: 10.1080/20013078.2018.1428004
221. Eichenberger RM, Ryan S, Jones L, Buitrago G, Polster R, Montes de Oca M, et al. Hookworm secreted extracellular vesicles interact with

host cells and prevent inducible colitis in mice. *Front Immunol.* (2018) 9:850. doi: 10.3389/fimmu.2018.00850
222. Leoni G, Neumann PA, Kamaly N, Quiros M, Nishio H, Jones HR, et al. Annexin A1-containing extracellular vesicles and polymeric nanoparticles promote epithelial wound repair. *J Clin Invest.* (2015) 125:1215–27. doi: 10.1172/JCI76693
223. Samborski P, Grzymisławski M. The role of HSP70 heat shock proteins in the pathogenesis and treatment of inflammatory bowel diseases. *Adv Clin Exp Med.* (2015) 24:525–30. doi: 10.17219/acem/44144
224. Reddy VS, Madala SK, Trinath J, Reddy GB. Extracellular small heat shock proteins: exosomal biogenesis and function. *Cell Stress Chaperones.* (2018) 23:441–54. doi: 10.1007/s12192-017-0856-z
225. Robertson C, Booth SA, Beniac DR, Coulthart MB, Booth TF, McNicol A. Cellular prion protein is released on exosomes from activated platelets. *Blood.* (2006) 107:3907–11. doi: 10.1182/blood-2005-02-0802
226. Petit CS, Barreau F, Besnier L, Gandille P, Riveau B, Chateau D, et al. Requirement of cellular prion protein for intestinal barrier function and mislocalization in patients with inflammatory bowel disease. *Gastroenterology.* (2012) 143:122–32.e15. doi: 10.1053/j.gastro.2012.03.029
227. Vautrin A, Manchon L, Garcel A, Campos N, Lapasset L, Laaref AM, et al. Both anti-inflammatory and antiviral properties of novel drug candidate ABX464 are mediated by modulation of RNA splicing. *Sci Rep.* (2019) 9:792. doi: 10.1038/s41598-018-37813-y
228. Begon-Pescia C, Mielle J, Campose N, Chebli K, Manchon L, Santo J, et al. ABX464, a novel drug in the field of inflammation, increases MIR-124 and modulates macrophages and T-cell functions. *Ann Rheum Dis.* (2020) 79:317–8. doi: 10.1136/annrheumdis-2020-eular.4959
229. Tazi J, Begon-Pescia C, Campos N, Apolit C, Garcel A, Scherrer D. Specific and selective induction of miR-124 in immune cells by the quinoline ABX464: a transformative therapy for inflammatory diseases. *Drug Discovery Today.* (2021) 26:1030–9. doi: 10.1016/j.drudis.2020.12.019
230. Vermeire S, Hebuterne X, Napora P, Wisniewska-Jarosinska M, Kiss G, Bourreille A, et al. ABX464 is safe and efficacious in a proof-of-concept study in ulcerative colitis patients. *J Crohns Colitis.* (2019) 13:S14–5. doi: 10.1093/ecco-jcc/jjy222.020
231. Verstockt B, Ferrante M, Vermeire S, Van Assche G. New treatment options for inflammatory bowel diseases. *J Gastroenterol.* (2018) 53:585–90. doi: 10.1007/s00535-018-1449-z
232. Monteleone G, Neurath MF, Ardizzone S, Di Sabatino A, Fantini MC, Castiglione F, et al. Mongersen, an oral SMAD7 antisense oligonucleotide, and Crohn's disease. *N Engl J Med.* (2015) 372:1104–13. doi: 10.1056/NEJMoa1407250
233. Fay NC, Muthusamy BP, Nyugen LP, Desai RC, Taverner A, MacKay J, et al. A novel fusion of IL-10 engineered to traffic across intestinal epithelium to treat colitis. *J Immunol.* (2020) 205:3191–204. doi: 10.4049/jimmunol.2000848
234. Bischoff SC, Escher J, Hébuterne X, Kłek S, Krznaric Z, Schneider S, et al. ESPEN practical guideline: clinical nutrition in inflammatory bowel disease. *Clin Nutr.* (2020) 39:632–53. doi: 10.1016/j.clnu.2019.11.002
235. Papa A, Gerardi V, Marzo M, Felice C, Rapaccini GL, Gasbarrini A. Venous thromboembolism in patients with inflammatory bowel disease: focus on prevention and treatment. *World J Gastroenterol.* (2014) 20:3173–9. doi: 10.3748/wjg.v20.i12.3173
236. Lee D, Albenberg L, Compher C, Baldassano R, Piccoli D, Lewis JD, et al. Diet in the pathogenesis and treatment of inflammatory bowel diseases. *Gastroenterology.* (2015) 148:1087–106. doi: 10.1053/j.gastro.2015.01.007
237. Tilg H, Moschen AR. Food, immunity, and the microbiome. *Gastroenterology.* (2015) 148:1107–19. doi: 10.1053/j.gastro.2014.12.036
238. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hébert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. *Public Health Nutr.* (2014) 17:1689–96. doi: 10.1017/S1368980013002115
239. Hébert JR, Shivappa N, Wirth MD, Hussey JR, Hurley TG. Perspective: the dietary inflammatory index (DII)-lessons learned, improvements made, and future directions. *Adv Nutr.* (2019) 10:185–95. doi: 10.1093/advances/nmy071
240. Lewis JD, Abreu MT. Diet as a trigger or therapy for inflammatory bowel diseases. *Gastroenterology.* (2017) 152:398–414.e6. doi: 10.1053/j.gastro.2016.10.019
241. Lamers CR, de Roos NM, Witteman BJM. The association between inflammatory potential of diet and disease activity: results from a cross-sectional study in patients with inflammatory bowel disease. *BMC Gastroenterol.* (2020) 20:316. doi: 10.1186/s12876-020-01435-4
242. Mirmiran P, Moslehi N, Morshedzadeh N, Shivappa N, Hébert JR, Farsi F, et al. Does the inflammatory potential of diet affect disease activity in patients with inflammatory bowel disease? *Nutr J.* (2019) 18:65. doi: 10.1186/s12937-019-0492-9
243. Weisshof R, Chermesh I. Micronutrient deficiencies in inflammatory bowel disease. *Curr Opin Clin Nutr Metab Care.* (2015) 18:576–81. doi: 10.1097/MCO.0000000000000226
244. Reich KM, Fedorak RN, Madsen K, Kroeker KI. Vitamin D improves inflammatory bowel disease outcomes: basic science and clinical review. *World J Gastroenterol.* (2014) 20:4934–47. doi: 10.3748/wjg.v20.i17.4934
245. Brotherton CS, Martin CA, Long MD, Kappelman MD, Sandler RS. Avoidance of fiber is associated with greater risk of crohn's disease flare in a 6-month period. *Clin Gastroenterol Hepatol.* (2016) 14:1130–6. doi: 10.1016/j.cgh.2015.12.029
246. Limketkai BN, Iheozor-Ejiofor Z, Gjuladin-Hellon T, Parian A, Matarese LE, Bracewell K, et al. Dietary interventions for induction and maintenance of remission in inflammatory bowel disease. *Cochrane Database Syst Rev.* (2019) 2:Cd012839. doi: 10.1002/14651858.CD012839.pub2
247. Albenberg L, Brensinger CM, Wu Q, Gilroy E, Kappelman MD, Sandler RS, et al. A diet low in red and processed meat does not reduce rate of Crohn's disease flares. *Gastroenterology.* (2019) 157:128–136.e5. doi: 10.1053/j.gastro.2019.03.015
248. Cox SR, Lindsay JO, Fromentin S, Stagg AJ, McCarthy NE, Galleron N, et al. Effects of low FODMAP diet on symptoms, fecal microbiome, and markers of inflammation in patients with quiescent inflammatory bowel disease in a randomized trial. *Gastroenterology.* (2020) 158:176–88.e7. doi: 10.1053/j.gastro.2019.09.024
249. Dong H, Rowland I, Yaqoob P. Comparative effects of six probiotic strains on immune function in vitro. *Br J Nutr.* (2012) 108:459–70. doi: 10.1017/S0007114511005824
250. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci USA.* (2008) 105:16731–6. doi: 10.1073/pnas.0804812105
251. Gu P, Feagins LA. Dining with inflammatory bowel disease: a review of the literature on diet in the pathogenesis and management of IBD. *Inflamm Bowel Dis.* (2020) 26:181–91. doi: 10.1093/ibd/izz268
252. Hou JK, Abraham B, El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. *Am J Gastroenterol.* (2011) 106:563–73. doi: 10.1038/ajg.2011.44
253. Vrdoljak J, Vilović M, Živković PM, Tadin Hadjina I, Rušić D, Bukić J, et al. Mediterranean diet adherence and dietary attitudes in patients with inflammatory bowel disease. *Nutrients.* (2020) 12:3429. doi: 10.3390/nu12113429
254. Johnson GJ, Cosnes J, Mansfield JC. Review article: smoking cessation as primary therapy to modify the course of Crohn's disease. *Aliment Pharmacol Ther.* (2005) 21:921–31. doi: 10.1111/j.1365-2036.2005.02424.x
255. Novak G, Stevens T, Van Viegen T, Bossuyt P, Štabuc B, Jeyarajah J, et al. Evaluation of optimal biopsy location for assessment of histological activity, transcriptomic and immunohistochemical analyses in patients with active Crohn's disease. *Aliment Pharmacol Ther.* (2019) 49:1401–9. doi: 10.1111/apt.15250
256. Pai RK, Jairath V, Vande Casteele N, Rieder F, Parker CE, Lauwers GY. The emerging role of histologic disease activity assessment in ulcerative colitis. *Gastrointest Endosc.* (2018) 88:887–98. doi: 10.1016/j.gie.2018.08.018
257. Colombel JF, D'Haens G, Lee WJ, Petersson J, Panaccione R. Outcomes and strategies to support a treat-to-target approach in inflammatory bowel disease: a systematic review.

J Crohns Colitis. (2020) 14:254–66. doi: 10.1093/ecco-jcc/jjz131

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Epidemiology and Risk Factors of Portal Venous System Thrombosis in Patients With Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis

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Background: Patients with inflammatory bowel disease (IBD) may be at risk of developing portal venous system thrombosis (PVST) with worse outcomes. This study aims to explore the prevalence, incidence, and risk factors of PVST among patients with IBD.

Methods: PubMed, Embase, and Cochrane Library databases were searched. All the eligible studies were divided according to the history of colorectal surgery. Only the prevalence of PVST in patients with IBD was pooled if the history of colorectal surgery was unclear. The incidence of PVST in patients with IBD after colorectal surgery was pooled if the history of colorectal surgery was clear. Prevalence, incidence, and risk factors of PVST were pooled by only a random-effects model. Subgroup analyses were performed in patients undergoing imaging examinations. Odds ratios (ORs) with 95% CIs were calculated.

Results: A total of 36 studies with 143,659 patients with IBD were included. Among the studies where the history of colorectal surgery was unclear, the prevalence of PVST was 0.99, 1.45, and 0.40% in ulcerative colitis (UC), Crohn's disease (CD), and unclassified IBD, respectively. Among the studies where all the patients underwent colorectal surgery, the incidence of PVST was 6.95, 2.55, and 3.95% in UC, CD, and unclassified IBD after colorectal surgery, respectively. Both the prevalence and incidence of PVST became higher in patients with IBD undergoing imaging examinations. Preoperative corticosteroids therapy (OR = 3.112, 95% CI: 1.017–9.525; $p = 0.047$) and urgent surgery (OR = 1.799, 95% CI: 1.079–2.998; $p = 0.024$) are significant risk factors of PVST in patients with IBD after colorectal surgery. The mortality of patients with IBD with PVST after colorectal surgery was 4.31% (34/789).

Conclusion: PVST is not rare, but potentially lethal in patients with IBD after colorectal surgery. More severe IBD, indicated by preoperative corticosteroids and urgent surgery, is associated with a higher risk of PVST after colorectal surgery. Therefore, screening

for PVST by imaging examinations and antithrombotic prophylaxis in high-risk patients should be actively considered.

Systematic Review Registration: Registered on PROSPERO, Identifier: CRD42020159579.

Keywords: portal venous system thrombosis, epidemiology, risk factor, inflammatory bowel disease, meta-analysis

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic and progressive inflammatory disease of the gastrointestinal tract, mainly consisting of ulcerative colitis (UC) and Crohn's disease (CD). In recent years, there has been a rising trend of IBD worldwide, which further increases the economic burden of illness among individual patients, their families, and healthcare systems (1) as well as morbidity and mortality (2). Patients with IBD can experience disease progression from inflammation to stricture or penetration/fistulization (3). Its related complications can also result in poor quality of life (4) and negative emotional impact (5).

Recently, it has been observed that IBD has significant secondary effects on the coagulation cascade, including initiation and propagation of coagulation activation, inhibition of fibrinolysis, and downregulation of physiological anticoagulation pathways (6), which lead to coagulation abnormalities, such as increased levels of coagulation factors V and VIII, platelet count, and fibrin, and a decreased level of antithrombin (7). Patients with IBD have a higher risk of venous thromboembolism (VTE) (8), further increasing their morbidity and mortality (9).

Portal venous system thrombosis (PVST) mainly refers to the development of thrombosis within the portal vein, mesenteric vein, and splenic vein (10). Its clinical manifestations can include complications of acute intestinal ischemia (11), hematemesis or melena from esophagogastric variceal bleeding (12), even multiple organ dysfunction, and death (13). Intraperitoneal inflammation, including pancreatitis (14) and IBD (15), is one of the most common local risk factors for PVST.

It is important for physicians to understand the epidemiology and risk factors of PVST in patients with IBD since such information is potentially helpful to assess and manage this complication in high-risk patients. In this study, this systematic review and meta-analysis aimed to explore the prevalence, incidence, and risk factors of PVST in patients with IBD.

METHODS

This meta-analysis was performed following the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) (16) and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (17) statements. The MOOSE and the PRISMA checklists are shown in **Supplementary Materials**.

Registration

This study was registered in International prospective register of systematic reviews (PROSPERO) with a registration number of CRD42020159579.

Search Strategy

All articles concerning PVST in patients with IBD were searched through PubMed, Embase, and Cochrane Library databases. Search terms were as follows: ("portal" or "splenic" or "mesenteric" or "portomesenteric" or "portosplenomesenteric") and ("vein" or "venous" or "vascular") and ("thrombosis" or "thrombi" or "thrombus" or "thrombotic" or "thrombosed" or "thromboembolism" or "thromboembolic" or "embolism" or "emboli" or "embolization" or "occluded" or "occlusion" or "occlusive" or "obstructed" or "obstructive" or "obstruction") and ("inflammatory bowel disease" or "IBD" or "Crohn's disease" or "CD" or "ulcerative colitis" or "UC" or "colitis"). The last retrieval was performed on November 3, 2021.

Selection Criteria

Eligible studies were included according to the following criteria: (1) patients should be diagnosed with IBD; (2) the number of PVST in patients with IBD can be extracted to calculate the prevalence; (3) the number of PVST after a diagnosis of IBD or colorectal surgery for IBD can be extracted to calculate the incidence; and (4) risk factors associated with the development of PVST in patients with IBD can be extracted. Exclusion criteria were as follows: (1) duplicate article; (2) comment, note, or letter; (3) guideline, consensus, or report; (4) review or meta-analysis; (5) case report; (6) experimental or animal study; (7) full text could not be obtained; (8) patients with IBD were not included; (9) PVST was not evaluated in patients with IBD; (10) relevant data could not be extracted; and (11) overlapping data.

Definitions

PVST was defined as thrombus occurring in the portal venous system including portal, mesenteric, and splenic vein. The cohort study was defined as the occurrence of PVST events in patients with IBD during follow-up. Cross-sectional study was defined as the presence of PVST events in patients with IBD at a fixed time point. Prevalence of PVST referred to all the PVST conditions in patients with IBD by collecting the data from cohort and cross-sectional studies. Incidence of PVST referred to new onset of PVST events after a diagnosis of IBD or colorectal surgery for IBD by collecting the data from cohort studies.

Data Extraction

We extracted the following information: first author, publication year, publication type, region, study design, enrollment period, source of case, severity of IBD, history of colorectal surgery, use of antithrombotic drugs, number of patients with IBD, number of patients with IBD who underwent imaging examination, and number of patients with IBD who developed PVST. The characteristics of patients with PVST were further

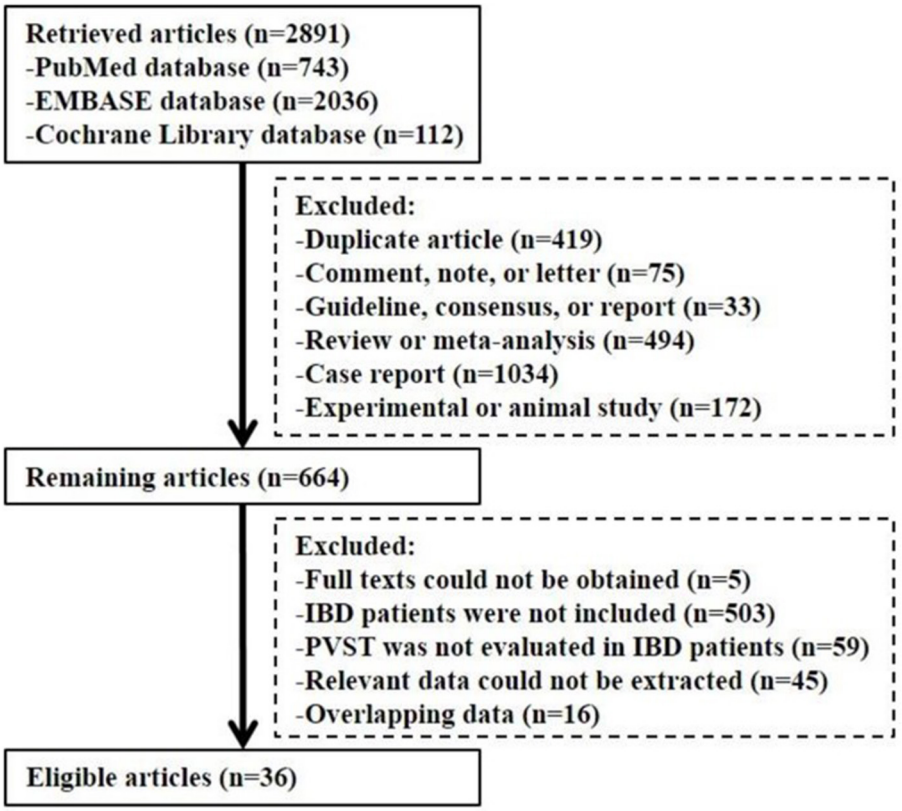

FIGURE 1 | Flowchart of study inclusion.

summarized including gender, location of PVST, main clinical presentation, interval from colorectal surgery to diagnosis of PVST, hematological abnormality, treatment selection, and outcome.

Study Quality

The Newcastle–Ottawa Scale (NOS) was used to evaluate the quality of cohort studies, in which 0–3, 4–6, and 7–9 stars represent low, moderate, and high quality, respectively (18). An 11-item checklist recommended by the Agency for Healthcare Research and Quality (AHRQ) was used to evaluate the quality of cross-sectional studies, in which a score of 0–3, 4–7, and 8–11 represent low, moderate, and high quality, respectively (19).

Statistical Analysis

All the meta-analyses were conducted by the R software version 3.6.2 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria) and Stata/SE software version 12.0 for Windows (Stata Corporation LP, College Station, Texas, USA). We pooled the prevalence, incidence, and risk factors of PVST in patients with IBD by a random-effects model. Odds ratio (OR) with 95% CI was calculated, if any. I^2 and p-value were calculated by inconsistency test to assess the heterogeneity among studies. $I^2 > 50\%$ and/or $p < 0.1$ were considered to have a statistically significant heterogeneity. Publication bias was evaluated by Egger's test. $p < 0.1$ was considered as a statistically significant publication bias. Subgroup analyses were performed in terms of region (Europe vs. North America vs. Asia vs. South America vs. Africa), study design (population-based cohort vs. hospital-based cohort vs. cross-sectional study), severity of IBD (exacerbated or refractory), use of antithrombotic drugs, whether the indications of imaging examinations for PVST were mentioned (yes vs. unclear), patients with IBD who underwent imaging examinations, and study quality (high vs. moderate). Meta-regression analyses were performed by the abovementioned covariates to explore the sources of heterogeneity. Sensitivity analyses were also performed by sequentially excluding one study in one turn.

RESULTS

Study Selection

Overall, 2,891 articles were retrieved. Finally, 36 studies were included (**Figure 1**). The study quality is given in **Supplementary Tables 1**, **2**.

Studies Where the Information With Respect to Colorectal Surgery Was Unclear

Characteristics of Included Studies

A total of 18 studies where the information with respect to colorectal surgery was unclear can be used to explore the prevalence of PVST in patients with IBD (**Table 1**) (20–37).

TABLE 1 | Characteristics of studies where the information with respect to colorectal surgery was unclear.

References	Publication type	Region	Study design	Enrollment period	Source of Pts.	No. Pts. PVST/all Pts.	Percentage of Pts. With PVST %	No. Pts. PVST/Pts. who underwent imaging examinations	Study quality
Ashamalla et al. (20)	Conference abstract	USA	Cross-Sectional	2010–2014	Two tertiary hospitals of the Northwell health system[a]	132/810 (IBD)	16.30	132/810 (IBD)	Moderate
Banerjee et al. (21)	Conference abstract	India	Cross-Sectional	2004.12–2010.03	Asian Institute of Gastroenterology in Hyderabad	7/569 (UC)	1.23	N/A	Moderate
Blonski et al. (22)	Conference abstract	USA	Cross-Sectional	1997.01–2011.10	Gastroenterology Division for University of Pennsylvania	10/14,674 (IBD)	0.07	N/A	Moderate
Bonnivard et al. (23)	Conference abstract	France	Hospital-Based cohort	2000.01–2012.06	Two French hospitals[a]	1/210 (IBD)	0.48	N/A	High
Bruining et al. (24)	Full text	USA	Hospital-Based cohort	2004.08–2005.10	Miles and Shirley Fiterman Center for Digestive Diseases in Rochester, Minnesota	6/357 (CD)	1.68	6/357 (CD)	Moderate
Campos et al. (25)	Conference abstract	Portugal	Cross-Sectional	2006.08–2013.05	Central Hospital for University of Coimbra	1/774 (IBD)	0.13	N/A	Moderate
Gutta et al. (26)	Conference abstract	USA	Cross-Sectional	2010.01–2014.12	Truman Medical Center for University of Missouri-Kansas City	5/2,408 (IBD)	0.21	N/A	Moderate
Heffley et al. (27)	Conference abstract	UK	Cross-Sectional	2015.04–2015.12	A tertiary care referral center of university[a]	2/84 (IBD)	2.38	N/A	Moderate
Kopylov et al. (28)	Full text	Israel	Hospital-Based cohort	2005–2010	Chaim Sheba Medical Center	6/460 (CD)	1.30	N/A	Moderate
Leustean et al. (29)	Conference abstract	Romania	Cross-Sectional	2012.06–2017.06	St Spiridon Hospital in Lasi	1/238 (IBD)	0.42	N/A	Moderate
Mouelhi et al. (30)	Conference abstract	Tunisia	Cross-Sectional	2000.01–2015.01	Charles Nicolle Hospital in Tunis	2/295 (IBD)	0.68	N/A	Moderate
Papay et al. (31)	Full text	Austria	Hospital-Based cohort	2006.06–2008.12	Fourteen participating centers in Austria	7/2784 (IBD)	0.25	N/A	High
Ribas Andrade et al. (32)	Conference abstract	Brazil	Hospital-Based cohort	2010.01–2015.10	Clinical Hospital for University of São Paulo	8/781 (CD)	1.02	N/A	Moderate
Sabban et al. (33)	Conference abstract	Argentina	Hospital-Based cohort	1996–2007	Hospital Italiano in Buenos Aires	1/51 (IBD)	1.96	N/A	High
Soteriadou et al. (34)	Conference abstract	UK	Hospital-Based cohort	2009.06–2012.12	Pennine Acute Hospital NHS Trust in Manchester	2/385 (CD)	0.52	2/385 (CD)	Moderate
Talbot et al. (35)	Full text	USA	Hospital-Based cohort	1970.01–1980.12	Mayo Clinic in Rochester, Minnesota	8/7,199 (IBD)	0.11	N/A	Moderate
Vegh et al. (36)	Full text	Hungary	Population-Based cohort	1977.01–2012.12	Five general hospitals and gastroenterology outpatient units in Veszprem province	1/1,060 (UC) 1/648 (CD)	0.09 0.15	N/A	High
Violi et al. (37)	Full text	Switzerland	Hospital-Based cohort	2006.07–2011.06	Swiss IBD Study Cohort at Lausanne University Hospital	8/39 (UC) 35/121 (CD)	20.51 28.93	8/39 (UC) 35/121 (CD)	High

[a] *Detailed information of hospitals cannot be found.*

PVST, portal venous system thrombosis; Pts, patients; USA, United States of America; N/A, not applicable; UC, ulcerative colitis; CD, Crohn's disease; IBD, inflammatory bowel disease; UK, United Kingdom.

Characteristics of Patients With PVST

Overall, 244 of 33,947 patients with IBD had PVST (20–37). Among them, PVST was located at the main portal vein and mesenteric vein and its branches in 106 (43.44%) and 147 (60.25%) patients, respectively; bowel stenosis, perianal fistula, internal fistula, and perianal abscess were observed in 24 (9.84%), ten (4.10%), seven (2.87%), and seven (2.87%) patients, respectively, and three (1.23%) patients died during follow-up (**Supplementary Table 3**).

Ulcerative Colitis

A total of three studies evaluated patients with UC (21, 36, 37). The pooled prevalence of PVST in patients with UC was 0.99% (**Table 2**). One study reported a detailed number of patients who underwent imaging examinations for PVST (37), with a prevalence of 20.51%. Meta-regression and sensitivity analyses were not performed due to a small number of included studies.

Crohn's Disease

A total of six studies evaluated patients with CD (24, 28, 32, 34, 36, 37). The pooled prevalence of PVST in patients with CD was 1.45% (**Table 2**). Three studies reported a detailed number of patients who underwent imaging examinations for PVST (24, 34, 37), with a pooled prevalence of 6.23%. Meta-regression (**Supplementary Table 4**) and sensitivity analyses (**Supplementary Table 5**) did not identify any source of heterogeneity.

Unclassified IBD

A total of 11 studies evaluated patients with unclassified IBD (20, 22, 23, 25–27, 29–31, 33, 35). The pooled prevalence of PVST in patients with unclassified IBD was 0.40% (**Table 2**). One study reported a detailed number of patients who underwent imaging examinations for PVST (20), with a prevalence of 16.30%. Meta-regression analyses indicated that the severity of IBD ($p < 0.001$) and whether the detailed number of patients undergoing imaging examinations was reported ($p < 0.001$) might be potential sources of heterogeneity (**Supplementary Table 4**). Sensitivity analyses found that the heterogeneity became non-significant after excluding the study by Ashamalla et al. (20) ($I^2 = 21.80\%$; $p = 0.2423$; **Supplementary Table 5**).

Risk Factors of PVST in Patients With IBD

A total of two studies demonstrated that disease duration and colorectal surgery might be significant risk factors of PVST (22), but not age, sex, body mass index (BMI), IBD location, corticosteroids therapy, smoking, or family history of IBD (37).

Studies Where the Information With Respect to Colorectal Surgery Was Clear

Characteristics of Included Studies

A total of 18 studies where the information with respect to colorectal surgery was clear were used to explore the incidence of PVST in patients with IBD after colorectal surgery (**Table 3**) (38–55).

Characteristics of Patients With PVST

Overall, 789 of 109,712 patients with IBD developed PVST after colorectal surgery (38–55). Among them, PVST was located at the main portal vein and mesenteric vein and its branches in 20 (2.53%) and 38 (4.82%) patients, respectively; abdominal pain, prolonged ileus, wound infection, and dehydration/sodium depletion was observed in 46 (5.83%), 22 (2.79%), 11 (1.39%), and eight (1.01%) patients, respectively. The interval from surgery to diagnosis of PVST was within 30 days and over 30 days in 45 (5.70%) and two (0.25%) patients, respectively; and 34 (4.31%) patients died during follow-up (**Supplementary Table 6**).

Ulcerative Colitis After Colorectal Surgery

A total of eight studies evaluated patients with UC (38, 39, 41, 43, 45, 51–53). The pooled incidence of PVST in patients with UC after colorectal surgery was 6.95% (**Table 4**). Three studies reported on the number of patients who underwent imaging examinations for PVST (39, 43, 45) in detail, with a pooled incidence of 38.33%. Meta-regression analyses indicated that whether the detailed number of patients undergoing imaging examinations was reported ($p = 0.043$) might be a potential source of heterogeneity (**Supplementary Table 7**). Sensitivity analyses did not identify any source of heterogeneity (**Supplementary Table 8**).

Crohn's Disease After Colorectal Surgery

A total of two studies evaluated patients with CD (38, 51). The pooled incidence of PVST in patients with CD after colorectal surgery was 2.55% (**Table 4**). Neither study reported on the number of patients who underwent imaging examinations for PVST in detail. Subgroup, meta-regression, and sensitivity analyses were not performed due to a small number of included studies.

Unclassified IBD After Colorectal Surgery

A total of ten studies evaluated patients with unclassified IBD (40, 42, 44, 46–50, 54, 55). The pooled incidence of PVST in patients with unclassified IBD after colorectal surgery was 3.95% (**Table 4**). Two studies reported the detailed number of patients who underwent imaging examinations for PVST (42, 44), with a pooled incidence of 17.17%. Meta-regression analyses indicated that use of antithrombotic drugs ($p = 0.008$), whether the indications of imaging examinations for PVST were mentioned ($p = 0.007$), and whether the detailed number of patients undergoing imaging examinations was reported ($p = 0.010$) might be potential sources of heterogeneity (**Supplementary Table 7**). Sensitivity analyses found that the heterogeneity became nonsignificant after excluding the study by Gu et al. (44) ($I^2 = 0\%$; $p = 0.7378$) or Murphy et al. (49) ($I^2 = 28.10\%$; $p = 0.1946$) (**Supplementary Table 8**).

Comparison of Incidence of PVST After Colorectal Surgery for IBD and Non-IBD Diseases

A total of two studies included patients who underwent colorectal surgery for IBD, cancer, diverticulitis, and polyps (38, 51). Meta-analyses demonstrated that the incidence of PVST after colorectal surgery was significantly higher in patients with UC than patients

TABLE 2 | Prevalence of PVST in patients with IBD in whom the information with respect to colorectal surgery was unclear.

Groups	No. studies	Range	Pooled proportion using random-effects model	Heterogeneity		Publication bias Egger test (*P*-value)
				I^2	*P*-value	
UC	3	0.0009–0.2051	0.0099 (95% CI 0–0.0274)	87.30%	0.0004	0.0115
Region (Europe vs. Asia)						
Europe	2	0.0009–0.2051	0.0928 (95% CI 0–0.2919)	90.00%	0.0016	N/A
Asia	1	N/A	0.0123 (95% CI 0.0050–0.0252)	N/A	N/A	N/A
Study design (population-based cohort vs. hospital-based cohort vs. cross-sectional)						
Population-Based cohort	1	N/A	0.0009 (95% CI 0–0.0052)	N/A	N/A	N/A
Hospital-Based cohort	1	N/A	0.2051 (95% CI 0.0930–0.3646)	N/A	N/A	N/A
Cross-Sectional	1	N/A	0.0123 (95% CI 0.0050–0.0252)	N/A	N/A	N/A
Whether the detailed number of patients who underwent imaging examinations was reported (Yes)						
Yes	1	N/A	0.2051[a] (95% CI 0.0930–0.3646)	N/A	N/A	N/A
Study quality (high vs. moderate)						
High	2	0.0009–0.2051	0.0928 (95% CI 0–0.2919)	90.00%	0.0016	N/A
Moderate	1	N/A	0.0123 (95% CI 0.0050–0.0252)	N/A	N/A	N/A
CD	6	0.0015–0.2893	0.0145 (95% CI 0.0026–0.0263)	91.60%	<0.0001	0.0136
Region (Europe vs. North America vs. Asia vs. South America)						
Europe	3	0.0015–0.2893	0.0273 (95% CI 0.0015–0.0531)	95.90%	<0.0001	0.2412
North America	1	N/A	0.0168 (95% CI 0.0062–0.0362)	N/A	N/A	N/A
Asia	1	N/A	0.0130 (95% CI 0.0048–0.0282)	N/A	N/A	N/A
South America	1	N/A	0.0102 (95% CI 0.0044–0.0201)	N/A	N/A	N/A
Study design (population-based cohort vs. hospital-based cohort)						
Population-Based cohort	1	N/A	0.0015 (95% CI 0–0.0086)	N/A	N/A	N/A
Hospital-Based cohort	5	0.0052–0.2893	0.0222 (95% CI 0.0047–0.0397)	91.80%	<0.0001	0.0145
Whether the detailed number of patients who underwent imaging examinations was reported (Yes)						
Yes	3	0.0052–0.2893	0.0623 (95% CI 0.0158–0.1088)	95.90%	<0.0001	0.1340
Study quality (high vs. moderate)						
High	2	0.0015–0.2893	0.1425 (95% CI 0–0.4243)	97.90%	<0.0001	N/A
Moderate	4	0.0052–0.0168	0.0096 (95% CI 0.0053–0.0139)	0.80%	0.3880	0.1904
Unclassified IBD	11	0.0007–0.1630	0.0040 (95% CI 0.0014–0.0067)	94.00%	<0.0001	0.0651
Region (Europe vs. North America vs. South America vs. Africa)						
Europe	5	0.0013–0.0238	0.0023 (95% CI 0.0008–0.0037)	0%	0.5911	0.1334

(Continued)

TABLE 2 | Continued

Groups	No. studies	Range	Pooled proportion using random-effects model	Heterogeneity		Publication bias Egger test (*P*-value)
				I^2	*P*-value	
North America	4	0.0007–0.1630	0.0052 (95% CI 0.0011–0.0093)	98.10%	<0.0001	0.1531
South America	1	N/A	0.0196 (95% CI 0.0005–0.1045)	N/A	N/A	N/A
Africa	1	N/A	0.0068 (95% CI 0.0008–0.0243)	N/A	N/A	N/A
Study design (hospital-based cohort vs. cross-sectional)						
Hospital-Based cohort	4	0.0011–0.0196	0.0015 (95% CI 0.0005–0.0024)	8.40%	0.3512	0.1096
Cross-Sectional	7	0.0007–0.1630	0.0105 (95% CI 0.0044–0.0165)	96.30%	<0.0001	0.1525
Severity of IBD (exacerbation)						
Exacerbation	1	N/A	0.1630 (95% CI 0.1382–0.1902)	N/A	N/A	N/A
Whether the detailed number of patients who underwent imaging examinations was reported (Yes)						
Yes	1	N/A	0.1630[a] (95% CI 0.1382–0.1902)	N/A	N/A	N/A
Study quality (high vs. moderate)						
High	3	0.0025–0.0196	0.0026 (95% CI 0.0008–0.0045)	0%	0.6124	0.1316
Moderate	8	0.0007–0.1630	0.0045 (95% CI 0.0013–0.0076)	95.70%	<0.0001	0.1132

[a]*Pooled prevalence of PVST in patients who underwent imaging examinations.*

PVST, portal venous system thrombosis; IBD, inflammatory bowel disease; UC, ulcerative colitis; N/A, not applicable; CD, Crohn's disease.

with non-IBD (OR, 4.41; 95% CI, 2.35–8.29; $p < 0.01$), but the incidence of PVST after colorectal surgery was not significantly different between patients with CD and non-IBD (OR, 1.71; 95% CI, 0.25–11.73; $p = 0.59$).

Risk Factors of PVST in Patients With IBD After Colorectal Surgery

A total of six studies demonstrated that age (44), BMI (49), corticosteroids therapy (44), preoperative C-reactive protein (CRP) (45), preoperative albumin (44), surgical approach (49), type of surgery (44), and urgent reoperation (45) were significant risk factors of PVST in patients with IBD after colorectal surgery on the univariate analysis (**Supplementary Table 9**). Two studies demonstrated that corticosteroid therapy (44), preoperative CRP (45), and type of surgery (44) were significant risk factors of PVST in patients with IBD after colorectal surgery on the multivariate analysis (**Supplementary Table 10**). Meta-analyses found that corticosteroid therapy (38, 39, 44, 45) and urgent surgery (44, 45) were significantly associated with PVST in patients with IBD after colorectal surgery, but not male gender (37, 44, 45), left-sided colitis (44, 45), extensive colitis (44, 45), severe colitis (38, 44), immunomodulators therapy (38, 44, 45), biologics therapy (38, 44, 45), history of thromboembolic disease (44, 45), or smoking (37, 44, 45) (**Figure 2**).

DISCUSSION

To the best of our knowledge, this is the first systematic review with a meta-analysis exploring the epidemiology of PVST in patients with IBD and evaluating its risk factors. Our major findings are as follows: first, among the patients with IBD in whom the history of colorectal surgery was unclear, the prevalence of PVST was 0.99, 1.45, and 0.40% in UC, CD, and unclassified IBD, respectively. Notably, the prevalence of PVST became higher in patients with IBD who underwent imaging examinations. Second, among the patients with IBD who underwent colorectal surgery, the incidence of PVST was 6.95, 2.55, and 3.95% in UC, CD, and unclassified IBD, respectively. Notably, the incidence became higher in patients with IBD who underwent imaging examinations after colorectal surgery. Third, the use of preoperative corticosteroids and urgent surgery are significant risk factors of PVST in patients with IBD who underwent colorectal surgery.

IBD is associated with a hypercoagulable state, which enhances the risk of thrombosis. Indeed, 1.01 to 2.14% of patients with IBD have VTE (56, 57). Several major mechanisms for explaining the association between PVST and IBD are as follows (**Supplementary Figure 1**). First, ulceration and loss of integrity of the normal mucosal barrier in the bowel may lead to microbial invasion or translocation into the portal venous system, leading to pylephlebitis and increasing the risk

TABLE 3 | Characteristics of studies where all the included patients underwent colorectal surgery.

References	Publication type	Region	Study design	Enrollment period	Source of Pts.	No. Pts. PVST/all Pts.	Percentage of Pts. With PVST %	No. Pts. PVST/ Pts. who underwent imaging examinations	Study quality
Allaix et al. (38)	Full text	USA	Hospital-Based cohort	2002.06–2012.06	Surgery Department for University of Chicago Medical Center in Illinois	23/447 (UC) 11/304 (CD)	5.15 3.62	N/A	High
Ball et al. (39)	Full text	Canada	Hospital-Based cohort	1997.01–2002.12	Foothills Medical Center for University of Calgary in Alberta	11/112 (UC)	9.82	11/28 (UC)	Moderate
Bence et al. (40)	Full text	USA	Hospital-Based cohort	2010.01–2016.06	Four tertiary care children's hospitals	9/276 (IBD)	3.26	N/A	High
Feuerstein et al. (41)	Conference abstract	USA	Hospital-Based cohort	2002.01–2014.01	Beth Israel Deaconess Medical Center in Boston, Massachusetts	8/259 (UC)	3.09	N/A	High
Fichera et al. (42)	Full text	USA	Hospital-Based cohort	1999.01–2001.12	Mount Sinai Hospital in New York	4/83 (IBD)	4.82	4/14 (IBD)	Moderate
Gonzales et al. (43)	Conference abstract	USA	Hospital-Based cohort	2003.06–2008.01	Department of Surgery for Boston University School of Medicine in Massachusetts	20/85 (UC)	23.53	20/33 (UC)	Moderate
Gu et al. (44)	Full text	USA	Hospital-Based cohort	2006–2012	Department of Colorectal Surgery for Cleveland Clinic in Ohio	36/521 (IBD)	6.91	36/216 (IBD)	High
Kayal et al. (45)	Full text	USA	Hospital-Based cohort	2010.01–2016.12	Mount Sinai Hospital in New York	36/434 (UC)	8.29	36/205 (UC)	High
Mathis et al. (46)	Conference abstract	USA	Hospital-Based cohort	2007.09–2012.08	Mayo Clinic in Rochester, Minnesota	4/63 (IBD)	6.35	N/A	Moderate
Mathis et al. (47)	Full text	USA	Hospital-Based cohort	1994–2005	Mayo Clinic in Rochester, Minnesota	3/100 (IBD)	3.00	N/A	High
Medress and Fleshner (48)	Full text	USA	Hospital-Based cohort	2001.08–2006.08	Cedars-Sinai Medical Center in Los Angeles, California	5/202 (IBD)	2.48	N/A	Moderate
Murphy et al. (49)	Conference abstract	USA	Hospital-Based cohort	2008.01–2012.07	Two clinic institution in Boston, Massachusetts[a]	26/1,014 (IBD)	2.56	N/A	Moderate
Naik et al. (50)	Conference abstract	USA	Hospital-Based cohort	2008–2010	Gastroenterology and Hepatology Division for Medical College of Wisconsin in Milwaukee	7/131 (IBD)	5.34	N/A	Moderate
Robinson et al. (51)	Full text	USA	Hospital-Based cohort	2007.01–2012.12	Mayo Clinic in Phoenix, Arizona	11/125(UC) 1/78 (CD)	8.80 1.28	N/A	High
Syed et al. (52)	Full text	USA	Population based cohort	1999–2020.04	Explorys database of IBM, New York	570/105,410 (UC)	0.54	N/A	High
Vaidya et al. (53)	Conference abstract	USA	Hospital-Based cohort	2018.06–2019.07	Department of Colorectal Surgery for Cleveland Clinic in Ohio	1/18 (UC)	5.56	N/A	Moderate
Weisshof et al. (54)	Full text	USA	Hospital-Based cohort	2010.01–2018.03	IBD Center for University of Chicago in Illinois	2/24 (IBD)	8.33	N/A	Moderate
Zaghiyan et al. (55)	Full text	USA	Hospital-Based cohort	2010.01–2010.08	Cedars-Sinai Medical Center in Los Angeles, California	1/26 (IBD)	3.85	N/A	Moderate

[a] *Detailed information of hospitals cannot be found.*

Pts, patients; PVST, portal venous system thrombosis; USA, United States of America; UC, ulcerative colitis; CD, Crohn's disease; N/A, not applicable; IBD, inflammatory bowel disease.

TABLE 4 | Incidence of PVST in patients with IBD after colorectal surgery.

Groups	No. studies	Range	Pooled proportion using random-effects model	Heterogeneity		Publication bias Egger test (*P*-value)
				I^2	*P*-value	
UC	8	0.0054–0.2353	0.0695 (95% CI 0.0355–0.1036)	93.40%	<0.0001	0.0013
Region (USA vs. Canada)						
USA	7	0.0054–0.2353	0.0654 (95% CI 0.0300–0.1007)	93.70%	<0.0001	0.0045
Canada	1	N/A	0.0982 (95% CI 0.0501–0.1689)	N/A	N/A	N/A
Study design (population-based cohort vs. hospital-based cohort)						
Population-Based cohort	1	N/A	0.0054 (95% CI 0.0050–0.0059)	N/A	N/A	N/A
Hospital-Based cohort	7	0.0309–0.2353	0.0789 (95% CI 0.0480–0.1099)	79.00%	<0.0001	0.0899
Severity of UC (refractory)						
Refractory	1	N/A	0.0829 (95% CI 0.0588–0.1130)	N/A	N/A	N/A
Use of antithrombotic drugs (yes)						
Yes	1	N/A	0.0841[a] (95% CI 0.0596–0.1145)	N/A	N/A	N/A
Whether the indications of imaging examinations for PVST were mentioned (yes vs. unclear)						
Yes	6	0.0309–0.2353	0.0810 (95% CI 0.0482–0.1137)	82.50%	<0.0001	0.0348
Unclear	2	0.0054–0.0556	0.0054 (95% CI 0.0050–0.0059)	0%	0.3530	N/A
Whether the detailed number of patients who underwent imaging examinations was reported (yes)						
Yes	3	0.1756–0.6061	0.3833[b] (95% CI 0.1058–0.6609)	92.50%	<0.0001	0.2530
Study quality (high vs. moderate)						
High	5	0.0054–0.0880	0.0486 (95% CI 0.0147–0.0825)	94.30%	<0.0001	0.0134
Moderate	3	0.0556–0.2353	0.1298 (95% CI 0.0330–0.2266)	75.90%	0.0158	0.8334
CD	2	0.0128–0.0362	0.0255 (95% CI 0.0027–0.0483)	49.30%	0.1604	N/A
Unclassified IBD	10	0.0248–0.0833	0.0395 (95% CI 0.0269–0.0521)	45.90%	0.0548	0.1038
Severity of IBD (refractory)						
Refractory	4	0.0248–0.0833	0.0523 (95% CI 0.0206–0.0839)	65.80%	0.0325	0.7107
Use of antithrombotic drugs (yes)						
Yes	2	0.0534–0.0691	0.0653[a] (95% CI 0.0463–0.0843)	0%	0.4878	N/A
Whether the indications of imaging examinations for PVST were mentioned (yes vs. unclear)						
Yes	3	0.0482–0.0691	0.0628 (95% CI 0.0453–0.0804)	0%	0.6267	0.0324
Unclear	7	0.0248–0.0833	0.0278 (95% CI 0.0200–0.0356)	0%	0.8232	0.0222
Whether the detailed number of patients who underwent imaging examinations was reported (yes)						
Yes	2	0.1667–0.2857	0.1717[b] (95% CI 0.1231–0.2203)	0%	0.3346	N/A

(Continued)

TABLE 4 | Continued

Groups	No. studies	Range	Pooled proportion using random-effects model	Heterogeneity		Publication bias Egger test (*P*-value)
				I^2	*P*-value	
Study quality (high vs. moderate)						
High	3	0.0300–0.0691	0.0451 (95% CI 0.0188–0.0714)	70.40%	0.0341	0.7808
Moderate	7	0.0248–0.0833	0.0288 (95% CI 0.0204–0.0371)	0%	0.5349	0.0120

[a]*Pooled incidence of PVST in patients who received antithrombotic drugs.*
[b]*Pooled incidence of PVST in patients who underwent imaging examinations.*
PVST, portal venous system thrombosis; IBD, inflammatory bowel disease; UC, ulcerative colitis; USA, United States of America; N/A, not applicable; CD, Crohn's disease.

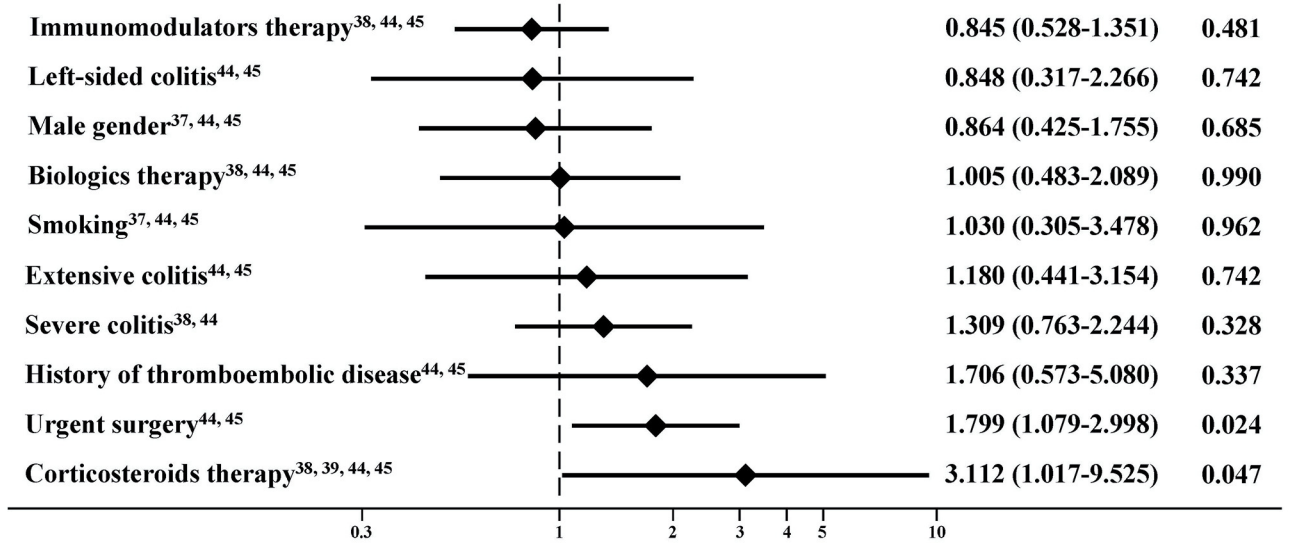

FIGURE 2 | Risk factors of portal venous system thrombosis (PVST) in patients with inflammatory bowel disease (IBD) after colorectal surgery.

of PVST (7). Second, the deficiency of anticoagulants, such as protein S, is related to IBD (58). Protein S deficiency is associated with a high risk of VTE (59) and PVST (60). Third, fibrinogen, which may contribute to the development of PVST (61), is increased in active IBD (62). Fourth, tissue plasminogen activator (t-PA) is released from storage sites in vascular endothelial cells as a result of inflammation in patients with IBD (6). An increase in t-PA level is counteracted by a delayed, but sustained increase in plasminogen activator inhibitor-1 (PAI-1) (63), thereby decreasing fibrinolysis (64). Fifth, homocysteine level is significantly higher in patients with IBD than healthy controls (65). Hyperhomocysteinemia can cause hypercoagulability, by increasing tissue factor and factor V levels, reducing t-PA level, and deactivating protein C (66). Sixth, increased platelet count (67) and decreased mean platelet volume (68) in patients with IBD could increase the thrombotic potential risk. Seventh, the initiation and progression of colitis are mainly caused by neutrophil extracellular traps (NETs), which could induce platelet activation to promote thrombotic tendency (69). Studies concerning NETs in the pathogenesis of PVST are scarce, but evidence on the critical role of NETs in thrombosis is comprehensive (70). NETs are released together with peptidylarginine deiminase type IV into the extracellular milieu, leading to thrombus formation in mesenteric venules in mice (71).

Computed tomography (CT) and magnetic resonance imaging (MRI) play an important role in the diagnosis and assessment of non-malignant PVST (72, 73). However, they are not routinely performed in patients with IBD, which potentially underestimates the actual epidemiology of PVST. This study suggested that the prevalence of PVST and incidence of PVST after colorectal surgery would be increased in patients who

underwent imaging examinations. Collectively, CT or MRI may be considered in patients with IBD at a high risk of developing PVST.

Corticosteroids are a major treatment option for IBD (74), but they potentiate the risk of VTE in patients with IBD regardless of colorectal surgery (75). The reasons for this association may include the following: first, corticosteroids could enhance the activity of the PAI-1 gene in cell cultures through a corticosteroid-responsive element with enhancer-like properties (76). Activation of the *PAI-1* gene could increase the PAI-1 level, thereby reducing the t-PA level and impairing the fibrinolytic activity (76). Therefore, corticosteroid-induced alterations in fibrinolysis may contribute to a hypercoagulable state. Second, corticosteroids use may be a surrogate marker for more severe disease status. Usually, mild cases can be treated with derivatives of 5-aminosalicylic acid, but severe cases achieve disease remission by corticosteroids (56). Patients with aggressive disease often suffer from abdominal pain or frequent diarrheal stool per day and need bed rest and relative immobility, which lead to a stronger prothrombotic state (77). Thus, some investigators speculate that an increased risk of VTE may be due to the disease activity, rather than corticosteroid use itself. Contrarily, others consider that patients with IBD treated with corticosteroids are more likely to experience a disease flare, thereby increasing the risk of VTE (78). Higgins et al. showed that corticosteroids themselves increased VTE risk regardless of inflammatory conditions (79). Indeed, there is also an increased risk of VTE in general patients and healthy volunteers receiving corticosteroids in the absence of inflammation (80, 81). In our meta-analysis, the preoperative use of corticosteroids seems to be associated with PVST after colorectal surgery (OR = 3.112; $p = 0.047$). Because the use of corticosteroids is often indispensable in patients with IBD (82), anticoagulation should be considered for the prophylaxis of VTE during the period of corticosteroid use (83). However, based on our meta-analysis, the pooled incidence of PVST in patients with UC after colorectal surgery is not lower in patients receiving antithrombotic drugs. Of course, it should be acknowledged that a direct comparison between anticoagulation vs. no anticoagulation is lacking in such patients. Therefore, further studies should explore the role of anticoagulation for the prevention of PVST in patients with IBD receiving corticosteroids.

A high risk of postoperative thromboembolic complications has been observed in patients with IBD undergoing colorectal surgery (84). Colorectal surgery has been identified as a risk factor of VTE (85). However, it is still unclear whether an increased risk of VTE among patients with IBD is specifically attributed to colectomy, disease severity necessitating colectomy, or their combination. Our meta-analysis also demonstrated that the pooled incidence of PVST was obviously higher in patients with UC who underwent colorectal surgery than those in whom the information regarding colorectal surgery was unclear (6.95 vs. 0.99%), suggesting a higher probability of PVST after colorectal surgery. Thrombosis in the portal venous system is associated with a borderline intrinsically hypercoagulable environment, which may result from direct surgical trauma to the colic veins (42). Additionally, the incidence of PVST after colorectal surgery is higher in patients with UC than patients with CD. This may be explained by the fact that patients with UC undergoing colorectal surgery have a larger inflammatory burden, but patients with CD undergo surgery mainly due to stenotic or fistulizing complications (86). Patients with UC undergoing urgent surgery have an over 5-fold increased odds of VTE, despite postoperative heparin (87). This is because patients usually have a flare of IBD at the time of urgent surgery, leading to a prominent risk of VTE (56). Our meta-analysis also demonstrated that urgent surgery might be a risk factor of PVST. Taken together, thromboprophylaxis in surgical patients with IBD should be adopted according to the specific guidelines (88).

This study has some other limitations. First, the heterogeneity among studies was significant in most meta-analyses. It might be from the enrollment period and follow-up duration. However, the source of heterogeneity cannot be identified by subgroup and meta-regression analyses. Second, the specific type of IBD, severity of IBD, number of patients with IBD who underwent imaging examinations, and history of colorectal surgery for IBD were unclear in some studies. Third, there is a high incidence of PVST after colorectal surgery (89). However, among the included studies, no relevant data can be extracted to compare the proportion of PVST between patients with IBD who underwent and did not undergo colorectal surgery.

In conclusion, there is an increased risk of PVST in patients with IBD. Corticosteroids therapy and urgent colorectal surgery both suggest that more severe IBD seems to increase the risk of PVST in patients with IBD. Imaging examinations should be recommended to improve the detection rate of PVST, especially in high-risk patients. Further large-scale prospective1 studies are necessary to clarify the prediction and prevention of PVST in patients with IBD in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

HL contributes to the methodology, formal analysis, investigation, data curation, writing—original draft, and writing—review and editing. ZB, FM, and YW contributes to the formal analysis, investigation, data curation, writing—original draft, and writing—review and editing. LL contributes to the formal analysis, investigation, data curation, and writing—review and editing. AS contributes to the writing—review and editing. EY contributes to the writing—review and editing. XG contributes to the investigation, writing—review and editing, and supervision. XQ contributes to the conceptualization, methodology, formal analysis, investigation, data curation, writing—original draft, writing—review and editing, supervision,

and project administration. All authors have made an intellectual contribution to the manuscript and approved the submission of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.744505/full#supplementary-material

Supplementary Figure 1 | Mechanisms of the association between PVST and IBD.

Supplementary Table 1 | Quality of cohort studies. Q1, Representativeness of the exposed cohort; Q2, Selection of the non-exposed cohort; Q3, Ascertainment of exposure; Q4, Demonstration that outcome of interest was not present at start of study; Q5, Comparability of cohorts on the basis of the design or analysis; Q6, Assessment of outcome; Q7, Was follow-up long enough for outcomes to occur; Q8, Adequacy of follow up of cohorts.

Supplementary Table 2 | Quality of cross-sectional studies. Q1, Define the source of information (survey, record review); Q2, List inclusion and exclusion criteria for exposed and unexposed subjects (cases and controls) or refer to previous publications; Q3, Indicate time period used for identifying patients; Q4, Indicate whether or not subjects were consecutive if not population-based; Q5, Indicate if evaluators of subjective components of study were masked to other aspects of the status of the participants; Q6, Describe any assessments undertaken for quality assurance purposes (e.g., test/retest of primary outcome measurements); Q7, Explain any patient exclusions from analysis; Q8, Describe how confounding was assessed and/or controlled; Q9, If applicable, explain how missing data were handled in the analysis; Q10, Summarize patient response rates and completeness of data collection; Q11, Clarify what follow-up, if any, was expected and the percentage of patients for which incomplete data or follow-up was obtained. Y, Yes; U, Unclear; N, No.

Supplementary Table 3 | Characteristics of included patients with PVST in whom the information regarding colorectal surgery was unclear ($n = 244$). [a]PVST was not located in one position. PVST, Portal venous system thrombosis; Pts, Patients.

Supplementary Table 4 | Results of meta-regression analyses regarding the prevalence of PVST in IBD patients in whom the information regarding colorectal surgery was unclear. PVST, Portal venous system thrombosis; IBD, Inflammatory bowel disease; CD, Crohn's disease.

Supplementary Table 5 | Sensitivity analyses in studies where the information regarding colorectal surgery was unclear. CI, Confidence interval; CD, Crohn's disease; IBD, Inflammatory bowel disease.

Supplementary Table 6 | Characteristics of included patients with PVST after colorectal surgery ($n = 789$). [a]PVST was not located in one position. [b]The hematological abnormalities in the four patients were thrombocytosis, G20210A prothrombin mutation, antithrombin III mutation and anti-phospholipid syndrome, respectively. PVST, Portal venous system thrombosis; Pts, Patients.

Supplementary Table 7 | Results of meta-regression analyses regarding the incidence of PVST in patients after colorectal surgery. PVST, Portal venous system thrombosis; UC, Ulcerative colitis; USA, United States of America; IBD: Inflammatory bowel disease.

Supplementary Table 8 | Sensitivity analyses in studies where the information regarding colorectal surgery was clear. CI, Confidence interval; UC, Ulcerative colitis; IBD, Inflammatory bowel disease.

Supplementary Table 9 | Univariate analysis of risk factors for PVST in IBD patients after colorectal surgery. PVST, Portal venous system thrombosis; IBD, Inflammatory bowel disease; TAC, Total abdominal colectomy; CP, Completion proctectomy; TPC, Total proctocolectomy; IPAA, Ileal pouch-anal anastomosis; RPC, Restorative proctocolectomy; ASA, American Society of Anesthesiologists classification.

Supplementary Table 10 | Multivariate analysis of risk factors for PVST in IBD patients after colorectal surgery. PVST, Portal venous system thrombosis; IBD, Inflammatory bowel disease; RPC, Restorative proctocolectomy; IPAA, Ileal pouch-anal anastomosis; CP, Completion proctectomy; TAC, Total abdominal colectomy.

REFERENCES

1. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet.* (2018) 390:2769–78. doi: 10.1016/S0140-6736(17)32448-0
2. Mowat C, Cole A, Windsor A, Ahmad T, Arnott I, Driscoll R, et al. Guidelines for the management of inflammatory bowel disease in adults. *Gut.* (2011) 60:571–607. doi: 10.1136/gut.2010.224154
3. Mak WY, Zhao M, Ng SC, Burisch J. The epidemiology of inflammatory bowel disease: east meets west. *J Gastroenterol Hepatol.* (2020) 35:380–9. doi: 10.1111/jgh.14872
4. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology.* (2012) 142:46–54. doi: 10.1053/j.gastro.2011.10.001
5. Cohen BL, Zoëga H, Shah SA, Leleiko N, Lidofsky S, Bright R, et al. Fatigue is highly associated with poor health-related quality of life, disability and depression in newly-diagnosed patients with inflammatory bowel disease, independent of disease activity. *Aliment Pharmacol Ther.* (2014) 39:811–22. doi: 10.1111/apt.12659
6. Levi M, van der Poll T, Büller HR. Bidirectional relation between inflammation and coagulation. *Circulation.* (2004) 109:2698–704. doi: 10.1161/01.CIR.0000131660.51520.9A
7. Navaneethan U, Shen B. Hepatopancreatobiliary manifestations and complications associated with inflammatory bowel disease. *Inflamm Bowel Dis.* (2010) 16:1598–619. doi: 10.1002/ibd.21219
8. Murthy SK, Nguyen GC. Venous thromboembolism in inflammatory bowel disease: an epidemiological review. *Am J Gastroenterol.* (2011) 106:713–8. doi: 10.1038/ajg.2011.53
9. Purnak T, Yuksel O. Overview of venous thrombosis in inflammatory bowel disease. *Inflamm Bowel Dis.* (2015) 21:1195–203. doi: 10.1097/MIB.0000000000000274
10. Qi X. Portal vein thrombosis: recent advance. *Adv Exp Med Biol.* (2017) 906:229–39. doi: 10.1007/5584_2016_118
11. Plessier A, Darwish-Murad S, Hernandez-Guerra M, Consigny Y, Fabris F, Trebicka J, et al. Acute portal vein thrombosis unrelated to cirrhosis: a prospective multicenter follow-up study. *Hepatology.* (2010) 51:210–8. doi: 10.1002/hep.23259
12. Noronha Ferreira C, Seijo S, Plessier A, Silva-Junior G, Turon F, Rautou PE, et al. Natural history and management of esophagogastric varices in chronic noncirrhotic, nontumoral portal vein thrombosis. *Hepatology.* (2016) 63:1640–50. doi: 10.1002/hep.28466
13. Valla D. Splanchnic vein thrombosis. *Semin Thromb Hemost.* (2015) 41:494–502. doi: 10.1055/s-0035-1550439
14. Xu W, Qi X, Chen J, Su C, Guo X. Prevalence of splanchnic vein thrombosis in pancreatitis: a systematic review and meta-analysis of observational studies. *Gastroenterol Res Pract.* (2015) 2015:245460. doi: 10.1155/2015/245460

15. Intagliata NM, Caldwell SH, Tripodi A. Diagnosis, development, and treatment of portal vein thrombosis in patients with and without cirrhosis. *Gastroenterology.* (2019) 156:1582–99. doi: 10.1053/j.gastro.2019.01.265
16. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of observational studies in epidemiology (MOOSE) group. *JAMA.* (2000) 283:2008–12. doi: 10.1001/jama.283.15.2008
17. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* (2009) 6:e1000097. doi: 10.1371/journal.pmed.1000097
18. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses.* Ottawa Health Research Institute (2014). Available online at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (accessed November, 2021).
19. Rostom A, Dubé C, Cranney A. Celiac disease. In: *Appendix D. Quality Assessment Forms.* Rockville, MD: Agency for Healthcare Research and Quality (US); Evidence Reports/Technology Assessments. (2004) Available online at: http://www.ncbi.nlm.nih.gov/books/NBK35156 (accessed November, 2021).
20. Ashamalla M, Gao Y, Grimaldi G, Keith S. Prevalence and outcomes of portomesenteric vein thrombosis during IBD exacerbation. *Gastroenterology.* (2019) 156:S386. doi: 10.1016/S0016-5085(19)37817-5
21. Banerjee R, Mansard MJ, Kalapala R, Tandan M, Rao GV, Reddy DN. The demographic spectrum of IBD in India: a prospective analysis. *J Gastroenterol Hepatol.* (2011) 26:85. doi: 10.1111/j.1440-1746.2011.06898.x
22. Blonski W, Lin MV, Weiner M, Tierney A, Marchioni RM, Buchner A, et al. Cerebral venous and portal vein thrombosis in patients with inflammatory bowel disease(IBD). *Gastroenterology.* (2012) 142:S787. doi: 10.1016/S0016-5085(12)63054-6
23. Bonnivard S, Cadiot G, Berlot AH, Brixi H. Prevalence, causes and impact of abnormal liver biochemistries in patients with inflammatory bowel disease. *Gastroenterology.* (2015) 148:S455. doi: 10.1016/S0016-5085(15)31535-3
24. Bruining DH, Siddiki HA, Fletcher JG, Tremaine WJ, Sandborn WJ, Loftus Jr EV. Prevalence of penetrating disease and extraintestinal manifestations of Crohn's disease detected with CT enterography. *Inflamm Bowel Dis.* (2008) 14:1701–6. doi: 10.1002/ibd.20529
25. Campos S, Oliveira A, Portela F, Sofia C. The thromboembolism in inflammatory bowel disease - apropos of 28 cases. *J Crohns Colitis.* (2015) 9:S125–S6. doi: 10.1093/ecco-jcc/jju027.214
26. Gutta A, Redd MK, Shah R, Jeepalyam S, Yousef O, Clarkston WK. Inflammatory bowel disease is associated with an increased risk of arterial and venous thrombosis in a tertiary hospital-based patient cohort. *Gastroenterology.* (2016) 150:S562. doi: 10.1016/S0016-5085(16)31919-9
27. Heffley JD, Iskandar H, Dhere TA. A comparison of hospitalizations of African-American and caucasian inflammatory bowel disease (IBD) patients in a tertiary care referral center. *Gastroenterology.* (2017) 152:S440–S1. doi: 10.1016/S0016-5085(17)31685-2
28. Kopylov U, Amitai MM, Lubetsky A, Eliakim R, Chowers Y, Ben-Horin S. Clinical and radiographic presentation of superior mesenteric vein thrombosis in Crohn's disease: a single center experience. *J Crohns Colitis.* (2012) 6:543–9. doi: 10.1016/j.crohns.2011.10.013
29. Leustean AM, Dorobat A, Popa I, Bejenariu I, Prelipcean CC, Mihai C. Inflammatory bowel disease and vascular complications. *J Gastrointestin Liver Dis.* (2018) 27:13.
30. Mouelhi L, Daboussi O, Ben Khelifa M, Houissa F, El Jery K, Said Y, et al. Venous thromboembolism with inflammatory bowel disease. *J Crohns Colitis.* (2016) 10:S173. doi: 10.1093/ecco-jcc/jjw019.285
31. Papay P, Miehsler W, Tilg H, Petritsch W, Reinisch W, Mayer A, et al. Clinical presentation of venous thromboembolism in inflammatory bowel disease. *J Crohns Colitis.* (2013) 7:723–9. doi: 10.1016/j.crohns.2012.10.008
32. Ribas Andrade A, Leite Barros L, Azevedo M, Sipahi A, Zonetti De Arruda Leite A. Clinical features of thrombosis in patients with crohn's disease followed at the clinical hospital of the university of São Paulo, Brazil. *J Crohns Colitis.* (2016) 10:S445. doi: 10.1093/ecco-jcc/jjw019.793
33. Sabban JC, Gallo J, Caicedo VY, Busoni V, Dagostino D, Etchevers J, et al. Long term follow up of children with inflammatory bowel disease with different age at onset in a latinoamerican center. *J Pediatr Gastroenterol Nutr.* (2017) 65:S154. doi: 10.1097/MPG.0000000000001805
34. Soteriadou S, White K, Filobbos R, Limdi JK. MR enterography is a useful test in the investigation of small bowel disease. *Gut.* (2013) 62:A212. doi: 10.1136/gutjnl-2013-304907.488
35. Talbot RW, Heppell J, Dozois RR, Beart Jr RW. Vascular complications of inflammatory bowel disease. *Mayo Clin Proc.* (1986) 61:140–5. doi: 10.1016/S0025-6196(12)65200-8
36. Vegh Z, Golovics PA, Lovasz BD, Kurti Z, Gecse KB, Szita I, et al. Low incidence of venous thromboembolism in inflammatory bowel diseases: prevalence and predictors from a population-based inception cohort. *Scand J Gastroenterol.* (2014) 50:306–11. doi: 10.3109/00365521.2014.985708
37. Violi NV, Schoepfer AM, Fournier N, Guiu B, Bize P, Denys A, et al. Prevalence and clinical importance of mesenteric venous thrombosis in the swiss inflammatory bowel disease cohort. *Am J Roentgenol.* (2014) 203:62–9. doi: 10.2214/AJR.13.12447
38. Allaix ME, Krane MK, Zoccali M, Umanskiy K, Hurst R, Fichera A. Postoperative portomesenteric venous thrombosis: lessons learned from 1,069 consecutive laparoscopic colorectal resections. *World J Surg.* (2014) 38:976–84. doi: 10.1007/s00268-013-2336-7
39. Ball CG, MacLean AR, Buie WD, Smith DF, Raber EL. Portal vein thrombi after ileal pouch-anal anastomosis: its incidence and association with pouchitis. *Surg Today.* (2007) 37:552–7. doi: 10.1007/s00595-006-3470-8
40. Bence CM, Traynor MD, Polites SF, Ha D, Muenks P, St. Peter SD, et al. The incidence of venous thromboembolism in children following colorectal resection for inflammatory bowel disease: a multi-center study. *J Pediatr Surg.* (2020) 55:2387–92. doi: 10.1016/j.jpedsurg.2020.02.020
41. Feuerstein JD, Lerner A, Alvarez D, Curran T, Cataldo TE, Falchuk KR, et al. Symptomatic venous thromboembolism is a rare following elective and non-elective surgery for ulcerative colitis. *Gastroenterology.* (2017) 152:S782–S3. doi: 10.1016/S0016-5085(17)32712-9
42. Fichera A, Cicchiello LA, Mendelson DS, Greenstein AJ, Heimann TM. Superior mesenteric vein thrombosis after colectomy for inflammatory bowel disease: a not uncommon cause of postoperative acute abdominal pain. *Dis Colon Rectum.* (2003) 46:643–8. doi: 10.1007/s10350-004-6625-y
43. Gonzales KD, Gupta A, Tkacz JN, Stucchi A, Soto J, Sentovich SM, et al. Portal venous thrombus (PVT) following restorative proctocolectomy and ileal pouch-anal anastomosis (IPAA) for ulcerative colitis (UC): does the laparoscopic approach increase the risk of PVT? *Gastroenterology.* (2010) 138:S869. doi: 10.1016/S0016-5085(10)64012-7
44. Gu J, Stocchi L, Gorgun E, Remzi FH. Risk factors associated with portomesenteric venous thrombosis in patients undergoing restorative proctocolectomy for medically refractory ulcerative colitis. *Colorectal Dis.* (2016) 18:393–9. doi: 10.1111/codi.13275
45. Kayal M, Radcliffe M, Plietz M, Rosman A, Greenstein A, Khaitov S, et al. Portomesenteric venous thrombosis in patients undergoing surgery for medically refractory ulcerative colitis. *Inflamm Bowel Dis.* (2020) 26:283–8. doi: 10.1093/ibd/izz169
46. Mathis K, Khanna S, Pardi D, Dozois E. Is concomitant clostridium difficile infection and inflammatory bowel disease associated with poor shortterm outcomes following colectomy? *Dis Colon Rectum.* (2013) 56:e185.
47. Mathis KL, Benavente-Chenhalls LA, Dozois EJ, Wolff BG, Larson DW. Short- and long-term surgical outcomes in patients undergoing proctocolectomy with ileal pouch-anal anastomosis in the setting of primary sclerosing cholangitis. *Dis Colon Rectum.* (2011) 54:787–92. doi: 10.1007/DCR.0b013e318217eea7
48. Medress Z, Fleshner PR. Can we predict unplanned hospital readmission after colectomy for ulcerative colitis and indeterminate colitis? *Am Surg.* (2007) 73:998–1001. doi: 10.1177/000313480707301016
49. Murphy M, Irani J, Shellito P, Bleday R. Portomesenteric venous thrombosis and inflammatory bowel disease: does surgical approach matter? *Dis Colon Rectum.* (2013) 56:e192–e3.
50. Naik AS, Zadvornova Y, Lundeen SJ, Stein DJ, Venu N, Otterson MF, et al. Abdominal venous thrombosis following inflammatory bowel disease related

surgeries while on dalteparin prophylaxis. Case series. *Gastroenterology.* (2011) 140:S432. doi: 10.1016/S0016-5085(11)61772-1
51. Robinson KA, O'Donnell ME, Pearson D, Scott Kriegshauser J, Odeleye M, Kalkbrenner K, et al. Portomesenteric venous thrombosis following major colon and rectal surgery: incidence and risk factors. *Surg Endosc.* (2015) 29:1071–9. doi: 10.1007/s00464-014-3788-z
52. Syed A, Seoud T, Carleton NM, Thakkar S, Kiran RP, Shen B. Association between portal vein thrombosis and pouchitis in patients with ulcerative colitis. *Dig Dis Sci*. (2021). doi: 10.1007/s10620-021-06969-5. [Epub ahead of print].
53. Vaidya P, Lee A, Lightner A, lipman J, Hull T, Steele S, et al. Preoperative hypercoagulable thromboelastography profiles are associated with post-operative venous thromboembolism in inflammatory bowel disease patients. *Gastroenterology.* (2020) 158:S35–6. doi: 10.1016/S0016-5085(20)34544-3
54. Weisshof R, Ollech JE, El Jurdi K, Yvellez OV, Cohen RD, Sakuraba A, et al. Ciclosporin therapy after infliximab failure in hospitalized patients with acute severe colitis is effective and safe. *J Crohns Colitis.* (2019) 13:1105–10. doi: 10.1093/ecco-jcc/jjz032
55. Zaghiyan K, Melmed G, Murrell Z, Fleshner P. Safety and feasibility of using low-dose perioperative intravenous steroids in inflammatory bowel disease patients undergoing major colorectal surgery: a pilot study. *Surgery.* (2012) 152:158–63. doi: 10.1016/j.surg.2012.02.019
56. Grainge MJ, West J, Card TR. Venous thromboembolism during active disease and remission in inflammatory bowel disease: a cohort study. *Lancet.* (2010) 375:657–63. doi: 10.1016/S0140-6736(09)61963-2
57. McCurdy JD, Kuenzig ME, Smith G, Spruin S, Murthy SK, Carrier M, et al. Risk of venous thromboembolism after hospital discharge in patients with inflammatory bowel disease: a population-based study. *Inflamm Bowel Dis.* (2020) 26:1761–8. doi: 10.1093/ibd/izaa002
58. Koutroubakis IE, Sfiridaki A, Mouzas IA, Maladaki A, Kapsoritakis A, Roussomoustakaki M, et al. Resistance to activated protein C and low levels of free protein S in Greek patients with inflammatory bowel disease. *Am J Gastroenterol.* (2000) 95:190–4. doi: 10.1111/j.1572-0241.2000.01683.x
59. Andrade AR, Barros LL, Azevedo MFC, Carlos AS, Damião A, Sipahi AM, et al. Risk of thrombosis and mortality in inflammatory bowel disease. *Clin Transl Gastroen.* (2018) 9:142. doi: 10.1038/s41424-018-0013-8
60. Qi X, De Stefano V, Wang J, Bai M, Yang Z, Han G, et al. Prevalence of inherited antithrombin, protein C, and protein S deficiencies in portal vein system thrombosis and budd-chiari syndrome: a systematic review and meta-analysis of observational studies. *J Gastroenterol Hepatol.* (2013) 28:432–42. doi: 10.1111/jgh.12085
61. Smalberg JH, Koehler E, Darwish Murad S, Plessier A, Seijo S, Trebicka J, et al. Fibrinogen γ' and variation in fibrinogen gamma genes in the etiology of portal vein thrombosis. *Thromb Haemost.* (2013) 109:558–60. doi: 10.1160/TH12-08-0632
62. Lagrange J, Lacolley P, Wahl D, Peyrin-Biroulet L, Regnault V. shedding light on hemostasis in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol.* (2020) 19:1088–97.e6. doi: 10.1016/j.cgh.2019.12.043
63. van der Poll T, Levi M, Büller HR, van Deventer SJ, de Boer JP, Hack CE, et al. Fibrinolytic response to tumor necrosis factor in healthy subjects. *J Exp Med.* (1991) 174:729–32. doi: 10.1084/jem.174.3.729
64. Kaiko GE, Chen F, Lai CW, Chiang IL, Perrigoue J, Stojmirović A, et al. PAI-1 augments mucosal damage in colitis. *Sci Transl Med.* (2019) 11:eaat0852. doi: 10.1126/scitranslmed.aat0852
65. Oussalah A, Guéant JL, Peyrin-Biroulet L. Meta-analysis: hyperhomocysteinaemia in inflammatory bowel diseases. *Aliment Pharm Ther.* (2011) 34:1173–84. doi: 10.1111/j.1365-2036.2011.04864.x
66. Owczarek D, Cibor D, Głowacki MK, Rodacki T, Mach T. Inflammatory bowel disease: epidemiology, pathology and risk factors for hypercoagulability. *World J Gastroenterol.* (2014) 20:53–63. doi: 10.3748/wjg.v20.i1.53
67. Andoh A, Yoshida T, Yagi Y, Bamba S, Hata K, Tsujikawa T, et al. Increased aggregation response of platelets in patients with inflammatory bowel disease. *J Gastroenterol.* (2006) 41:47–54. doi: 10.1007/s00535-005-1721-x
68. Kapsoritakis AN, Koukourakis MI, Sfiridaki A, Potamianos SP, Kosmadaki MG, Koutroubakis IE, et al. Mean platelet volume: a useful marker of inflammatory bowel disease activity. *Am J Gastroenterol.* (2001) 96:776–81. doi: 10.1111/j.1572-0241.2001.03621.x
69. Li T, Wang C, Liu Y, Li B, Zhang W, Wang L, et al. Neutrophil extracellular traps induce intestinal damage and thrombotic tendency in inflammatory bowel disease. *J Crohns Colitis.* (2020) 14:240–53. doi: 10.1093/ecco-jcc/jjz132
70. Jiménez-Alcázar M, Kim N, Fuchs TA. Circulating extracellular DNA: cause or consequence of thrombosis? *Semin Thromb Hemost.* (2017) 43:553–61. doi: 10.1055/s-0036-1597284
71. Sorvillo N, Mizurini DM, Coxon C, Martinod K, Tilvawala R, Cherpokova D, et al. Plasma peptidylarginine deiminase iv promotes vwf-platelet string formation and accelerates thrombosis after vessel injury. *Circ Res.* (2019) 125:507–19. doi: 10.1161/CIRCRESAHA.118.314571
72. Qi X, Han G, He C, Yin Z, Guo W, Niu J, et al. CT features of non-malignant portal vein thrombosis: a pictorial review. *Clin Res Hepatol Gastroenterolog.* (2012) 36:561–8. doi: 10.1016/j.clinre.2012.05.021
73. Tirumani SH, Shanbhogue AK, Vikram R, Prasad SR, Menias CO. Imaging of the porta hepatis: spectrum of disease. *Radiographics.* (2014) 34:73–92. doi: 10.1148/rg.341125190
74. Feuerstein JD, Isaacs KL, Schneider Y, Siddique SM, Falck-Ytter Y, Singh S. AGA clinical practice guidelines on the management of moderate to severe ulcerative colitis. *Gastroenterology.* (2020) 158:1450–61. doi: 10.1053/j.gastro.2020.01.006
75. Sarlos P, Szemes K, Hegyi P, Garami A, Szabo I, Illes A, et al. Steroid but not biological therapy elevates the risk of venous thromboembolic events in inflammatory bowel disease: a meta-analysis. *J Crohns Colitis.* (2018) 12:489–98. doi: 10.1093/ecco-jcc/jjx162
76. van Zaane B, Nur E, Squizzato A, Gerdes VE, Büller HR, Dekkers OM, et al. Systematic review on the effect of glucocorticoid use on procoagulant, anti-coagulant and fibrinolytic factors. *J Thromb Haemost.* (2010) 8:2483–93. doi: 10.1111/j.1538-7836.2010.04034.x
77. Wallaert JB, De Martino RR, Marsicovetere PS, Goodney PP, Finlayson SRG, Murray JJ, et al. Venous thromboembolism after surgery for inflammatory bowel disease: are there modifiable risk factors? Data from ACS NSQIP. *Dis Colon Rectum.* (2012) 55:1138–44. doi: 10.1097/DCR.0b013e3182698f60
78. Dai C, Jiang M, Cao Q. Steroids and the risk of venous thromboembolic events in inflammatory bowel disease. *J Crohns Colitis.* (2018) 12:627. doi: 10.1093/ecco-jcc/jjx183
79. Higgins PD, Skup M, Mulani PM, Lin J, Chao J. Increased risk of venous thromboembolic events with corticosteroid vs biologic therapy for inflammatory bowel disease. *Clin Gastroenterol Hepatol.* (2015) 13:316–21. doi: 10.1016/j.cgh.2014.07.017
80. Johannesdottir SA, Horváth-Puhó E, Dekkers OM, Cannegieter SC, Jørgensen JO, Ehrenstein V, et al. Use of glucocorticoids and risk of venous thromboembolism: a nationwide population-based case-control study. *JAMA internal medicine.* (2013) 173:743–52. doi: 10.1001/jamainternmed.2013.122
81. Brotman DJ, Girod JP, Posch A, Jani JT, Patel JV, Gupta M, et al. Effects of short-term glucocorticoids on hemostatic factors in healthy volunteers. *Thromb Res.* (2006) 118:247–52. doi: 10.1016/j.thromres.2005.06.006
82. Herrinton LJ, Liu L, Fireman B, Lewis JD, Allison JE, Flowers N, et al. Time trends in therapies and outcomes for adult inflammatory bowel disease, Northern California, 1998-2005. *Gastroenterology.* (2009) 137:502–11. doi: 10.1053/j.gastro.2009.04.063
83. Nguyen GC, Elnahas A, Jackson TD. The impact of preoperative steroid use on short-term outcomes following surgery for inflammatory bowel disease. *J Crohns Colitis.* (2014) 8:1661–7. doi: 10.1016/j.crohns.2014.07.007
84. Ali F, Al-Kindi SG, Blank JJ, Peterson CY, Ludwig KA, Ridolfi TJ. Elevated venous thromboembolism risk following colectomy for IBD is equal to those for colorectal cancer for ninety days after surgery. *Dis Colon Rectum.* (2018) 61:375–81. doi: 10.1097/DCR.0000000000001036
85. Kohoutova D, Moravkova P, Kruzliak P, Bures J. Thromboembolic complications in inflammatory bowel disease. *J Thromb Thrombolysis.* (2015) 39:489–98. doi: 10.1007/s11239-014-1129-7
86. Alatri A, Schoepfer A, Fournier N, Engelberger RP, Safroneeva E, Vavricka S, et al. Prevalence and risk factors for venous thromboembolic complications in the Swiss inflammatory bowel disease cohort. *Scand J Gastroenterol.* (2016) 51:1200–5. doi: 10.1080/00365521.2016.1185464
87. Kaplan GG, Lim A, Seow CH, Moran GW, Ghosh S, Leung Y, et al. Colectomy is a risk factor for venous thromboembolism in ulcerative colitis. *World J Gastroenterol.* (2015) 21:1251–60. doi: 10.3748/wjg.v21.i4.1251

88. Fleming F, Gaertner W, Ternent CA, Finlayson E, Herzig D, Paquette IM, et al. The American society of colon and rectal surgeons clinical practice guideline for the prevention of venous thromboembolic disease in colorectal surgery. *Dis Colon Rectum*. (2018) 61:14–20. doi: 10.1097/DCR.0000000000000982
89. Shimada N, Ohge H, Kitagawa H, Yoshimura K, Shigemoto N, Uegami S, et al. High incidence of postoperative silent venous thromboembolism in ulcerative colitis: a retrospective observational study. *BMC Surg.* (2021) 21:247. doi: 10.1186/s12893-021-01250-y

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Cell-Matrix Interactions Contribute to Barrier Function in Human Colon Organoids

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The importance of cell-matrix adhesion to barrier control in the colon is unclear. The goals of the present study were to: (i) determine if disruption of colon epithelial cell interactions with the extracellular matrix alters permeability control measurement and (ii) determine if increasing the elaboration of protein components of cell-matrix adhesion complexes can mitigate the effects of cell-matrix disruption. Human colon organoids were interrogated for transepithelial electrical resistance (TEER) under control conditions and in the presence of Aquamin®, a multi-mineral product. A function-blocking antibody directed at the C-terminal region of the laminin α chain was used in parallel. The effects of Aquamin® on cell-matrix adhesion protein expression were determined in a proteomic screen and by Western blotting. Aquamin® increased the expression of multiple basement membrane, hemidesmosomal and focal adhesion proteins as well as keratin 8 and 18. TEER values were higher in the presence of Aquamin® than they were under control conditions. The blocking antibody reduced TEER values under both conditions but was most effective in the absence of Aquamin®, where expression of cell-matrix adhesion proteins was lower to begin with. These findings provide evidence that cell-matrix interactions contribute to barrier control in the colon.

Keywords: Aquamin®, basement membrane, cell-matrix adhesion, cell-cell junction, colonoid, gut barrier, laminin, proteomics

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INTRODUCTION

Functional defects in the gastrointestinal tract barrier have been documented in inflammatory conditions of the bowel, including both ulcerative colitis (UC) and Crohn's Disease (1–6). Barrier defects have also been described in irritable bowel syndrome (7) and noted in celiac disease (5) and as a consequence of acute bacterial infection (8). Barrier defects have also been seen in obesity related to high-fat and high-sugar diets (9, 10) and, thus, may contribute to chronic, systemic inflammation. Finally, gastrointestinal discomfort associated with chronic environmental stress may reflect barrier dysfunction (11). In these situations, inflammatory injury to the intestinal wall contributes to barrier break-down. At the same time, however, preexisting barrier defects, leading to permeation of bacteria, bacterial products, food allergens and toxins into the mucosal wall, may promote inflammation in the gastrointestinal tract (4).

Tight junctions are the epithelial cell surface structures that mediate permeability control—at least in so far as soluble factors are concerned (12–18). Desmosomes are responsible for tissue cohesion and strength (19, 20). While not directly involved in regulating transepithelial passage of small molecules, an effective barrier in mechanically-active tissue depends on tissue cohesion. In a recent study, it was demonstrated that Aquamin®, a calcium-, magnesium-, and trace element-rich, multi-mineral product obtained from marine red algae (21), strongly up-regulated desmosomal proteins and increased the number of desmosomes in human colon tissue (obtained from normal healthy subjects and UC patients) in organoid culture but had little effect on tight junctional elements (22–24). In parallel with these desmosomal changes, tissue cohesion was increased. In addition, electrical resistance across a monolayer of organoid-derived cells was also increased (23). The same multi-mineral intervention that increased desmosomes also up-regulated expression of other moieties that contribute to the permeability barrier. Among these were cadherin family members (adherens junction components), carcinoembryonic antigen cell adhesion molecules (CEACAM), mucins and trefoils.

In the present study, we have used a proteomic screen to assess the expression of proteins involved in cell-matrix interactions in human colon organoid culture derived from either normal colon tissue or UC disease-affected tissue. Studies by other investigators have utilized immunohistochemical methods to show basement membrane defects in UC and Crohn's disease as well as in other inflammatory conditions of the bowel (25–28). While these findings suggest a role for cell-basement interactions in barrier function, how these interactions influence gastrointestinal barrier function, *per se*, has not been studied. Here it is shown that which proteins are affecting cell-basement membrane interactions through both focal adhesions and desmosomes in response to Aquamin®. Further, it has demonstrated a role of an antibody to the major cell adhesion domain in the laminin α-chain on transepithelial electrical resistance (TEER) in human colon organoid-derived monolayer. In contrast, the effect of treatment with the same antibody is evaluated on tissue cohesion/tissue strength. The findings presented here directly address the role of cell-matrix interactions in barrier function.

MATERIALS AND METHODS

In vitro Intervention - Aquamin®

This is a calcium-rich, magnesium-rich, trace element-rich multi-mineral product obtained from the skeletal remains of the red marine algae, *Lithothamnion sp* (21) (Marigot Ltd, Cork, Ireland). Aquamin® contains calcium and magnesium in a molar ratio of approximately 12:1 along with measurable levels of 72 other trace minerals (essentially all of the trace elements algae fronds accumulate from the deep ocean water). The same single batch of Aquamin® Soluble that was used in the previous colon organoid studies (22–24) was used for this study. **Supplementary File S1** describes the complete mineral/trace element composition of the multi-mineral product—Aquamin®.

Anti-laminin Antibodies and Other Reagents

The known laminin heterotrimers contain a globular region in the C-terminal end of the molecule made up of five modules. Cell-binding sites are located here (29–31). A mouse monoclonal antibody (IgG1 clone) reactive against epitopes within this region (present in all of the individual α chain members) was used for functional blockade. This antibody (clone #P3H9-2; R&D Systems) has been demonstrated to detect antigen in a variety of epithelia and has been shown to inhibit cell proliferation of both rat and human epithelial cells (32). A control mouse monoclonal IgG1 immunoglobulin was used in parallel with the anti-laminin antibody for comparison. A rabbit polyclonal antibody (Invitrogen; PA5-27271) prepared against a recombinant protein fragment from the human laminin β1 chain was used in Western blotting. A monoclonal antibody recognizing a human actin epitope (Cell Signaling Technology; 5125S) was used as control.

Organoid Culture (From Normal Colon or UC Biopsies)

Colon tissue in organoid culture was available from our previous studies (22–24). The Institutional Review Board at the University of Michigan Medical School approved the tissue collection and use protocol (IRBMED protocols: HUM00076276 and HUM00102771). Subjects provided written informed consent prior to flexible sigmoidoscopy and biopsy collection. This study was conducted according to the principles stated in the Declaration of Helsinki. For the present work, cryopreserved colon organoid samples (from healthy subjects) were put into culture and expanded over a 3-4 week period with weekly subculture during the expansion period (23). Growth medium consisted of a 50:50 mix of Advanced DMEM (Gibco) and the same base media that had been conditioned by the previous growth of L-cells engineered to provide a source of recombinant Wnt3a, R-spondin-3, and Noggin—referred to as L-WRN conditioned medium (33). The growth medium formulation also contained 100 ng/ml human recombinant epidermal growth factor (EGF) (R&D) as the major growth-supporting peptide and also contained 10 μM Y27632 (Tocris), 500 nM A83-01 (Tocris), 10μM SB202190 (Sigma), 2.5 μM CHIR99021 (Tocris), 1X B-27 without vitamin A (Invitrogen), 1 mM N-Acetyl-L-cysteine, 10 mM HEPES (Invitrogen), 2 mM Glutamax (Invitrogen), and 100 μg/ml Primocin (InvivoGen). Since L-WRN medium was supplemented with 20% fetal bovine serum, after 1:1 dilution, the final serum concentration of the growth medium was 10%. After expansion, organoids were used to assess TEER or tissue cohesion as described below. TEER assessments were carried out in either differentiation medium or in a mix of KGM Gold and growth medium.

Differentiation Medium

Differentiation medium consisted of a mix of Advanced DMEM and F12 media. This formulation lacked Wnt3a and R-spondin-3 but was supplemented with EGF (50 ng/ml) along with Gastrin (10 nM, Sigma), Noggin (50 ng/ml, R&D), and Y27632 (2.5 μM, Tocris). AlbuMAX® (Gibco), a lipid-rich Bovine Serum Albumin (BSA), was used as a component of the

medium to replace serum. The final calcium concentration in complete differentiation medium was 1.04 mM. This medium was used as a positive control to test monolayer integrity by TEER assessment.

KGM Gold-Growth Medium Mix

KGM Gold is a serum-free, calcium-free medium designed for epithelial cell growth (Lonza) during experimental phase. When KGM Gold was mixed with the growth medium at a 1:4 dilution, the serum concentration decreased to 2.5% and the calcium concentration equaled to 0.25 mM (and this mix was used as a control).

Assessment of Electrical Resistance Across the Organoid-Derived Cell Monolayer

TEER assessments were carried out in the Translational Tissue Modeling (TTML) Laboratory using a standard operating procedure developed in the TTML for organoid evaluation (34). Briefly, colon organoids (from three healthy subjects) were dissociated into small cell aggregates (<40 μm in size) and plated onto collagen IV (Sigma)-coated polyethylene terephthalate filters (0.4 μm pore size, 0.33 cm^2, in transwell filter support, Costar) at 200,000 individual organoids per well in growth medium. After seeding in growth medium, organoids were allowed to attach to the transwell insert filters and incubated without further treatment for 1 day. Then growth medium was replaced with either differentiation medium alone (for initial assessment) or with the KGM Gold-growth medium mix with or without Aquamin®. When Aquamin® was included, it was added at 0.51 mg/ml; an amount to bring the final calcium concentration to 1.5 mM.

The function-blocking anti-laminin antibody (an antibody to the major cell adhesion domain in the laminin α-chain) was included at the start of the treatment period at 25 μg/ml. Fresh culture medium and antibody were provided every 2 days during the assay period. A control mouse IgG was used at the same concentration for comparison. Electrical resistance values were determined using an epithelial volt/ohm meter (EVOM2, World Precision Instruments) and STX2 series chopstick electrodes as described previously (23).

Histochemical Staining and Light Microscopy

After finishing electrical resistance measurements, transwell insert filters with organoid-derived monolayer cells still attached were prepared for light microscopy. The transwell insert filters were fixed for 1 h in 10% buffered formalin. Following this, insert filters were paraffin-embedded, sectioned and stained with hematoxylin and eosin. The stained specimens were visualized by light microscopy. Slides were digitally scanned using the Aperio AT2 brightfield whole slide scanner (Leica Biosystems) at a resolution of 0.5 μm per pixel with 20X objective. Quantitation was performed using Aperio ImageScope by measuring the gap between the epithelial layer and the transwell insert membrane at 20× magnification.

Western Blotting

After finishing electrical resistance measurements on Day 3, organoid-derived monolayer cells were harvested for protein. Briefly, insert wells were washed gently with PBS, then subjected to extraction using RIPA buffer (89901; Thermo Scientific). Organoid-derived monolayer cells were lysed by repetitive pipetting in the buffer, followed by incubation for 10 min on ice. Non-soluble cellular debris was removed by centrifugation at 14,000 x g for 10 min and protein was quantified using a BCA assay (23227; Pierce). Samples were heated for 10 min at 70°C in NuPage LDS sample buffer and then run on 3-8% Tris-Acetate gels using NuPage MOPS running buffer under reducing conditions. Proteins were then transferred onto nitrocellulose membranes, blocked with 5% non-fat dry milk and probed with the primary and appropriate secondary antibodies. Secondary antibodies were used at 1:5,000 for all membranes. β-actin was used as a loading control in each assay. SuperSignal WestPico Plus (34577; Thermo Scientific) detection reagent was used and bands were visualized by exposing the membranes on CL-XPosure Film (34090; Thermo Scientific) and developing the films using Konica Minolta SRX-101A. Relative band density was determined using ImageJ gel analysis tools.

Confocal Fluorescence Microscopy

After finishing electrical resistance measurements, some of the transwell insert filters were prepared for confocal fluorescence microscopy and stained with an antibody to occludin for the purpose of visualizing the cell layer. The filters with cells still attached were fixed for 15 min at −20°C in methanol. They were then washed three times in PBS before blocking in 3% BSA (A8806; Sigma) in PBS for 1 h. Following this, cells were stained with an antibody to occludin (331594; Invitrogen) 1:400 for 1 h in 1% BSA in PBS. Stained cells were rinsed three times (5 min each) in PBS, stained with DAPI for 5 min to identify nuclei and washed an additional three times with PBS. Finally, the filters with cells still attached were gently cut from the transwell inserts and mounted apical side up on Superfrost Plus glass slides (Fisher Scientific, Pittsburgh, PA) with Prolong Gold (P36930; Life Technologies Molecular Probes). The stained specimens were visualized and imaged with a Leica Inverted SP5X Confocal Microscope System (University of Michigan Medical School Biomedical Research Core Facility).

Organoid Cohesion Assay

Organoid cohesion was assessed by employing healthy colon-derived organoids from three subjects as described previously (23). Briefly, after establishment and culture expansion, healthy colon organoids were incubated in KGM Gold-growth medium with or without the same anti-laminin antibody (25 μg/ml) as described above. Treatment was for seven days with fresh medium and antibody added at days 2 and 4. Over the course of the 7-day treatment period, individual organoids increased in size. At the end of the incubation period, phase-contrast microscopy (Hoffman Modulation Contrast—Olympus IX70 with a DP71 digital camera) was used to capture images in

order to measure the size of multiple individual organoids (53-104 individual organoids per condition). Then organoids were separated from the Matrigel and fragmented with mechanical force alone by pipetting the entire pellet 30x through an uncut 200 microliter pipet tip. After washing 3x in PBS, organoids were re-cultured in fresh Matrigel. One day after establishment, multiple organoids were again examined under phase-contrast microscopy and sized. For both pre-harvest and post-harvest samples, phase-contrast images were analyzed using area measurements in Adobe Photoshop (CC version 19.1.5). Average organoid size-reduction (i.e., the difference in organoid size between pre- and post-harvest) was determined by dividing the average post-harvest surface area by the average pre-harvest area.

Differential Proteomic Analysis

Proteomic assessment was conducted at the Proteomics Resource Facility (PRF) in the Department of Pathology at the University of Michigan using mass spectrometry (MS)-based tandem mass tag (TMT) analysis (ThermoFisher Scientific). The complete details for the experimental conditions, protocols and analysis methodology can be found in previously published reports (22, 24). Briefly, colon organoids (normal healthy subjects and subjects with UC) were exposed to 2mM EDTA for 15 min to dissolve and completely remove Matrigel and then exposed to Radioimmuno-precipitation assay (RIPA)—lysis and extraction buffer (Thermo Scientific, Rockford, IL) for protein isolation. Fifty micrograms of organoid protein from each condition were digested with trypsin and individually labeled with isobaric mass tags. Labeled peptides were fractionated using 2D-LC (basic pH reverse phase separation followed by acidic pH reverse-phase) and analyzed on a high-resolution, tribrid mass spectrometer (Orbitrap Fusion Tribrid, ThermoFisher Scientific) using conditions optimized in the PRF. MultiNotch MS3 was employed to obtain accurate quantitation of the identified proteins/peptides. Data analysis involved peptide filtering to retain only those that passed $\leq 2\%$ false discovery rate (FDR) threshold of detection. Quantitation was performed using high-quality MS3 spectra. Differential protein expression values (fold-change) for proteins of interest in each treatment group were compared to protein values of the respective control group. Proteins were identified using Universal Protein Resource (UniProt) databases (Uniprot.org). Reactome version 78—a pathway analysis database was used to recognize associated pathways for species "*Homo sapiens*" (reactome.org) by providing the entities detected in the proteomic data sets (both from normal and UC data sets). Reactome is a curated and peer-reviewed database of pathways and reactions in human biology. Reactome database identifies possible reactions with all annotated proteins present and active simultaneously in a cell. Pathway over-representation analysis is performed by overlaying an experimental dataset on these annotations (35). Additionally, STRING database—v11.5 (string-db.org) was utilized to conduct enrichment analyses and to identify protein-protein interactions among the proteins. For proteomic enrichment analysis, STRING employs Gene Ontology (GO) knowledgebase and provide information related to molecular functions, biological processes and cellular components involved.

For the purpose of the present study, we accessed two existing data sets—one generated from colon organoids of four healthy subjects and the other generated from colon organoid tissue of three ulcerative colitis patients in remission. In each case, organoids grown in the KGM Gold-growth medium mix were compared to organoids grown in the same medium supplemented with Aquamin® at levels providing 1.5-3.0 mM calcium. Protein expression levels with Aquamin® were compared to protein-expression levels in the control to obtain fold-change ratios for individual proteins of interest with each subject separately. Following this, data from individual subjects were merged and analyzed as groups ($n = 4$ healthy and $n = 3$ UC in remission). For comparison purposes, the data presented here include only the maximum response. The complete proteomics data sets are available at the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD020244 (for UC derived colon organoids) and identifier PXD026923 (for normal colon organoids).

Statistical Analysis

Means and standard deviations were obtained for discrete values obtained in the TEER assessment and cohesion assays as well as from expression level changes for individual proteins in proteomic assessment). Data generated in this way were analyzed by ANOVA followed by paired *t*-test (two-tailed) for comparison using GraphPad Prism version 8.3. For the pathways analysis, the significant data were based on the overrepresentation analysis (hypergeometric distribution) using Reactome database. A binomial test was used to calculate the probability for each result, and the *p*-values were corrected for the multiple testing (Benjamini–Hochberg procedure) that arose from evaluating the submitted list of identifiers against every pathway. A high-level of FDR stringency (<1%) was used and the whole genome statistical background was assumed for STRING analysis. A p-value < 0.05 was considered significant.

RESULTS

Aquamin® Up-Regulates Basement Membrane Components, Proteins Associated With Hemidesmosome Formation and Keratins

Findings from the proteomic assessment based on data from four healthy subjects and data from three subjects with UC are shown in **Figure 1**. In both data sets (assessed independently), strong up-regulation of several laminin chains (α1, β1, β2, and γ1) (components of laminin 111 and 121) along with nidogen-1, the basement membrane-specific heparin sulfate proteoglycan (HSPG-2, perlecan) and one of the chains of type IV collagen (α2) was seen in response to Aquamin®, regardless of the tissue type. These proteins are the major constituents of the basement membrane (36, 37). They mediate cell-matrix attachments (focal adhesions) in epithelial cells (30, 31, 36, 37). Also detected in the proteomic analysis (**Figure 1**) were laminin α3, β3, and

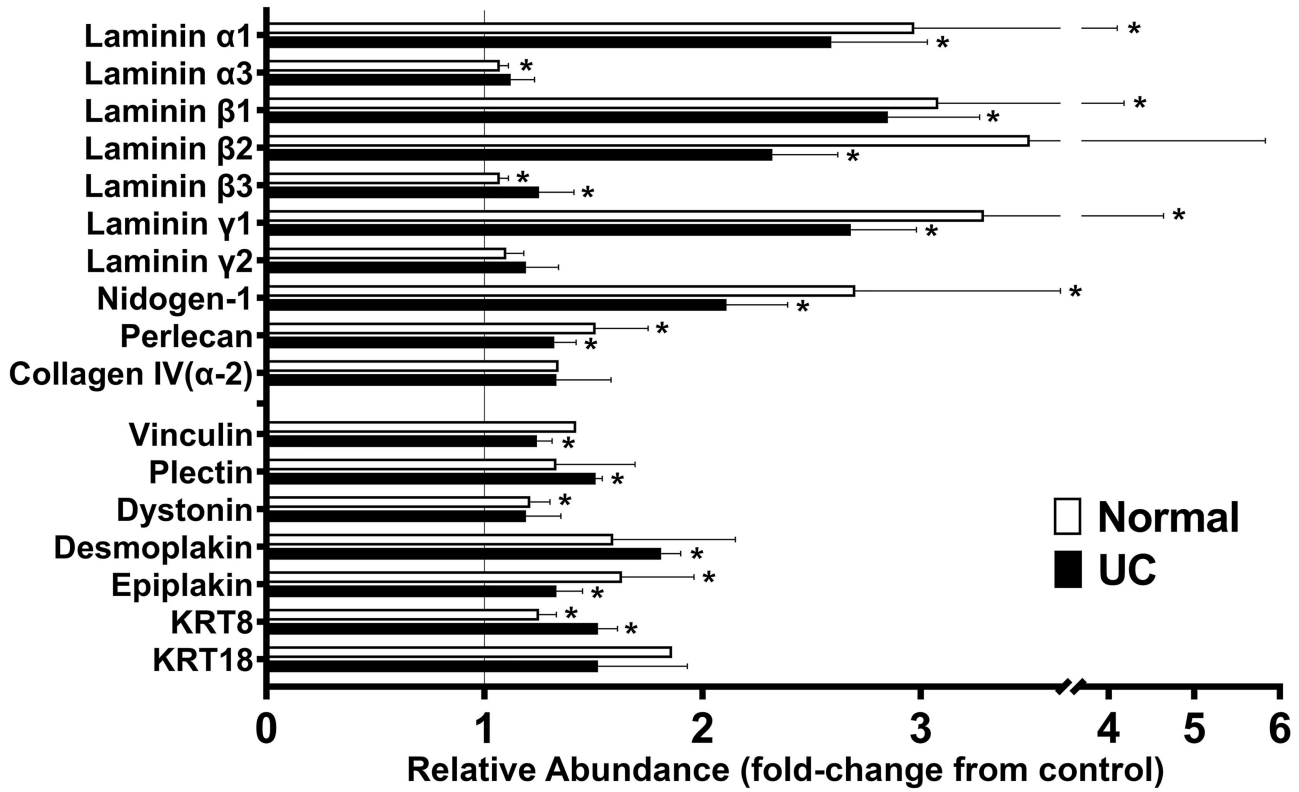

FIGURE 1 | Effects of Aquamin® on expression of proteins involved in cell-matrix interactions: Proteomic screen. Organoid cultures were established with colon biopsies from four healthy subjects and three with ulcerative colitis in remission. Organoids were cultured in the presence or absence of Aquamin® as described in the Materials and Methods section. At the end of the incubation period, protein was isolated from the cultures and assessed in the proteomic screen. Values from respective controls were set at 1.0 and the values from Aquamin®-treated samples reflect a ratio (increase or decrease fold-change) relative to respective control. Values shown reflect mean (±SD) fold-change from respective control in colon organoids from normal subjects ($N = 4$) and subjects with UC in remission ($N = 3$). Methods for generating the data sets were described in detail in past reports (22, 24). Data for individual proteins were compared for statistical differences using the student *t*-test. Asterisk (*) indicates a difference from respective control at $p < 0.05$.

γ2 chains (components of laminin 332 or laminin-5 in the older terminology). This laminin isoform is a major component of hemidesmosomes (38, 39). While these laminin chains did not demonstrate an increase in response to Aquamin® in colon organoids, several other hemidesmosomal proteins were detected, and a subset of these (dystonin, plectin, desmoplakin, and epiplakin) were also increased by Aquamin® as compared to control (**Figure 1**). The plakins are critical linkers between laminin in the hemidesmosomes and intermediate filaments (40, 41). Similarly, additional hemidesmosomal components (BP180 or Collagen Type XVII α1 Chain, and CD151) were also detected in both datasets. The abundance ratios for BP180 (1.14 ± 0.19-fold in normal vs. 1.21 ± 0-fold in UC) and CD151 (0.94 ± 0.07-fold in normal vs. 0.96 ± 0.06-fold in UC) did not increase with Aquamin®. In addition, three proteins that serve as connectors between focal adhesions and the actin cytoskeleton (talin, vinculin and α-actinin) were detected. Vinculin was modestly up-regulated in both data sets (**Figure 1**).

While this study did not address differentiation-related proteins, *per se*, since we have previously reported on this (22, 24), we noted that keratin 8 and keratin 18 (components of intermediate filaments in gastrointestinal epithelial cells) (42) were increased in response to Aquamin® (**Figure 1**). With keratin 8, expression was increased 1.25 ± 0.01-fold and 1.52 ± 0.09-fold in the normal and UC data sets, respectively. With keratin 18, values were 1.52 ± 0.41-fold and 1.86 ± 0.00-fold. Of interest, recent studies have demonstrated that mutations in Keratin 8/18 in colonic epithelial cells are associated with loss of permeability control in inflammatory bowel disease (43). Similarly, acute bowel inflammation has been shown to reduce Keratin 8/18 expression; levels were restored upon improvement in disease status as assessed by both clinical and endoscopic parameters (44).

In addition to the findings presented above, other proteins of interest were searched for in the protein screen. Subunits of laminin-binding integrins (α3, α6, β1, and β4) (45) were present, but not significantly altered (ranged from 0.90 to 0.96-fold-change) with Aquamin® as compared to control (not shown). Among other moieties that have been reported to interact with laminin, both dystroglycan and syndecan were slightly down-regulated, sulfatide was unchanged and oncofetal

TABLE 1 | Top pathways associated with the proteins presented in **Figure 1**.

Pathway name	Entities p-value	Entities FDR	Mapped entities
Laminin interactions	1.11×10^{-16}	5.55×10^{-15}	COL4A2;LAMA1;LAMA3;LAMB1;LAMB2;LAMB3; LAMC1;LAMC2;HSPG2;NID1
Non-integrin membrane-ECM interactions	1.11×10^{-16}	5.55×10^{-15}	COL4A2;LAMA1;LAMA3;LAMB1;LAMB2;LAMB3; LAMC1;LAMC2;HSPG2
Extracellular matrix organization	2.22×10^{-15}	7.33×10^{-14}	COL4A2;DST;LAMA1;LAMA3;LAMB1;LAMB2;LAMB3; LAMC1;LAMC2;HSPG2;NID1;PLEC
MET activates PTK2 signaling	3.00×10^{-14}	7.49×10^{-13}	LAMA1;LAMA3;LAMB1;LAMB2;LAMB3;LAMC1;LAMC2
MET promotes cell motility	2.64×10^{-13}	5.29×10^{-12}	LAMA1;LAMA3;LAMB1;LAMB2;LAMB3;LAMC1;LAMC2
Type I hemidesmosome assembly	7.62×10^{-12}	1.22×10^{-10}	DST;LAMA3;LAMB3;LAMC2;PLEC
ECM proteoglycans	1.93×10^{-11}	2.70×10^{-10}	COL4A2;LAMA1;LAMA3;LAMB1;LAMB2;LAMC1; HSPG2
Degradation of the extracellular matrix	2.27×10^{-11}	2.73×10^{-10}	COL4A2;LAMA3;LAMB1;LAMB2;LAMB3;LAMC1; LAMC2;NID1;HSPG2
Signaling by MET	2.75×10^{-11}	3.03×10^{-10}	LAMA1;LAMA3;LAMB1;LAMB2;LAMB3;LAMC1;LAMC2
Assembly of collagen fibrils and other multimeric structures	4.49×10^{-10}	4.49×10^{-9}	COL4A2;DST;LAMA3;LAMB3;LAMC2;PLEC
Collagen formation	4.51×10^{-9}	4.06×10^{-8}	COL4A2;DST;LAMA3;LAMB3;LAMC2;PLEC
Anchoring fibril formation	9.57×10^{-9}	7.66×10^{-8}	COL4A2;LAMA3;LAMB3;LAMC2
Cell junction organization	2.88×10^{-7}	2.02×10^{-6}	DST;LAMA3;LAMB3;LAMC2;PLEC
Signaling by receptor tyrosine kinases	7.83×10^{-7}	5.48×10^{-6}	COL4A2;LAMA1;LAMA3;LAMB1;LAMB2;LAMB3; LAMC1;LAMC2
Cell-cell communication	1.57×10^{-6}	9.39×10^{-6}	DST;LAMA3;LAMB3;LAMC2;PLEC
Signal transduction	0.001	0.003	COL4A2;DSP;DST;LAMA1;LAMA3;LAMB1;LAMB2; LAMB3;LAMC1;LAMC2;VCL
Post-translational protein phosphorylation	0.001	0.003	LAMB1;LAMB2;LAMC1
L1CAM interactions	0.001	0.004	LAMA1;LAMB1;LAMB2;LAMC1
Formation of the cornified envelope	0.001	0.004	DSP;KRT8;KRT18
Keratinization	0.005	0.014	DSP;KRT8;KRT18

*The pathway analysis was conducted by Reactome database (v78) for species "Homo sapiens" employing the entities presented in **Figure 1**. These significant data (with a p-value/FDR < 0.05) are based on the overrepresentation analysis (hypergeometric distribution). A binomial test is used to calculate the probability for each result, and the p-values are corrected for the multiple testing (Benjamini–Hochberg procedure) that arises from evaluating the submitted list of identifiers against every pathway. FDR, False discovery rate.*

antigen/immature laminin receptor OFA(iLRP)/67-kD laminin receptor was not detected.

As part of the analysis, we searched for the pathways associated with the proteins presented in **Figure 1** using Reactome. The top 20 pathways with the involved entities are presented in **Table 1**. Laminin interactions, extracellular matrix organization and type I hemidesmosome assembly were among the top pathways (**Table 1**). To check the protein-protein interaction (PPI) of the moieties shown in **Figure 1**, we used the STRING database, and the PPI enrichment *p*-value was $<1.0 \times 10^{-16}$. **Supplementary Figure 1** showed these strong protein-protein interactions among these proteins. Lastly, we have shown the GO-based enrichment data in **Supplementary Table 1**. There were 38 biological processes, 7 molecular functions, and 31 cellular components involved based on these annotations (**Supplementary Table 1**). These data further demonstrated the involvement of these proteins in various cell-cell and cell-matrix adhesion-related processes as suggested by the pathways analysis. Basement membrane, laminin complex, extracellular region, extracellular space, anchoring junction and extracellular exosome are some of the top cellular components involved (**Supplementary Table 1**).

Western blotting with an antibody to the laminin β1 chain (most highly up-regulated of all the laminin chains detected in the proteomic screen) was used to confirm laminin up-regulation. **Figure 2** shows the remarkable increase in laminin β1 expression in Aquamin®-treated organoids as compared to control. The complete film along with the nitrocellulose blot are presented in the **Supplementary Figure 2**.

TEER Values in Cell Monolayers Established From Organoids: Effects of Aquamin® and Anti-laminin Treatment

Preliminary studies were carried out (following the standard operating procedure) in the TTML. For these studies, organoids were plated on transwell insert filters in growth medium. One day later, growth medium was replaced with a formulation optimized in the TTML for assessing electrical resistance (34). This formulation, referred to as differentiation medium, was described in the Materials and Methods section. TEER values

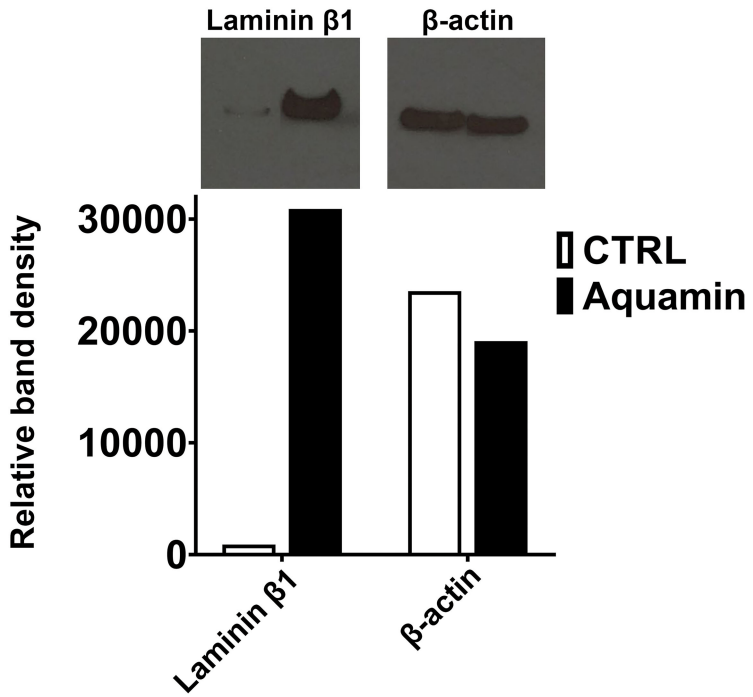

FIGURE 2 | Effects of Aquamin® on expression of Laminin β1: Western blotting. Protein isolated from control and Aquamin®-treated (healthy normal subjects) colon organoid-derived monolayer cells was assessed for laminin β1 expression by Western blotting as described in the Materials and Methods section. 10 μg of protein from each condition was used. β-actin was assessed in parallel (as a loading control). Band quantitation was done using ImageJ software. Relative band density is presented for laminin β1 and β-actin.

were determined daily beginning on the next day. Results are shown in **Figure 3**. **Figure 3A** demonstrates that under conditions optimized to promote electrical resistance, TEER values were low during the first 2 days after treatment, rose precipitously at day-3, remained elevated through day-6 (except with a slight decrease every day) and declined thereafter. A combination of antibody to occludin (tight junctional protein) and DAPI (nuclear stain) was used to illuminate organoids and cell outgrowth from the organoids on the transwell filters at day-2 and day-5. As shown in the inserts in **Figure 3A**, intact cell-cell borders could be seen between cells in the organoids, themselves, by day-2. However, cell outgrowth from the organoids did not completely cover the transwell insert filter surface at this time (accounting for the lack of electrical resistance). Coverage of the filter surface was complete by day-5. The effects of the function-blocking antibody—anti-laminin α3 (25 μg/ml) on electrical resistance in differentiation medium are shown in **Figure 3B**. A modest decrease in TEER values was observed at days-3,−4, and−5 (9-17% decrease; not statistically significant). Lower antibody concentrations were not effective.

In parallel, electrical resistance was assessed in the KGM Gold-growth medium mix. Similar to what was observed in differentiation medium, TEER values were low on day 1 and day 2 ($<100\ \Omega$ x cm^2) but rose sharply such that maximum values were observed on day-3 (1,700-1,900 Ω x cm^2), depending on experiment. Values remained elevated through day-5 and then fell (not shown).

At the completion of TEER assessment (on day-3) in differentiation medium, transwell insert filters with cells still attached were fixed in 10% buffered formalin, stained with hematoxylin and eosin and examined at the light microscopic level (**Figure 3B** insert). It can be seen that under control conditions (IgG-treated cells) or in the same medium with anti-laminin antibody, the filter surface was covered with a complete monolayer of cells. However, in the presence of the anti-laminin antibody, focal areas where cells had detached from the underlying substrate could be observed. In these areas, cell-cell attachments remained intact such the structure had the appearance of a tiny blister. When these visible gaps in the detached monolayer were digitally quantified, the gaps decreased from 16 ± 18 μm in the presence of anti-laminin antibody to 5 ± 7 μm under control conditions.

Based on the outcome of the preliminary studies, KGM Gold-growth medium was used in subsequent experiments. Anti-laminin antibody was included at a final concentration of 25 μg/ml and electrical resistance was determined at day-3.

Following the preliminary studies described above, human colon organoids were plated on transwell filters in growth medium. One day later, growth medium was replaced with the KGM Gold-growth medium mix (0.25 mM calcium; final concentration). In some wells, Aquamin® was added to bring the final calcium level to 1.5 mM and provide the additional trace elements that make up the marine algae product. Electrical resistance across the cell layer was assessed as described

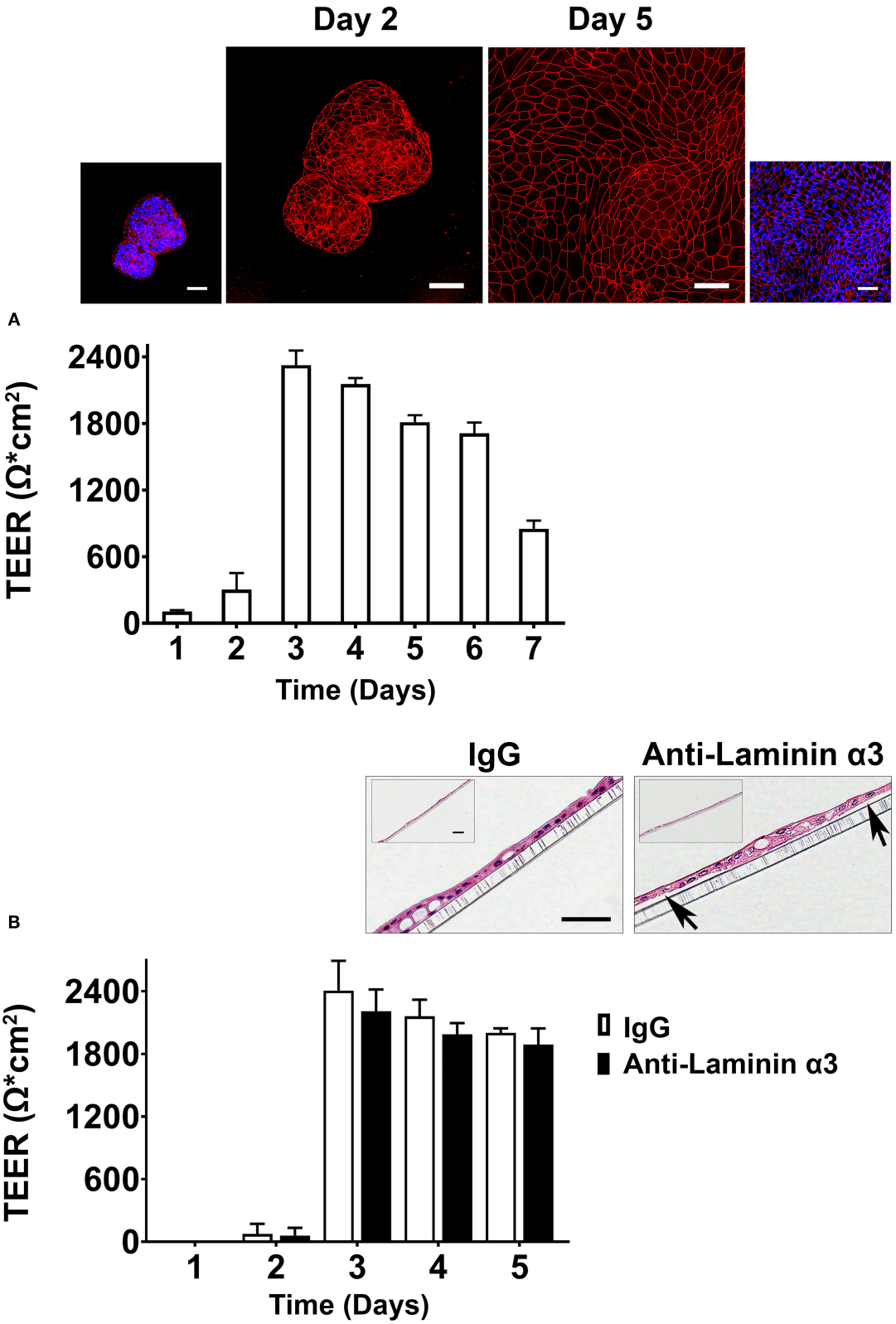

FIGURE 3 | Transepithelial electrical resistance in differentiation medium (Preliminary assessment). **(A)** Time-dependent changes in TEER values. Values shown are means and standard deviations based on four separate experiments with four samples (individual transwell insert filters seeded with healthy colon organoid-derived monolayer cells) per data point at each time-point in each experiment. Insert: Confocal fluorescent microscopic (max-projected) images of organoids and

(Continued)

FIGURE 3 | organoid-derived cells on transwell inserts stained after the day-2 and day-5 readings with antibody to occludin and with a combination of antibody to occludin and DAPI. Scale bars = 50 μm. **(B)** Effects of anti-laminin antibody on TEER values. Values shown are means and standard deviations based on two separate experiments with 4 samples (individual transwell insert filters seeded with healthy colon organoid-derived monolayer cells) per data point at each time-point in each experiment. Insert: hematoxylin and eosin-stained images of the cell monolayers still attached to the transwell inserts from IgG-treated and anti-laminin-treated wells. Arrows in the anti-laminin-treated image show areas where cell detachment from the underlying transwell insert was visible. Scale bar = 100 μm (small) and 50 μm (Large).

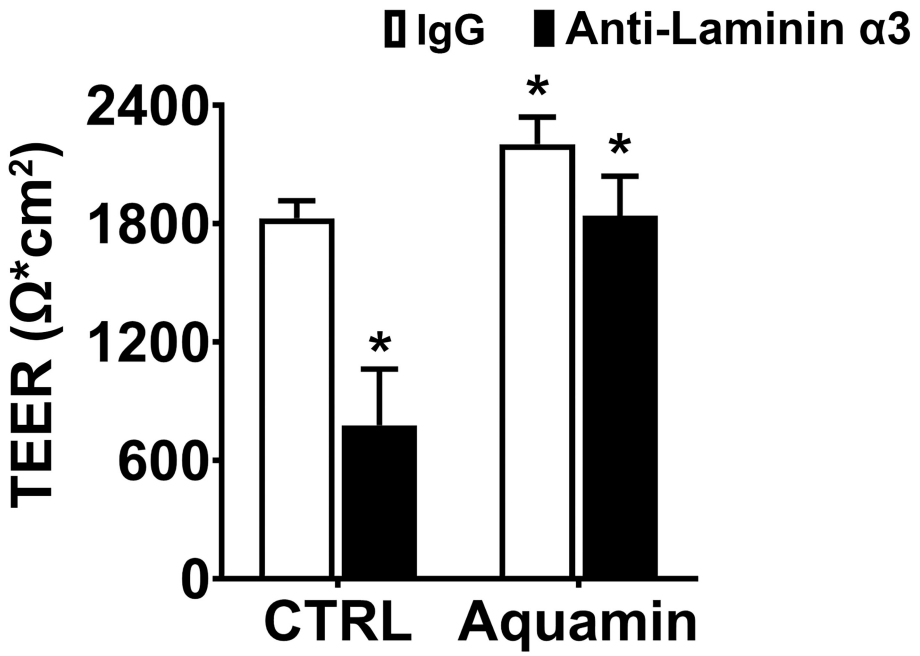

FIGURE 4 | Transepithelial electrical resistance in KGM Gold-growth medium with or without Aquamin® and with or without anti-laminin antibody. TEER values shown are means and standard deviations based on three separate experiments with four samples (individual transwell insert filters seeded with healthy colon organoid-derived monolayer cells) per data point in each experiment. Data were compared for statistical differences using ANOVA followed by unpaired-group comparisons. Asterisk (*) above the open Aquamin® bar indicates a difference from control at $p < 0.05$. Asterisks (*) above the closed bars indicates difference from respective IgG control at $p < 0.05$.

above on day-3). In the unsupplemented KGM Gold-growth medium mix, a TEER value of 1,828 Ω x cm^2 was achieved as compared to 2,325 Ω x cm^2 in differentiation medium (21.5% decrease) while the TEER value in Aquamin® supplemented medium (2,214 Ω x cm^2) was virtually identical to that seen in differentiation medium (compare values in **Figure 4** with those in **Figure 3A**—Day 3). **Figure 4** also shows the effects of anti-laminin treatment on TEER values in the two conditions. In Aquamin-supplemented medium, TEER values were reduced by 16% with anti-laminin. This is comparable to what was seen in differentiation medium (compare values to those in **Figure 3B**). In unsupplemented KGM Gold-growth medium, where TEER values were lower to begin, the inclusion of anti-laminin antibody further reduced TEER values to 787 ± 288 Ω x cm^2 (57% decrease).

Effects of Anti-laminin Antibody on Organoid Cohesion

In our previous study, we demonstrated that treatment of human colon organoids with Aquamin® increased organoid cohesion in parallel with TEER values. Specifically, organoids maintained in KGM Gold-growth medium without Aquamin® fragmented into much smaller pieces than did organoids grown in the presence of Aquamin® and subjected to the same mechanical disruption protocol (23). We attributed increased cohesion in the presence of Aquamin® to the increase in desmosomes seen in parallel. This does not, of course, rule out the possible contribution of other adhesive interactions. To determine whether interactions involving laminin contributed to intra-organoid cohesion, colon organoids were maintained for 1 week in KGM Gold-growth medium with either IgG or the same anti-laminin antibody that reduced TEER values. At the end of the incubation period, cohesion was assessed as described in Methods and in our previous study (23). No detectable antibody effect on organoid cohesion was seen. Specifically, there was no difference between IgG-treated and anti-laminin-treated organoids in average organoid size after harvest and fragmentation (i.e., post- to pre-harvest ratio). This was 0.45 and 0.46 with IgG and anti-laminin antibody, respectively (**Figure 5**).

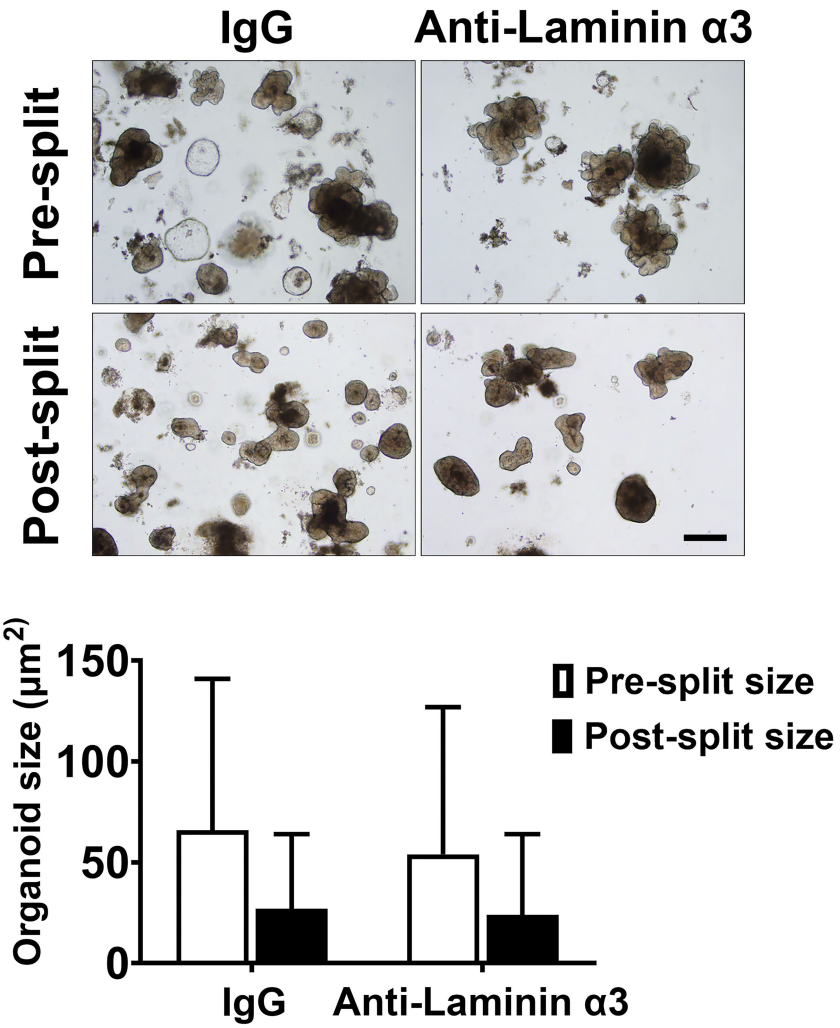

FIGURE 5 | Colon organoid cohesion in KGM Gold-growth medium mix: Effect of anti-laminin antibody. Colon organoids were maintained for 7 days in KGM Gold-growth medium with either IgG or anti-laminin. At the end of the incubation period, organoid cohesion was assessed as described in the Materials and Methods section. Values shown represent the change in organoid size (i.e., mean surface area ± SD of individual colon organoids based on two separate experiments with a minimum of 53-104 colon or-ganoids assessed individually per treatment group in both pre- and post-harvest cultures. Data were compared for statistical differences using ANOVA followed by unpaired-group comparisons. While the decrease in organoid size between post-harvest and pre-harvest organoids were statistically significant with either IgG or anti-laminin, the differences between anti-laminin and IgG were not different. Inset: Representative examples of organoid appearance immediately prior to harvest (upper) and 1 day after the harvested organoids had been reestablished in culture. Scale bar = 200 μm.

DISCUSSION

Most studies of barrier dysfunction in the gastrointestinal tract have focused on the structural components that regulate cell-cell interactions (i.e., desmosomes and, especially, tight junctions) (16–19), but basement membrane disruptions are also commonly observed (25–28). Experimental animal models of colitis, likewise, demonstrate basement membrane disruptions in inflamed colonic tissue (28, 46). In all of these settings, a loss or reduction in laminin immunoreactivity is commonly observed (25–28), although altered distribution of laminin forms has been reported as well, with some forms actually increasing (27). Laminin is not unique in being altered in inflammatory bowel conditions. Basement membrane collagens including type IV have been reported to be increased in inflamed bowel (28). Together, these past findings provide a picture of widespread cell-matrix disruption in the context of the inflamed colon. Although these changes are thought to be a consequence of the inflammatory process, anomalies have been noted in some patients with inflammatory bowel disease in the absence of acute tissue damage (4). Thus, preexisting basement membrane irregularities may contribute to inflammation, and not simply be the consequence of tissue injury. In support of this, a murine model in which laminin α-chain was overexpressed showed a decreased sensitivity to chemical-induced colitis (28). In another model, hemidesmosome disruption promoted colitis (46) in genetically manipulated animals.

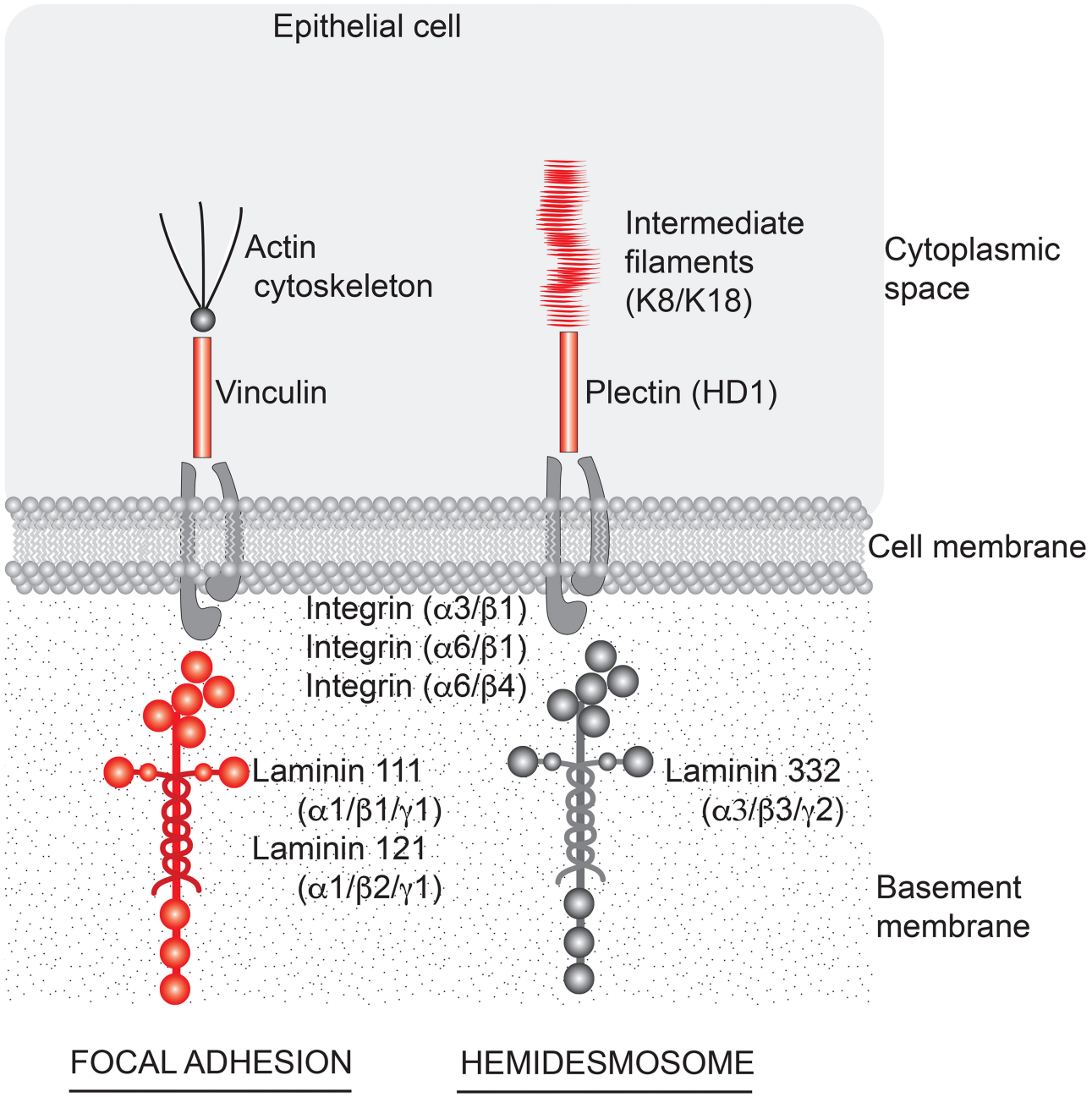

FIGURE 6 | Aquamin®-responsive cell-matrix adhesion structures in the colon. A cartoon depicting structures important to cell-matrix adhesion in the colon and components of those structures that are responsive to Aquamin® (shown in red). Based on the profile of proteins that are induced by Aquamin®, cell-matrix adhesion through both focal adhesions and hemidesmosomes could be affected. Laminin-binding integrins did not alter with Aquamin®.

Regardless of whether preexisting barrier defects in the gastrointestinal tract promote bowel inflammation or are simply the consequence of inflammation, improvement in barrier structure/function would seem to be of value. The findings presented here demonstrate that interfering with cell-basement membrane interactions reduces electrical resistance across the cell layer (a measure of permeability control) without a major effect on tissue cohesion in human colon organoid culture. Our findings also demonstrate that treating colon organoids with a multi-mineral supplement increases the elaboration of basement membrane proteins and hemidesmosomal/intermediate filament components while partially mitigating the consequences of interfering with cell-basement membrane interactions. As summarized graphically in the cartoon (**Figure 6**), the basement membrane, hemidesmosomal and intermediate filament proteins that are responsive to Aquamin® treatment could be expected

to have an effect on both focal adhesions and hemidesmosomes (47). In our previous studies, the same mineral supplement was shown to substantially increase desmosome formation along the lateral surface of adjacent epithelial cells in colon organoid culture without a major effect on tight junctions (22–24). Thus, while functioning tight junctions are directly responsible for permeability control, our past findings suggest that permeability control cannot be optimally maintained in a mechanically active tissue such as the colon when cell-cell cohesion (22–24) is disrupted. The data presented here extend this conclusion to cell-matrix interactions. These data suggest that cell-matrix interactions also play a contributing role in barrier function. It should be noted, of course, that cell-matrix interactions are complex and involve multiple cell surface and cytoskeletal proteins on the one hand, and several different matrix moieties on the other. The use of a single blocking antibody which could interfere, presumably, with laminin binding to many different partners precludes a more precise determination of the relative importance of different individual cell-matrix protein combinations to the overall result. This notion has been tested before by targeting a specific molecule (kalinin) using an antibody in keratinocyte cell culture, and concluding that that kalinin (i.e., laminin 332) is the critical component of basement membrane (48).

How Aquamin® functions to improve barrier structure/function is not fully understood. Many of the matrix-related proteins found to be up-regulated here are products of differentiation, and calcium, the most abundant mineral in the algae product (21), is the quintessential regulator of epithelial differentiation (49). While calcium is undoubtedly critical, many of the additional trace elements in Aquamin® have a higher affinity than calcium itself for the extracellular calcium-sensing receptor (50–52). They act like calcimimetic agents to "left-shift" the response to calcium. We believe that increasing the elaboration of critical barrier proteins is an important mechanism by which Aquamin® promotes barrier function. At the same time, calcium, magnesium and manganese are all critical to the protein-protein interactions that mediate cell-cell and cell-matrix adhesion interactions (53). Thus, the contribution of Aquamin® to barrier function likely extends beyond driving new protein production.

The studies carried out here made use of a sophisticated *ex vivo* culture system (colon tissue maintained in organoid culture) and comprise data from seven different subjects for proteomics, three different subjects for organoid-derived monolayer culture on transwell membrane and three different subjects for the tissue cohesion assay. This *ex vivo* system used here may have its limitation while lacking *in vivo* environment, but it provides a good substitute for colonic tissue to test interventions *ex vivo* (54). Still, whether the effects obtained *in vitro* have relevance to what occurs *in vivo* remains to be demonstrated. In an effort to begin addressing this issue, we have recently carried out a pilot phase trial in which 10 healthy subjects were treated with the same multi-mineral product (Aquamin®) used here. To summarize the results of this pilot study, there were no tolerability issues with daily Aquamin® ingestion over a 90-day period and no safety concerns (55, 56). Equally important, when Aquamin®-treated subjects were compared to subjects receiving placebo for the same period, we saw up-regulation of laminin chains along with increased levels of other basement membrane components and hemidesmosome moieties in colonic biopsies (56). Subjects receiving calcium alone (i.e., the most abundant mineral in Aquamin®) also demonstrated increases in several of the same molecules, but the degree of up-regulation with calcium alone was lower than that seen with Aquamin® (56).

As a follow-up, we are conducting a 180-day interventional trial with Aquamin® in UC patients (ClinicalTrials.gov: NCT03869905). In addition to evaluating therapeutic benefit, the same approaches used in the earlier trial with healthy individuals (immunohistology and proteomics) are being used to evaluate proteins changes in the colon over the course of intervention. In parallel, the urine lactulose/mannitol ratio (57) is being assessed to provide a direct measure of treatment effects on gastrointestinal permeability (ClinicalTrials.gov: NCT04855799). If successful, Aquamin® or a similarly formulated product could be used as a low-cost, low- to no-toxicity adjuvant therapy to improve gastrointestinal barrier function in individuals suffering from a variety of gastrointestinal maladies. At the very least, individuals with barrier defect-associated gastrointestinal conditions should be encouraged to include an adequate source of calcium and other minerals in their diet. Unfortunately, deficiencies in calcium and other critical mineral components are widespread throughout the world (58) and this is especially true for those consuming a Western-style diet (59, 60).

Finally, there is another group of diseases—epidermolysis bullosa and related conditions—that are manifestations of mutations in various basement membrane, desmosomal/hemidesmosomal and keratin genes (61). At the same time, there are case reports and studies that provide evidence of an association between bullous pemphigoid and inflammatory bowel disease (62–64). At this point, we can only speculate as to whether optimizing the expression of multiple cell-cell and cell-matrix adhesion molecules in an individual might overcome, at least in part, the consequences of a function-modifying mutation in one or another critical component. If this turns out to be the case, it could open the door to a new adjuvant therapeutic approach. While speculative for now, experimental models in which a hypothesis could be tested are available (65–67).

In summary, an intact barrier is required for healthy gastrointestinal function. While cell-cell adhesion structures are well-known participants in effective barrier function, the present study provides evidence that cell-matrix interactions are also important. These studies show, furthermore, that a multi-mineral natural product has the capacity to stimulate the production of cell-matrix adhesion moieties and, concomitantly, to improve barrier control.

DATA AVAILABILITY STATEMENT

The mass spectrometry proteomics datasets presented in this study can be found in online repositories – on ProteomeXchange Consortium (PRIDE partner repository) with identifier

PXD020244 (for UC-derived organoids) and identifier PXD026923 (for normal colon organoids).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board at the University of Michigan Medical School (IRBMED). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MA and JV: conceptualization, resources, writing—original draft preparation, and funding acquisition. MA, SM, and JV: methodology, validation, investigation, writing—review and editing. MA and SM: software, formal analysis, and data curation. MA: visualization, supervision, and project administration. All authors have read and agreed to this version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.838975/full#supplementary-material

Supplementary Data Sheet 1: S1 File | Mineral composition of Aquamin® Soluble.

Supplementary Data Sheet 2: Figure 1 | Protein-Protein interactions of proteins shown in **Figure 1**.

Supplementary Data Sheet 3: Figure 2 | Source documentation for Western blot data shown in **Figure 2**.

Supplementary Table 1 | GO-based STRING enrichment data.

REFERENCES

1. Salim SY, Söderholm JD. Importance of disrupted intestinal barrier in inflammatory bowel diseases. *Inflamm Bowel Dis.* (2011) 17:362-81. doi: 10.1002/ibd.21403
2. Antoni L, Nuding S, Wehkamp J, Stange EF. Intestinal barrier in inflammatory bowel disease. *World J Gastroenterol.* (2014) 20:1165-79. doi: 10.3748/wjg.v20.i5.1165
3. Lee JY, Wasinger VC, Yau YY, Chuang E, Yajnik V, Leong RW. Molecular pathophysiology of epithelial barrier dysfunction in inflammatory bowel diseases. *Proteomes.* (2018) 6:17. doi: 10.3390/proteomes6020017
4. Vivinus-Nébot M, Frin-Mathy G, Bzioueche H, Dainese R, Bernard G, Anty R, et al. Functional bowel symptoms in quiescent inflammatory bowel diseases: role of epithelial barrier disruption and low-grade inflammation. *Gut.* (2014) 63:744-52. doi: 10.1136/gutjnl-2012-304066
5. Pearson AD, Eastham EJ, Laker MF, Craft AW, Nelson R. Intestinal permeability in children with Crohn's disease and coeliac disease. *Br Med J.* (1982) 285:20-1. doi: 10.1136/bmj.285.6334.20
6. Luissint AC, Parkos CA, Nusrat A. Inflammation and the intestinal barrier: leukocyte-epithelial cell interactions, cell junction remodeling, and mucosal repair. *Gastroenterology.* (2016) 151:616-32. doi: 10.1053/j.gastro.2016.07.008
7. Dunlop SP, Hebden J, Campbell E, Naesdal J, Olbe L, Perkins AC, et al. Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. *Am J Gastroenterol.* (2006) 101:1288-94. doi: 10.1111/j.1572-0241.2006.00672.x
8. Flügel A, Schulze-Koops H, Heesemann J, Kühn K, Sorokin L, Burkhardt H, et al. Interaction of enteropathogenic Yersinia enterocolitica with complex basement membranes and the extracellular matrix proteins collagen type IV, laminin-1 and−2, and nidogen/entactin. *J Biol Chem.* (1994) 269:29732-8. doi: 10.1016/S0021-9258(18)43942-7
9. Moreira AP, Texeira TF, Ferreira AB, Peluzio Mdo C, Alfenas Rde C. Influence of a high-fat diet on gut microbiota, intestinal permeability and metabolic endotoxaemia. *Br J Nutr.* (2012) 108:801-9. doi: 10.1017/S0007114512001213
10. Thaiss CA, Levy M, Grosheva I, Zheng D, Soffer E, Blacher E, et al. Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. *Science.* (2018) 359:1376-83. doi: 10.1126/science.aar3318
11. Meddings JB, Swain MG. Environmental stress-induced gastrointestinal permeability is mediated by endogenous glucocorticoids in the rat. *Gastroenterology.* (2000) 119:1019-28. doi: 10.1053/gast.2000.18152
12. Clayburgh DR, Shen L, Turner JR. A porous defense: the leaky epithelial barrier in intestinal disease. *Lab Invest.* (2004) 84:282-91. doi: 10.1038/labinvest.3700050
13. Turner JR. Molecular basis of epithelial barrier regulation: from basic mechanisms to clinical application. *Am J Pathol.* (2006) 169:1901-9. doi: 10.2353/ajpath.2006.060681
14. Shen L, Su L, Turner JR. Mechanisms and functional implications of intestinal barrier defects. *Dig Dis.* (2009) 27:443-9. doi: 10.1159/000233282
15. Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol.* (2009) 9:799-809. doi: 10.1038/nri2653
16. Camilleri M, Madsen K, Spiller R, Greenwood-Van Meerveld B, Verne GN. Intestinal barrier function in health and gastrointestinal disease. *Neurogastroenterol Motil.* (2012) 24:503-12. doi: 10.1111/j.1365-2982.2012.01921.x
17. Cereijido M, Valdés J, Shoshani L, Contreras RG. Role of tight junctions in establishing and maintaining cell polarity. *Annu Rev Physiol.* (1998) 60:161-77. doi: 10.1146/annurev.physiol.60.1.161
18. Aijaz S, Balda MS, Matter K. Tight junctions: molecular architecture and function. *Int Rev Cytol.* (2006) 248:261-98. doi: 10.1016/S0074-7696(06)48005-0
19. Green KJ, Simpson CL. Desmosomes: new perspectives on a classic. *J Invest Dermatol.* (2007) 127:2499-515. doi: 10.1038/sj.jid.5701015
20. Kowalczyk AP, Green KJ. Structure, function, and regulation of desmosomes. *Prog Mol Biol Transl Sci.* (2013) 116:95-118. doi: 10.1016/B978-0-12-394311-8.00005-4

21. Adey WH, McKibbin DL. Studies on the maerl species Phymatolithon calcareum (Pallas) nov. comb. and Lithothamnium corallioides Crouan in the Ria de Vigo. *Botanical Marina.* (1970) 13:100–6. doi: 10.1515/botm.1970.13.2.100
22. Attili D, McClintock SD, Rizvi AH, Pandya S, Rehman H, Nadeem DM, et al. Calcium-induced differentiation in normal human colonoid cultures: Cell-cell / cell-matrix adhesion, barrier formation and tissue integrity. *PLoS ONE.* (2019) 14:e0215122. doi: 10.1371/journal.pone.0215122
23. McClintock SD, Attili D, Dame MK, Richter A, Silvestri SS, Berner MM, et al. Differentiation of human colon tissue in culture: effects of calcium on trans-epithelial electrical resistance and tissue cohesive properties. *PLoS ONE.* (2020) 15:e0222058. doi: 10.1371/journal.pone.0222058
24. Aslam MN, McClintock SD, Attili D, Pandya S, Rehman H, Nadeem DM, et al. Ulcerative colitis-derived colonoid culture: a multi-mineral-approach to improve barrier protein expression. *Front Cell Dev Biol.* (2020) 8:577221. doi: 10.3389/fcell.2020.577221
25. Schmehl K, Florian S, Jacobasch G, Salomon A, Körber J. Deficiency of epithelial basement membrane laminin in ulcerative colitis affected human colonic mucosa. *Int J Colorectal Dis.* (2000) 15:39-48. doi: 10.1007/s003840050006
26. Verbeke S, Gotteland M, Fernández M, Bremer J, Ríos G, Brunser O. Basement membrane and connective tissue proteins in intestinal mucosa of patients with coeliac disease. *J Clin Pathol.* (2002) 55:440-5. doi: 10.1136/jcp.55.6.440
27. Bouatrouss Y, Herring-Gillam FE, Gosselin J, Poisson J, Beaulieu JF. Altered expression of laminins in Crohn's disease small intestinal mucosa. *Am J Pathol.* (2000) 156:45-50. doi: 10.1016/S0002-9440(10)64704-9
28. Spenlé C, Lefebvre O, Lacroute J, Méchine-Neuville A, Barreau F, Blottière HM, et al. The laminin response in inflammatory bowel disease: protection or malignancy? *PLoS ONE.* (2014) 9:e111336. doi: 10.1371/journal.pone.0111336
29. Timpl R, Tisi D, Talts JF, Andac Z, Sasaki T, Hohenester E. Structure and function of laminin LG modules. *Matrix Biol.* (2000) 19:309-17. doi: 10.1016/S0945-053X(00)00072-X
30. Colognato H, Yurchenco PD. Form and function: the laminin family of heterotrimers. *Dev Dyn.* (2000) 218:213-34. doi: 10.1002/(SICI)1097-0177(200006)218:2<213::AID-DVDY1>3.0.CO;2-R
31. Turck N, Gross I, Gendry P, Stutzmann J, Freund JN, Kedinger M, et al. Laminin isoforms: biological roles and effects on the intracellular distribution of nuclear proteins in intestinal epithelial cells. *Exp Cell Res.* (2005) 303:494-503. doi: 10.1016/j.yexcr.2004.10.025
32. Gonzales M, Haan K, Baker SE, Fitchmun M, Todorov I, Weitzman S, et al. A cell signal pathway involving laminin-5, alpha3beta1 integrin, and mitogen-activated protein kinase can regulate epithelial cell proliferation. *Mol Biol Cell.* (1999) 10:259-70. doi: 10.1091/mbc.10.2.259
33. Miyoshi H, Stappenbeck TS. *In vitro* expansion and genetic modification of gastrointestinal stem cells in spheroid culture. *Nat Protoc.* (2013) 8:2471-82. doi: 10.1038/nprot.2013.153
34. Zou WY, Blutt SE, Crawford SE, Ettayebi K, Zeng XL, Saxena K, et al. Human intestinal enteroids: new models to study gastrointestinal virus infections. *Methods Mol Biol.* (2019) 1576:229-47. doi: 10.1007/7651_2017_1
35. Fabregat A, Sidiropoulos K, Viteri G, Forner O, Marin-Garcia P, Arnau V, et al. Reactome pathway analysis: a high-performance in-memory approach. *BMC Bioinformatics.* (2017) 18:142. doi: 10.1186/s12859-017-1559-2
36. Sekiguchi R, Yamada KM. Basement membranes in development and disease. *Curr Top Dev Biol.* (2018) 130:143-91. doi: 10.1016/bs.ctdb.2018.02.005
37. Pozzi A, Yurchenco PD, Iozzo RV. The nature and biology of basement membranes. *Matrix Biol.* (2017) 57-58:1-11. doi: 10.1016/j.matbio.2016.12.009
38. Baker SE, Hopkinson SB, Fitchmun M, Andreason GL, Frasier F, Plopper G, et al. Laminin-5 and hemidesmosomes: role of the alpha 3 chain subunit in hemidesmosome stability and assembly. *J Cell Sci.* (1996) 109:2509-20. doi: 10.1242/jcs.109.10.2509
39. Green KJ, Jones JC. Desmosomes and hemidesmosomes: structure and function of molecular components. *FASEB J.* (1996) 10:871-81. doi: 10.1096/fasebj.10.8.8666164
40. Chidgey M. *Plakin Proteins, Hemidesmosomes and Human Disease.* In: eLS editor. John Wiley & Sons (2012). doi: 10.1002/9780470015902.a0024527
41. Has C. Hemidesmosomes: how much plakins do they need? *Exp Dermatol.* (2016) 25:263-4. doi: 10.1111/exd.12939
42. Polari L, Alam CM, Nyström JH, Heikkilä T, Tayyab M, Baghestani S, et al. Keratin intermediate filaments in the colon: guardians of epithelial homeostasis. *Int J Biochem Cell Biol.* (2020) 129:105878. doi: 10.1016/j.biocel.2020.105878
43. Zupancic T, Stojan J, Lane EB, Komel R, Bedina-Zavec A, Liovic M. Intestinal cell barrier function in vitro is severely compromised by keratin 8 and 18 mutations identified in patients with inflammatory bowel disease. *PLoS ONE.* (2014) 9:e99398. doi: 10.1371/journal.pone.0099398
44. Corfe BM, Majumdar D, Assadsangabi A, Marsh AM, Cross SS, Connolly JB, et al. Inflammation decreases keratin level in ulcerative colitis; inadequate restoration associates with increased risk of colitis-associated cancer. *BMJ Open Gastroenterol.* (2015) 2:e000024. doi: 10.1136/bmjgast-2014-000024
45. Nishiuchi R, Takagi J, Hayashi M, Ido H, Yagi Y, Sanzen N, et al. Ligand-binding specificities of laminin-binding integrins: a comprehensive survey of laminin-integrin interactions using recombinant alpha3beta1, alpha6beta1, alpha7beta1 and alpha6beta4 integrins. *Matrix Biol.* (2006) 25:189-97. doi: 10.1016/j.matbio.2005.12.001
46. De Arcangelis A, Hamade H, Alpy F, Normand S, Bruyère E, Lefebvre O, et al. Hemidesmosome integrity protects the colon against colitis and colorectal cancer. *Gut.* (2017) 66:1748-60. doi: 10.1136/gutjnl-2015-310847
47. Stutzmann J, Bellissent-Waydelich A, Fontao L, Launay JF, Simon-Assmann P. Adhesion complexes implicated in intestinal epithelial cell-matrix interactions. *Microsc Res Tech.* (2000) 51:179-90. doi: 10.1002/1097-0029(20001015)51:2<179::AID-JEMT9>3.0.CO;2-4
48. Rousselle P, Lunstrum GP, Keene DR, Burgeson RE. Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. *J Cell Biol.* (1991) 114:567-76. doi: 10.1083/jcb.114.3.567
49. Hennings H, Holbrook KA. Calcium regulation of cell-cell contact and differentiation of epidermal cells in culture. An ultrastructural study. *Exp Cell Res.* (1983) 143:127-42. doi: 10.1016/0014-4827(83)90115-5
50. Huang Y, Zhou Y, Castiblanco A, Yang W, Brown EM, Yang JJ. Multiple Ca(2+)-binding sites in the extracellular domain of the Ca(2+)-sensing receptor corresponding to cooperative Ca(2+) response. *Biochemistry.* (2009) 48:388-98. doi: 10.1021/bi8014604
51. Singh N, Aslam MN, Varani J, Chakrabarty S. Induction of calcium sensing receptor in human colon cancer cells by calcium, vitamin D and aquamin: promotion of a more differentiated, less malignant and indolent phenotype. *Mol Carcinog.* (2015) 54:543-53. doi: 10.1002/mc.22123
52. Carrillo-López N, Fernández-Martín JL, Alvarez-Hernández D, González-Suárez I, Castro-Santos P, Román-García P, et al. Lanthanum activates calcium-sensing receptor and enhances sensitivity to calcium. *Nephrol Dial Transplant.* (2010) 25:2930-7. doi: 10.1093/ndt/gfq124
53. Tiwari S, Askari JA, Humphries MJ, Bulleid NJ. Divalent cations regulate the folding and activation status of integrins during their intracellular trafficking. *J Cell Sci.* (2011) 124:1672-80. doi: 10.1242/jcs.084483
54. Varani J, McClintock SD, Aslam MN. Organoid culture to study epithelial cell differentiation and barrier formation in the colon: bridging the gap between monolayer cell culture and human subject research. *In Vitro Cell Dev Biol Anim.* (2021) 57:174-90. doi: 10.1007/s11626-020-00534-6
55. Aslam MN, Bassis CM, Bergin IL, Knuver K, Zick SM, Sen A, et al. A calcium-rich multimineral intervention to modulate colonic microbial communities and metabolomic profiles in humans: results from a 90-day trial. *Cancer Prev Res.* (2020) 13:101-16. doi: 10.1158/1940-6207.CAPR-19-0325
56. Aslam MN, McClintock SD, Jawad-Makki MAH, Knuver K, Ahmad HM, Basrur V, et al. A multi-mineral intervention to modulate colonic mucosal protein profile: results from a 90-day trial in human subjects. *Nutrients.* (2021) 13:939. doi: 10.3390/nu13030939
57. Sequeira IR, Lentle RG, Kruger MC, Hurst RD. Standardising the lactulose mannitol test of gut permeability to minimise error and promote comparability. *PLoS ONE.* (2014) 9:e99256. doi: 10.1371/journal.pone.0099256
58. Balk EM, Adam GP, Langberg VN, Earley A, Clark P, Ebeling PR, et al. International osteoporosis foundation calcium steering committee. global dietary calcium intake among adults: a systematic review. *Osteoporos Int.* (2017) 28:3315-24. doi: 10.1007/s00198-017-4230-x

59. Aslam MN, Varani J. The western-style diet, calcium deficiency and chronic disease. *J Nutr Food Sci.* (2016) 6:3. doi: 10.4172/2155-9600.1000496
60. U.S. Department of Health and Human Services; U.S. Department of Agriculture. *2015-—2020 Dietary Guidelines for Americans.* 8th ed. Washington, DC: U.S. Department of Health and Human Services (2015).
61. Fine JD, Eady RA, Bauer EA, Bauer JW, Bruckner-Tuderman L, Heagerty A, et al. The classification of inherited epidermolysis bullosa (EB): report of the third international consensus meeting on diagnosis and classification of EB. *J Am Acad Dermatol.* (2008) 58:931-50. doi: 10.1016/j.jaad.2008.02.004
62. Sachsenberg-Studer EM, Runne U, Wehrmann T, Wolter M, Kriener S, Engels K, et al. Bullous colon lesions in a patient with bullous pemphigoid. *Gastrointest Endosc.* (2001) 54:104-8. doi: 10.1067/mge.2001.115472
63. Seo JW, Park J, Lee J, Kim MY, Choi HJ, Jeong HJ, et al. A case of pemphigus vulgaris associated with ulcerative colitis. *Intest Res.* (2018) 16:147-50. doi: 10.5217/ir.2018.16.1.147
64. Chen YJ, Juan CK, Chang YT, Wu CY, Ho HJ, Tseng HC. Association between inflammatory bowel disease and bullous pemphigoid: a population-based case-control study. *Sci Rep.* (2020) 10:12727. doi: 10.1038/s41598-020-69475-0
65. Natsuga K, Shinkuma S, Nishie W, Shimizu H. Animal models of epidermolysis bullosa. *Dermatol Clin.* (2010) 28:137-42. doi: 10.1016/j.det.2009.10.016
66. Bruckner-Tuderman L, McGrath JA, Robinson EC, Uitto J. Animal models of epidermolysis bullosa: update 2010. *J Invest Dermatol.* (2010) 130:1485-8. doi: 10.1038/jid.2010.75
67. Heimbach L, Li N, Diaz A, Liu Z. Experimental animal models of bullous pemphigoid. *G Ital Dermatol Venereol.* (2009) 144:423-31.

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Impact of the SARS-CoV-2 Delta Variant on the Psychological States and Health-Related Quality of Life in Patients With Crohn's Disease

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Background: Since the outbreak of the coronavirus disease 2019 (COVID-19) pandemic first reported in Wuhan, China, several research on the psychological impact of the pandemic on patients with Crohn's disease (CD) have been conducted. However, with the progression of the global pandemic and the emergence of the SARS-CoV-2 B.1.617.2 (Delta) variant, follow-up studies need to be performed to monitor the alterations of psychological status and health-related quality of life (HRQoL) among CD patients.

Aims: We aimed to evaluate the impact of the SARS-CoV-2 Delta variant on the mental health and life quality among the CD population and tried to explore potent risk factors.

Methods: This observational study included 153 CD patients who responded to our pre-designed self-reported questionnaire. Demographic, clinical, and psychological information were collected and analyzed.

Results: Quite a number of CD patients were confronted with different levels of anxiety and depression, with incidence of 28.10 and 31.37% for anxiety and depression, respectively. Compared with non-pandemic circumstances, the life quality of CD patients due to the present situation was more often compromised. Isolation [odds ratio (OR): 4.71, $P = 0.007$] was verified as a risk factor for anxiety while use of telemedicine could help relieve anxiety (OR: 0.22, $P < 0.001$). Worsening of symptoms (OR: 4.92, $P = 0.006$), isolation (OR: 5.75, $P = 0.005$), and drug withdrawn (OR: 2.66, $P = 0.026$) were identified to be independent factors for developing depression. Likewise, use of telemedicine (OR: 0.13, $P < 0.001$) was negatively related to depression. Considering life quality, vaccination (OR: 3.07, $P = 0.021$) together with no medication (OR: 7.73, $P = 0.010$) was relevant to better life quality while worsening of symptoms (OR: 0.09, $P = 0.034$) were an independent risk factor for impaired life quality.

Conclusion: Many CD patients suffered from symptoms of anxiety and depression and impaired life quality during the COVID-19 pandemic. Those in isolation or with worsening of symptoms and drug withdrawn were more prone to experience psychological stress. Individualized management such as drug delivery and telemedicine should be promoted to maintain control of mental health and life quality during the pandemic.

Keywords: COVID-19, SARS-CoV-2 Delta variant, Crohn's disease, mental health, health-related quality of life

INTRODUCTION

Since the outbreak of the coronavirus disease 2019 (COVID-19) pandemic first reported in Wuhan, China, numerous variants of concern (VoCs) of SARS-CoV-2 have been revealed, among which the Delta variant (B.1.617.2) is by far the dominant strain (1–3). First detected in India in December 2020, the Delta variant has now been detected nearly all of the globe (4). The Delta variant is characterized by mutations of the spike proteins such as T19R, T478K, Δ157-158, P681R, L452R, D614G, D950N, etc. (5). Several of these mutations may influence immune responses against the key antigenic regions of receptor-binding protein and deletion of part of the N-terminal domain while some mutations at the S1–S2 cleavage site appear to be associated with increased replication, which leads to higher viral loads and increased transmission (6, 7). It has been reported that the relative viral loads of the Delta variant of quarantine contact cases was significantly higher than the original lineage (8–10). With a remarkably elevated transmissibility (40–60% higher compared with the Alpha variant), the Delta variant has exerted a disastrous impact on the infection and mortality rates globally (11–13). In China, the Delta variant accounts for most of the new cases since the year of 2021. Previous research has implicated that the COVID-19 pandemic is likely to cause abnormality of emotion, cognition, behavior and quality of life, especially in population with chronic diseases such as systemic lupus erythematosus, rheumatoid arthritis and inflammatory bowel disease (IBD), in whom psychological distress and somatic diseases could influence mutually (14–16).

Crohn's disease (CD) is a subtype of IBD, which is featured by chronic and relapsing inflammation of the gastrointestinal tract (17). The etiology and pathogenesis of CD still remain unclear. It is commonly recognized that the interplay among genetic background, environment triggers, host microbiota, and immune response contributes to the initiation of CD (18). Importantly, due to the chronic and recurrent behavior of CD, psychological distress also plays an indispensable role throughout the disease course (19). Anxiety and depression are very common in CD patients, with a morbidity of anxiety and/or depression about 29–35% in the remission stage and this rate can rise to as high as 60–80% during relapses (20). Compared with the general population, the rate of psychological disorders such as anxiety and depression in CD patients, especially in the active stage, is 2–3 times higher (21). In turn, Frolkis et al. reported that depression is related to early clinical recurrence and disease severity in CD, which could be mitigated by treatment of depression (22). Several mechanisms underlying the psychological disorders in CD patients have been revealed, among which the theory of the brain-gut axis is the most wildly acknowledged. Briefly, there are abundant autonomic nervous plexus connections between the enteric nervous system (ENS) and central nervous system, which is also known as brain-gut axis. On one hand, the motility, sensory and secretory functions and pain thresholds of the gastrointestinal tract can be directly or indirectly affected by psychological and emotional stress through the brain-gut axis. In this process, substance P (SP), vasoactive intestinal peptides (VIP), various neuropeptides, neurotransmitters and hormones play a part. On the other hand, intestinal inflammation can also act on the central system by the production of pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), thus inducing the symptoms of anxiety or depression (23, 24).

Apart from psychological status, health-related quality of life (HRQoL) has become another concern in the management of CD patients (25). HRQoL is often compromised in CD patients especially during disease relapses (26). Additionally, factors independent of disease activity also contribute to the alteration of life quality (27). A recent study demonstrated that during the COVID-19 pandemic, HRQoL among CD patients was impaired but the underlying variables still awaits exploration (28).

In the current situation where social isolation and uncertainty are likely to occur, CD patients are facing an increasing risk of suffering from worsening anxiety and depression, which may cause relapse or escalation of CD activity. Previous studies conducted at the beginning of the pandemic suggested that COVID-19 exerted negative effects on psychological and disease outcomes among CD patients (28, 29). However, with the progression of the global pandemic and the emergence of the SARS-CoV-2 Delta variant, there have been changes in how patients with CD perceive and respond to the pandemic. For example, many patients have mastered protection skills against the virus and might have been vaccinated; some patients have learned how to manage CD under the pandemic, while another group of patients are more concerned of the Delta variant. These changes could lead to alterations in the psychological states and HRQoL under this kind of situation. Therefore, in this study, we aimed to evaluate the impact

Abbreviations: COVID-19, coronavirus disease 2019; CD, Crohn's disease; HRQoL, health-related quality of life; VoCs, variants of concern; IBD, inflammatory bowel disease; ENS, enteric nervous system; SP, substance P; VIP, vasoactive intestinal peptides; TNF-α, tumor necrosis factor α; ECCO, European Crohn's and Colitis Organization; HBI, Harvey-Bradshaw Disease Activity Index; GAD-7, Generalized Anxiety Disorder Scale 7; PHQ-9, Patient Health Questionnaire-9; IBDQ, Inflammatory Bowel Disease Questionnaire; SD, standard deviation; 5-ASA, 5-aminosalicylic acid; JAK, Janus kinase; IOIBD, International Organization for the Study of Inflammatory Bowel Disease.

of the SARS-CoV-2 Delta variant on the mental health and life quality among CD population and tried to explore potent risk factors, so as to modify variables that are feasible to intervention and set optimized management for susceptible CD patients.

MATERIALS AND METHODS

Design and Setting

This study is a cross-sectional, observational analysis using information of CD patients diagnosed and treated at Affiliated Hospital of Yangzhou University from 2012 to 2021. The study was conducted between 25 August and 15 September 2021. All protocols in the study were carried out in accordance with the principles of the Declaration of Helsinki. This study was approved by the Institutional Review Board and Ethics Committee of Affiliated Hospital of Yangzhou University (REC ref 2021-YKL06-09-006). Completion of the study questionnaire was regarded as informed consent.

Patients

Clinical staff at Department of Gastroenterology, Affiliated Hospital of Yangzhou University first screened potential participants that met the inclusion criteria of this study. Inclusion criteria consisted of (1) diagnosis with CD based on the criteria determined by the European Crohn's and Colitis Organization (ECCO) guidelines (30, 31) and (2) age >18 years. Exclusion criteria were comprised of the following: (1) history of mental diseases diagnosed prior to CD onset (e.g., mood disorders, schizophrenia, psychosis, obsessive-compulsive disorder, psychoactive substance abuse, post-traumatic or acute stress-disorder, and intellectual disability); (2) accepting pharmacological or psychological treatment for mental health problems at the time of study recruitment; (3) diagnosed with somatic diseases reported to have an impact on the psychological state (e.g., diabetes mellitus, thyroid dysfunction, heart failure, and renal insufficiency). Such patients were excluded for these conditions are potent confounding variables for psychological assessment. Also, those lacking sufficient data for our study were ruled out.

Measures

After recruitment of eligible patients, a pre-designed self-reported questionnaire was sent *via* an online survey platform. The questionnaire consists of four sections. The first section collects basic demographic and socioeconomical information of the CD patients including age, gender, education level, occupation, income level, etc. The second section focuses on the clinical characteristics of CD such as age at the time of the diagnosis, disease duration, involvement of perianal disease, presence of extraintestinal manifestations, history of CD related surgeries, medication, and current symptoms (especially worsening of symptoms). For each patient, the Harvey-Bradshaw Disease Activity Index (HBI) for CD was calculated by experienced gastroenterologists to assess the disease severity. A score of ≥5 was defined as existence of disease activity.

The third section assesses the psychological state and HRQoL of the participants. The Generalized Anxiety Disorder Scale 7 (GAD-7) was used to assess the frequency of the patients' anxiety in the past 2 weeks. It consists of seven questions and each question has four choices on a scale of 0 to 3, totaling 21 points. The higher the score is, the more serious the anxiety degree is. A score 0–4 points is within the normal range; 5–9 points indicate mild anxiety levels; 10–14 points indicate moderate anxiety levels while individuals scoring 15–21 points are considered to suffer from severe anxiety (32). With regards to depression, Patient Health Questionnaire-9 (PHQ-9) which is composed of nine depression-related items was adopted. Each item is scored on a scale of 0 to 3, with an overall score of 0 to 27. According to the score of PHQ-9, depression can be divided into five severity categories: minimal (0–4), mild (5–9), moderate (10–14), moderately severe (15–19), and severe (20–27) (33). To evaluate the HRQoL of the participants, the Inflammatory Bowel Disease Questionnaire (IBDQ) was applied (34). A total of 32 questions were included which reflected the intestinal symptoms, systemic symptoms, emotional functions, and social functions of the patients. The

TABLE 1 | Demographic characteristics of CD patients included in the study.

Variables	Mean ± SD or *n* (%)
Gender, *n* (%)	
Male	85 (55.56%)
Female	68 (44.44%)
Age, years (mean ± SD)	38.44 ± 13.00
Marital status, *n* (%)	
Married	105 (68.63%)
Single	42 (27.45%)
Divorced	6 (3.92%)
Occupation, *n* (%)	
Manual worker	32 (20.92%)
Mental worker	57 (37.25%)
Unemployed	26 (17.00%)
Retired	14 (9.15%)
Student	24 (15.69%)
Education level, *n* (%)	
Elementary	11 (7.19%)
High school	93 (60.78%)
Bachelor	44 (28.76%)
Post graduate	5 (3.27%)
Socio-economic status, *n* (%)	
Low	83 (54.25%)
Middle	32 (20.92%)
High	38 (24.84%)
Medical insurance coverage, *n* (%)	123 (80.39%)
Smoking	28 (17.95%)
Drinking	18 (11.54%)

SD, standard deviation.

total score ranges from 32 to 224, with a higher score indicating a better quality of life.

The fourth section of our questionnaire concerns issues around COVID-19 and the SARS-CoV-2 Delta variant and how patient care is influenced. To assess the participants' knowledge of the COVID-19 pandemic and the SARS-CoV-2 Delta variant, we designed nine questions related to the virus (**Supplementary Table 1**). With 1 point for each correct answer, the total score ranges from 0 to 9. Additionally, questions regarding the isolation status, drug withdrawn and vaccination are also included.

It was stressed that results the participants filled in would be made of the biggest value if they could give honest answers. Data extraction from the questionnaires was performed by two independent researchers for further analysis. In case of a dispute in the interpretation of the questionnaires, discussion among experienced experts in our department would be held to reach a consensus.

Statistical Analysis

Statistical analysis was performed with SPSS version 22.0 (IBM SPSS Statistics, United States) and GraphPad Prism 5 (GraphPad Software, United States). Continuous variables were expressed as mean ± standard deviation (SD) while categorical variables are presented as proportions. To detect potent factors underlying altered psychological stress and life quality, binary logistic regression was performed. In the univariable analysis, candidate predictors were screened with a criterion of $P \leq 0.10$. Then multivariable analysis was applied for further exploration to eliminate variables without statistical significance. A P-value < 0.05 was considered statistically significant in the multivariable analysis. The odds ratio (OR) was used as a measure of association between the variables and results of the psychological stress and life quality.

TABLE 2 | Disease characteristics and the SARS-CoV-2 Delta variant-related items.

Variables	Mean ± SD or *n* (%)
Disease duration, years (mean ± SD)	5.37 ± 4.81
Disease activity, *n* (%)	
Inactive (HBI < 5)	113 (75.16%)
Moderate active (5 ≤ HBI < 9)	33 (21.57%)
Severe active (HBI ≥ 9)	7 (4.58%)
Perianal involvement, *n* (%)	81 (52.94%)
History of surgery, *n* (%)	34 (22.22%)
Treatment, *n* (%)	
5-ASA	31 (20.26%)
Prednisolone	3 (1.96%)
Immunomodulators	56 (36.60%)
Biologics	80 (52.29%)
Traditional Chinese medicine	3 (1.96%)
No medication	15 (9.80%)
Isolation, *n* (%)	20 (13.07%)
Use of telemedicine, *n* (%)	86 (56.21%)
Vaccination, *n* (%)	55 (35.95%)
Knowledge of the Delta variant	7.46 ± 1.40
Drug withdrawn, *n* (%)	64 (41.83%)
Worsening of symptoms, *n* (%)	44 (28.76%)
Fear of contracting COVID-19, *n* (%)	62 (40.53%)
Stopped working, *n* (%)	41 (26.80%)
Willingness to turn to psychological help	21 (13.73%)

SD, standard deviation; HBI, Harvey-Bradshaw Disease Activity Index; 5-ASA, 5-aminosalicylic acid; COVID-19, coronavirus disease 2019.

RESULTS

Demographic Characteristics

As shown in **Supplementary Figure 1**, a total of 202 individuals responded to our questionnaire, of whom 49 were excluded from our analysis. Among these being excluded, 10 had a non-CD diagnosis; 23 previously suffered or were suffering from mental diseases at the time of our study; 8 were concomitant with other somatic diseases related to the psychological state, and the remaining 8 did not provide sufficient information. Basic characteristics are displayed in **Table 1**. The mean age of CD patients is 38.44 ± 13.00 years old, and the male: female ratio was 85:68. Sixty-nine percent were married, 27.45% single, and 3.92% divorced. In regard to the occupation: 20.92% were manual worker, 37.25% mental worker, 17.00% unemployed, 9.15% retired, and 15.69% student, and 18.90% retired. As for education level, 32.03% got a bachelor's degree or higher. Eighty percent were covered by medical insurance, and smokers and drinkers accounted for 17.95 and 11.54%, respectively.

Disease Characteristics and Life Changes Caused by the SARS-CoV-2 Delta Variant

The mean disease duration of CD patients is 5.37 ± 4.81 years (**Table 2**). According to the HBI score, 113 patients were sorted into the inactive group, while the rest 40 were classified as having disease activity (33 moderate and 14 severe). Concerning the IBD medication, 15 patients (9.80%) had not received prior medication, and the number of patients using 5-aminosalicylic acid (5-ASA), prednisolone, immunomodulators, biologics and traditional Chinese medicine is 31 (20.26%), 3 (1.96%), 56 (36.60%), 80 (52.29%), and 3 (1.96%), respectively. A total of 34 patients (22.22%) received surgical treatment. In 81 patients (52.94%), perianal involvement was reported.

During the lockdown, 44 patients (28.76%) experienced worsening of symptoms. Of those with exacerbated symptoms, 68% reported abdominal pain, and the rest mainly complained of diarrhea, bloody stool, fever, etc. (**Figure 1**). None of the included patients were infected with COVID-19, but 20 (13.07%) were isolated as close contacts. The shutdown led to drug withdrawn in 64 patients (41.83%) due to restricted accessibility to the hospital and pharmacies. Rates of drug withdrawn for different medications were shown in **Figure 2**. Additionally, to minimize the spread of SARS-CoV-2, IBD physicians in our institute tried to provide medical service *via* telemedicine, and this was utilized by 86 patients (56.21%). Owing to the strong advocation of the

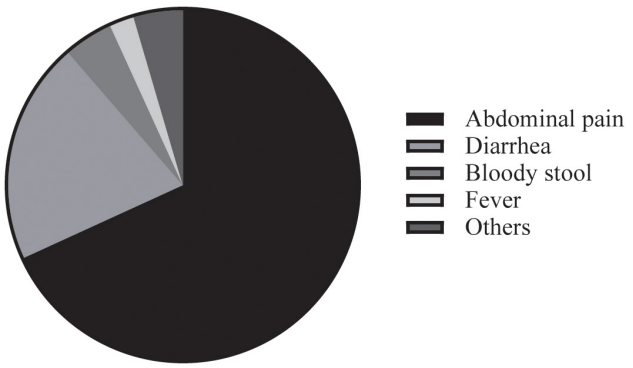

FIGURE 1 | Worsening symptoms during the COVID-19 pandemic.

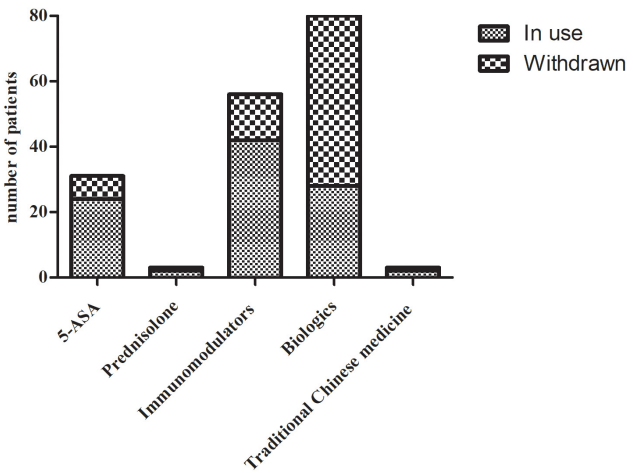

FIGURE 2 | Rates of drug withdrawn for different medications. 5-ASA, 5-aminosalicylic acid.

health authorities, 55 (35.95%) CD patients finished the COVID-19 vaccination, and the SARS-CoV-2 Delta variant is well known to CD patients (7.46 ± 1.40).

Anxiety, Depression, and Health-Related Quality of Life Among Crohn's Disease Patients During the SARS-CoV-2 Delta Variant Predominance

Using 5 as the cut-off value for the GAD-7 scale, the incidence of anxiety was 28.1% (43/153), with 34 in the mild group and 9 in the moderate group (**Table 3**). In terms of depression, 48 (31.37%) scored ≥5 for the PHQ-9 scale, which is regarded as the existence of depression, and among these, 36 (23.53%) presented mild depression, 10 (6.54%) moderate, and 2 (1.31%) moderately severe. During the closure, most of the CD patients (80.39%) maintained a good life quality (IBDQ score ≥209), while HRQoL of 30 patients (19.61%) was impaired. Among the various sources of psychological stress, drug withdrawn was the problem that bothers CD patients the most (41.83%), followed by fear of contracting SARS-CoV-2 Delta variant (62, 40.53%), worsening of symptoms (44, 28.76%) and suspension of work (41, 26.80%). During the pandemic, many cities in China have opened psychological counseling hotlines during the epidemic, but only a small proportion of our patients are aware of and are willing to turn to it for help (21, 13.73%).

Factors Associated With Anxiety, Depression, and Health-Related Quality of Life Among Crohn's Disease Patients During the SARS-CoV-2 Delta Variant Predominance

To explore the factors associated with anxiety in CD patients, we first applied univariable logistic regression to screen for potential variables. As shown in **Table 4**, a post graduate degree (OR: 40 [95% CI: 1.98–807.10], $P = 0.016$), higher disease activity (OR: 1.17 [95% CI: 1.02–1.34], $P = 0.030$), worsening of symptoms (OR: 2.69 [95% CI: 1.27–5.69], $P = 0.010$), isolation (OR: 4.94 [95% CI: 1.85–13.16], $P = 0.001$), better knowledge of the Delta variant (OR: 1.38 [95% CI: 1.03–1.85], $P = 0.031$) and drug withdrawn (OR: 1.94 [95% CI: 0.95–3.95], $P = 0.070$) were

positively related to the occurrence of anxiety, while utility of telemedicine (OR: 0.26 [95% CI: 0.12–0.54], $P < 0.001$) was associated with less anxiety. After adjustment by multivariant analysis, isolation (OR: 4.71 [95% CI: 1.54–14.44], $P = 0.007$) was verified as a risk factor for anxiety while telemedicine could help relieve anxiety (OR: 0.22 [95% CI: 0.09–0.52], $P < 0.001$).

With respect to depression, worsening of symptoms (OR: 4.92 [95% CI: 1.58–15.29], $P = 0.006$), isolation (OR: 5.75 [95% CI: 1.69–19.59], $P = 0.005$), and drug withdrawn (OR: 2.66 [95% CI: 1.12–6.31], $P = 0.026$) were identified to be independent factors for developing depression. Likewise, telemedicine (OR: 0.13 [95% CI: 0.05–0.32], $P < 0.001$) was negatively related to depression (**Table 5**).

Finally, we found that no medication (OR: 7.73 [95% CI: 1.65–36.27], $P = 0.010$) and vaccination (OR: 3.07 [95% CI: 1.19–7.93], $P = 0.021$) were relevant to better life quality while worsening of symptoms (OR: 0.09 [95% CI: 0.01–0.83], $P = 0.034$) was an independent risk factor for impaired life quality (**Table 6**).

DISCUSSION

In the current context of SARS-CoV-2 Delta variant predominance, patients suffering from CD have been forced to face dramatically altered psychological stress and life quality. In this study, we aimed to evaluate the level of anxiety and depression as well as HRQoL among CD patients and tried to explore potent risk factors, so as to optimize the management of patients with CD. As far as we know, this is the first study that focused on the impact of SARS-CoV-2 Delta variant on the mental health and life quality among the CD population. According to the analysis of our questionnaire, we found that quite a number of CD patients were confronted with different levels of anxiety and depression, with incidence of 28.10 and 31.37% for anxiety and depression, respectively. This result went in line with a previous conducted survey (29). Also, compared with non-pandemic circumstances, the life quality of CD patients due to the present situation was more often compromised (35).

Though we expected fear of contracting SARS-CoV-2 Delta variant to be a risk factor for psychological stress, no relevance was observed in our analysis. This could possibly be explained by the relatively extensive publicity on the knowledge of COVID-19, which was reflected by the high score on the perception of SARS-CoV-2 Delta variant. Also, the strictly limited traffic by the lockdown was thought to protect the citizens from contracting COVID-19, which might enhance the sense of security among CD patients.

Our findings demonstrated that isolation was closely related to both anxiety and depression. In the context of the epidemic, residents at a high risk of getting infected with COVID-19 (especially those having contacted with COVID-19 patients) were usually isolated, which on one hand stopped the patients from getting healthcare including going to the clinic, purchasing drugs, and maintaining biologics infusion and on the other hand, separated the patients from their loved ones. Hence, it is not surprising that isolation is an independent risk factor for anxiety/depression. In our study, 13.07% participants were isolated, which is in consistence with previous studies (29, 35, 36). These results implicated the necessity of offering timely psychological counseling to this high-risk population. Tremendous studies have shown that various psychological therapies could relieve psychological stress as well as alleviate disease activity in IBD patients (37, 38). In terms of this issue, many cities in China have opened psychological counseling hotlines during the epidemic, but only a small proportion of the patients are aware of and are willing to turn to it for help (21, 13.73%), suggesting that more attention need to be paid to the publicity of the psychological counseling hotlines.

In our study, worsening of symptoms showed significant relationships with depression and impaired HRQoL, which once again proved the bi-directional interaction of the brain-gut axis model (39). Growing evidence illustrated that depressive symptoms were strictly related to disease recurrence in IBD patients (40). Furthermore, higher psychological stress is verified as a predictor of lower life quality (41). These investigations together with our study remind our gastroenterologists of the importance of paying more attention to those patients with exacerbated symptoms and further addressing the knowledge of how to manage CD during pandemic in this setting of patients.

The clinical course of CD is characterized by periods of remissions with recurrent episodes, making it extremely crucial for the patients to adhere to continuous and long-term use of medication. In the present study, 64 participants (41.83%) were forced to discontinue their CD medications, among which the biologics infusion was the most affected. Our analysis showed that drug withdrawn was relevant to the occurrence of depression, which was in line with our expectation. It is widely acknowledged that 5-ASA and biologics are critic to CD treatment, and unreasonable withdrawn would trigger disease relapse (42). Hence, we recommend that CD patients should avoid drug discontinuation whenever possible. Good news is that to handle this issue, alternative options like drug delivery by

TABLE 3 | Level of anxiety, depression, and HRQoL among CD patients.

Variables	*n* (%)	Mean ± SD
Anxiety (GAD-7)		
No	110 (71.90%)	0.83 ± 1.20
Mild	34 (22.22%)	6.74 ± 1.31
Moderate	9(5.88%)	12.22 ± 1.64
Severe	0	/
Depression (PHQ-9)		
No	105 (68.63%)	1.24 ± 1.42
Mild	36 (23.53%)	7.17 ± 1.66
Moderate	10 (6.54%)	11.40 ± 1.58
Moderately severe	2 (1.31%)	17.00 ± 1.41
Severe	0	/
HRQoL (IBDQ)		
Low	123 (80.39%)	181.16 ± 22.00
High	30 (19.61%)	215.13 ± 4.60

SD, standard deviation; GAD-7, Generalized Anxiety Disorder Scale 7; PHQ-9, Patient Health Questionnaire-9; HRQoL, health-related quality of life; IBDQ, Inflammatory Bowel Disease Questionnaire.

TABLE 4 | Factors associated with elevated anxiety.

Variables	Univariable analysis			Multivariable analysis		
	P	OR	95% CI	*P*	OR	95% CI
Gender (female)	0.445	0.76	0.37–1.55			
Age	0.932	1.00	0.97–1.03			
Marital status, *n* (%)						
Married						
Single	0.163	0.54	0.22–1.29			
Divorced	0.328	2.28	0.44–11.92			
Occupation, *n* (%)						
Manual worker						
Mental worker	0.612	1.28	0.50–3.30			
Unemployed	0.596	1.35	0.44–4.13			
Retired	0.320	0.43	0.08–2.29			
Student	0.320	0.51	0.14–1.92			
Education level						
Elementary						
High school	0.269	3.29	0.40–27.07	0.522	2.05	0.23–18.59
Bachelor	0.134	5.17	0.60–44.32	0.456	2.37	0.25–23.00
Post graduate	0.016*	40	1.98–807.10	0.132	10.96	0.49–247.00
Socio-economic status						
Low						
Middle	0.675	0.82	0.32–2.08			
High	0.997	1.00	0.43–2.34			
Medical insurance coverage	0.518	1.36	0.54–3.45			
Smoking	0.952	1.03	0.42–2.55			
Drinking	0.600	1.32	0.46–3.79			
Disease duration	0.414	1.03	0.96–1.11			
Disease activity (HBI)	0.030*	1.17	1.02–1.34	0.770	1.03	0.85–1.25
Perianal involvement	0.525	1.26	0.62–2.55			
History of surgery	0.139	1.84	0.82–4.11			
Treatment						
5-ASA	0.750	0.87	0.35–2.12			
Prednisolone	0.999	0	/			
Immunomodulators	0.922	1.04	0.50–2.15			
Biologics	0.207	1.59	0.78–3.25			
Traditional Chinese medicine	0.839	1.29	0.11–14.56			
No medication	0.466	0.61	0.16–2.29			
Isolation	0.001*	4.94	1.85–13.16	0.007**	4.71	1.54–14.44
Use of telemedicine	<0.001*	0.26	0.12–0.54	0.001**	0.22	0.09–0.52
Vaccination	0.324	1.43	0.70–2.91			
Knowledge of the Delta variant	0.031*	1.38	1.03–1.85	0.156	1.27	0.91–1.78
Drug withdrawn	0.070*	1.94	0.95–3.95	0.291	1.58	0.68–3.69
Worsening of symptoms	0.010*	2.69	1.27–5.69	0.405	1.59	0.53–4.73
Fear of contracting COVID-19	0.958	0.98	0.48–2.02			
Stopped working	0.713	0.83	0.31–2.25			

OR, odds ratio; CI, confidence interval; HBI, Harvey-Bradshaw Disease Activity Index; 5-ASA, 5-aminosalicylic acid; COVID-19, coronavirus disease 2019.
**P < 0.10; **P < 0.05.*

hospitals have been provided, which could to a large extent solve the problem of drug withdrawn. For those on biologic treatment, we recommend that a subcutaneous dosage form such as adalimumab could be considered to replace intravenous infusion.

Apart from limited accessibility to CD drugs, many patients were also in face of difficulty in keeping in a regular contact with their treating physicians. Previous research showed that patients who talked to their healthcare providers felt more supported compared to those who did not, and they were at a lower risk of experiencing worsening of IBD symptoms, highlighting the importance of regular communication between patients and their gastroenterologists (43). To minimize the spread of

TABLE 5 | Factors associated with elevated depression.

Variables	Univariable analysis			Multivariable analysis		
	P	OR	95% CI	*P*	OR	95% CI
Gender (female)	0.063*	0.51	0.25–1.04	0.162	0.53	0.22–1.29
Age	0.314	0.99	0.96–1.01			
Marital status						
Married						
Single	0.823	1.10	0.51–2.34			
Divorced	0.457	0.44	0.05–3.88			
Occupation						
Manual worker						
Mental worker	0.193	1.86	0.73–4.72			
Unemployed	0.433	0.61	0.18–2.11			
Retired	0.635	0.70	0.16–3.10			
Student	0.932	1.05	0.33–3.39			
Education level						
Elementary						
High school	0.633	1.48	0.30–7.35			
Bachelor	0.115	3.75	0.73–19.39			
Post graduate	0.112	6.75	0.64–71.17			
Socio-economic status						
Low						
Middle	0.183	1.79	0.76–4.19			
High	0.663	1.20	0.52–2.78			
Medical insurance coverage	0.293	1.64	0.65–4.15			
Smoking	0.724	0.85	0.35–2.10			
Drinking	0.209	1.90	0.70–5.17			
Disease duration	0.667	1.02	0.95–1.09			
Disease activity (HBI)	0.006*	1.22	1.06–1.40	0.859	0.98	0.80–1.21
Perianal involvement	0.400	1.34	0.68–2.66			
History of surgery	0.165	1.75	0.79–3.86			
Treatment						
5-ASA	0.456	0.71	0.29–1.73			
Prednisolone	0.999	0	/			
Immunomodulators	0.110	1.77	0.88–3.56			
Biologics	0.312	1.43	0.72–2.84			
Traditional Chinese medicine	0.941	1.10	0.10–12.39			
No medication	0.325	0.52	0.14–1.92			
Isolation	0.001*	5.20	1.92–14.09	0.005**	5.75	1.69–19.59
Use of telemedicine	<0.001*	0.19	0.09–0.40	<0.001**	0.13	0.05–0.32
Vaccination	0.200	1.61	0.78–3.33			
Knowledge of the Delta variant	0.277	1.15	0.89–1.49			
Drug withdrawn	0.006*	2.68	1.33–5.41	0.026**	2.66	1.12–6.31
Worsening of symptoms	<0.001*	4.92	2.32–10.45	0.006**	4.92	1.58–15.29
Fear of contracting COVID-19	0.508	1.26	0.63–2.53			
Stopped working	0.101	2.10	0.87–5.12			

OR, odds ratio; CI, confidence interval; HBI, Harvey-Bradshaw Disease Activity Index; 5-ASA, 5-aminosalicylic acid; COVID-19, coronavirus disease 2019.
**P < 0.10; **P < 0.05.*

SARS-CoV-2, IBD physicians in our institute started to make greater use of telehealth service, which turned out to be received with high satisfaction by CD patients. In this study, the analysis revealed that those staying in contact with the gastroenterologists by telemedicine tend to have less anxiety/depression, which partially proved that the telehealth service we provided was a nice try and was worth promoting among CD patients in isolation. The definite role of telemedicine among CD patients during the COVID-19 pandemic needs to be verified in larger cohorts and the procedure awaits standardization, but we believe that in the near future it will become an indispensable alternative for more CD patients.

TABLE 6 | Factors associated with better life quality.

Variables	Univariable analysis			Multivariable analysis		
	P	OR	95% CI	*P*	OR	95% CI
Gender (female)	0.891	0.95	0.42–2.11			
Age	0.115	0.97	0.94–1.01			
Marital status, *n* (%)						
Married						
Single	0.034*	2.49	1.07–5.80	0.091	2.42	0.87–6.73
Divorced	0.925	1.11	1.22–10.16	0.780	1.41	0.13–15.75
Occupation						
Manual worker						
Mental worker	0.950	1.04	0.34–3.13			
Unemployed	0.736	0.79	0.20–3.15			
Retired	0.714	0.72	0.13–4.12			
Student	0.364	1.78	0.51–6.23			
Education level						
Elementary						
High school	0.350	2.74	0.33–22.70			
Bachelor	0.396	2.57	0.29–22.80			
Post graduate	0.999	0	/			
Socio-economic status						
Low						
Middle	0.326	0.56	0.17–1.80			
High	0.691	1.21	0.48–3.02			
Medical insurance coverage	0.952	0.97	0.36–2.64			
Smoking	0.191	1.87	0.73–4.80			
Drinking	0.357	1.69	0.55–5.18			
Disease duration	0.766	1.01	0.93–1.10			
Disease activity (HBI)	0.001*	0.59	0.43–0.81	0.069	0.73	0.51–1.03
Perianal involvement	0.444	1.37	0.61–3.04			
History of surgery	0.199	0.48	0.15–1.48			
Treatment						
5-ASA	0.131	0.38	0.11–1.34			
Prednisolone	0.554	2.09	0.18–23.80			
Immunomodulators	0.667	1.20	0.53–2.71			
Biologics	0.493	0.76	0.34–1.68			
Traditional Chinese medicine	0.554	2.09	0.18–23.80			
No medication	0.044*	3.17	1.03–9.73	0.010**	7.73	1.65–36.27
Isolation	0.597	0.69	1.19–2.54			
Use of telemedicine	0.094*	2.08	0.88–4.91	0.166	2.00	0.75–5.32
Vaccination	0.042*	2.32	1.03–5.24	0.021**	3.07	1.19–7.93
Knowledge of the Delta variant	0.303	1.18	0.86–1.60			
Drug withdrawn	0.523	0.77	0.34–1.74			
Worsening of symptoms	0.008*	0.06	0.01–0.49	0.034**	0.09	0.01–0.83
Fear of contracting COVID-19	0.104	0.48	0.20–1.16			
Stopped working	0.693	0.79	0.25–2.52			

OR, odds ratio; CI, confidence interval; HBI, Harvey-Bradshaw Disease Activity Index; 5-ASA, 5-aminosalicylic acid; COVID-19, coronavirus disease 2019.
**P < 0.10; **P < 0.05.*

Since the outbreak of the COVID-19 pandemic, tremendous efforts have been made into the research and development of effective vaccines. Up to now, several mRNA vaccines and inactivated vaccines have been approved for use in many countries and additional novel vaccines are emerging (44). It is well acknowledged that immune dysfunction is a key part during the onset of IBD and in IBD patients, the immune capacity is often compromised because of the application of immune-modifying treatment such as corticosteroids, immunomodulators and biologic agents [e.g., monoclonal antibodies for TNF-α, interleukin 12/23, integrin α4β7, and small molecules such as Janus kinase (JAK) inhibitors]. Additionally, there remain some doubts about the effectiveness of the vaccines against the Delta variant. On this basis, a number of patients are still in

hesitation to get vaccinated. In our study, only 55 CD patients (35.95%) reported to have been vaccinated. Though no relevance was found between vaccination and anxiety/depression, we did observe that the vaccinated population seemed to own higher life quality. Possible explanation for this association could be that those willing to get vaccinated were in good health and therefore they were not bothered by the above-mentioned doubts. Importantly, we also detected a desire among CD patients to get protection from vaccination. In fact, several researches on the use of vaccines have been carried out in IBD patients, which confirmed the safety and efficacy of the vaccines among this specific population (45–47). According to recommendations from International Organization for the Study of Inflammatory Bowel Disease (IOIBD), patients with IBD should be vaccinated against SARS-CoV-2 as early as possible, and vaccination should not be delayed due to the ongoing immunoregulatory therapy (48). Also, the already existed vaccines remain effective against the Delta variant (49). Hence, to relieve the anxiety and enhance immune defense of CD patients during the COVID-19 pandemic, we need to reassure our CD patients of SARS-CoV-2 vaccination.

The main limitations of this study mainly consisted of the relatively small sample because of the online property of the procedure. A total of 153 participants were recruited, which only accounted for about 50% of all CD patients that we can obtain by the electronic medical records. Also, since the questionnaire were self-reported, subjectivity is a non-negligible factor when we turn these findings into clinical practice. In some cases, the degree of anxiety/depression might be over-exaggerated or under-estimated, and the assessment of disease severity could also be inaccurate. Additionally, those without accessibility to the internet were naturally excluded from the survey, making this population a blind spot of our study. In can be inferred that this group of patients are probably older and might live in remote places, which indicates higher psychological stress due to worse knowledge about the SARS-CoV-2 Delta variant and ambiguity in their disease severity. In addition, it is very likely that the changes in mental anxiety were associated with changes in the number of SARS-CoV-2-infected patients, which could possibly be explained by consequent alterations in the administration of the mandatory confinement of the population. However, in our study, the survey was conducted only once, so the changes in mental anxiety could not be captured. To verify this hypothesis, consecutive studies should be conducted to monitor the changes in mental anxiety among CD patients. Hence, these factors should be taken into consideration when using the results of our study. Nevertheless, our study provides first-hand information about the incidence and risk factors of anxiety/depression and impaired life quality in CD patients in the context of the SARS-CoV-2 Delta variant dominance. Further prospective longitudinal studies are necessary to validate our findings.

CONCLUSION

During SARS-CoV-2 Delta variant predominance, many CD patients suffered from symptoms of anxiety and depression and impaired life quality. Those in isolation or with worsening of symptoms and drug withdrawn were more prone to experience psychological stress. Individualized management such as drug delivery and telemedicine should be promoted to maintain control of mental health and life quality during the pandemic.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board and Ethics Committee of Affiliated Hospital of Yangzhou University (REC ref 2021-YKL06-09-006). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JL and YS carried out the studies, participated in the experimental design and statistical analysis, and drafted the manuscript. XH and TZ participated in the analysis of the overall data. GY and WX were responsible for the second check of relevant data. YD participated in and supervised the whole study process. SH and MW critically revised important knowledge content. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.795889/full#supplementary-material

Supplementary Figure 1 | Flowchart of study process.

Supplementary Table 1 | Questionnaire assessing patients' knowledge of the COVID-19 pandemic and the SARS-CoV-2 Delta variant.

REFERENCES

1. Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM, et al. 2 variants, spike mutations and immune escape. *Nat Rev Microbiol.* (2021) 19:409–24. doi: 10.1038/s41579-021-00573-0
2. Vaidyanathan G. Coronavirus variants are spreading in India – what scientists know so far. *Nature.* (2021) 593:321–2. doi: 10.1038/d41586-021-01274-7
3. Wang P, Nair MS, Liu L, Iketani S, Luo Y, Guo Y, et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. *Nature.* (2021) 593:130–5.
4. Rubin EJ, Baden LR, Udwadia ZF, Morrissey S. Audio interview: India's covid-19 crisis. *N Engl J Med.* (2021) 384:e84. doi: 10.1056/NEJMe2107728
5. Tao K, Tzou PL, Nouhin J, Gupta RK, de Oliveira T, Kosakovsky Pond SL, et al. The biological and clinical significance of emerging SARS-CoV-2 variants. *Nat Rev Genet.* (2021) 22:757–73. doi: 10.1038/s41576-021-00408-x
6. Li Q, Wu J, Nie J, Zhang L, Hao H, Liu S, et al. The impact of mutations in SARS-CoV-2 spike on viral infectivity and antigenicity. *Cell.* (2020) 182:1284.e–94.e.
7. Chu H, Hu B, Huang X, Chai Y, Zhou D, Wang Y, et al. Host and viral determinants for efficient SARS-CoV-2 infection of the human lung. *Nat commun.* (2021) 12:134. doi: 10.1038/s41467-020-20457-w
8. Teyssou E, Delagrèverie H, Visseaux B, Lambert-Niclot S, Brichler S, Ferre V, et al. The delta SARS-CoV-2 variant has a higher viral load than the beta and the historical variants in nasopharyngeal samples from newly diagnosed COVID-19 patients. *J Infect.* (2021) 83:e1–3. doi: 10.1016/j.jinf.2021.08.027
9. Mikszewski A, Stabile L, Buonanno G, Morawska L. Increased close proximity airborne transmission of the SARS-CoV-2 delta variant. *Sci Total Environ.* (2021) 816:151499. doi: 10.1016/j.scitotenv.2021.151499
10. Mlcochova P, Kemp SA, Dhar MS, Papa G, Meng B, Ferreira I, et al. SARS-CoV-2 B.1.617.2 delta variant replication and immune evasion. *Nature* (2021) 599:114–9. doi: 10.1038/s41586-021-03944-y
11. Kannan SR, Spratt AN, Cohen AR, Naqvi SH, Chand HS, Quinn TP, et al. Evolutionary analysis of the delta and delta plus variants of the SARS-CoV-2 viruses. *J Autoimmun.* (2021) 124:102715. doi: 10.1016/j.jaut.2021.102715
12. Shah SA, Moore E, Robertson C, McMenamin J, Katikireddi SV, Simpson CR, et al. COVID-19 positive cases, hospitalisations, and deaths associated with the delta variant of concern. *Lancet Digit Health* (2021) 3:e539–41. doi: 10.1016/S2589-7500(21)00175-8
13. Herlihy R, Bamberg W, Burakoff A, Alden N, Severson R, Bush E, et al. Rapid increase in circulation of the SARS-CoV-2 B.1.617.2 (Delta) variant – mesa county, Colorado, April-June 2021. *MMWR Morb Mortal Wkly Rep.* (2021) 70:1084–7. doi: 10.15585/mmwr.mm7032e2
14. Lane JCE, Weaver J, Kostka K, Duarte-Salles T, Abrahao MTF, Alghoul H, et al. Risk of depression, suicide and psychosis with hydroxychloroquine treatment for rheumatoid arthritis: a multinational network cohort study. *Rheumatology (Oxford).* (2021) 60:3222–34. doi: 10.1093/rheumatology/keaa771
15. Yan AF, Sun X, Zheng J, Mi B, Zuo H, Ruan G, et al. Risk, behavior changes and health-related outcomes during COVID-19 pandemic: findingsamong adults with and without diabetesin China. *Diabetes Res Clin Pract.* (2020) 167:108350. doi: 10.1016/j.diabres.2020.108350
16. Ammitzbøll C, Andersen JB, Vils SR, Mistegaard CE, Mikkelsen S, Erikstrup C, et al. Isolation, behavioral changes and low seroprevalence of SARS-CoV-2 antibodies in patients with systemic lupus erythematosus or rheumatoid arthritis. *Arthritis Care Res (Hoboken).* (2021). doi: 10.1002/acr.24716 [Epub ahead of print].
17. Torres J, Mehandru S, Colombel JF, Peyrin-Biroulet L. Crohn's disease. *Lancet.* (2017) 389:1741–55.
18. Feuerstein JD, Cheifetz AS. Crohn disease: epidemiology, diagnosis, and management. *Mayo Clin Proc.* (2017) 92:1088–103. doi: 10.1016/j.mayocp.2017.04.010
19. Gaines LS, Slaughter JC, Horst SN, Schwartz DA, Beaulieu DB, Haman KL, et al. Association between affective-cognitive symptoms of depression and exacerbation of Crohn's disease. *Am J Gastroenterol.* (2016) 111:864–70. doi: 10.1038/ajg.2016.98
20. Thakur ER, Sansgiry S, Kramer JR, Waljee AK, Gaidos JK, Feagins LA, et al. The incidence and prevalence of anxiety, depression, and post-traumatic stress disorder in a national Cohort of US Veterans with inflammatory bowel disease. *Inflamm Bowel Dis.* (2020) 26:1423–8. doi: 10.1093/ibd/izz280
21. Neuendorf R, Harding A, Stello N, Hanes D, Wahbeh H. Depression and anxiety in patients with inflammatory bowel disease: a systematic review. *J Psychosom Res.* (2016) 87:70–80. doi: 10.1016/j.jpsychores.2016.06.001
22. Frolkis AD, Vallerand IA, Shaheen AA, Lowerison MW, Swain MG, Barnabe C, et al. Depression increases the risk of inflammatory bowel disease, which may be mitigated by the use of antidepressants in the treatment of depression. *Gut.* (2019) 68:1606–12. doi: 10.1136/gutjnl-2018-317182
23. Abautret-Daly Á, Dempsey E, Parra-Blanco A, Medina C, Harkin A. Gut-brain actions underlying comorbid anxiety and depression associated with inflammatory bowel disease. *Acta Neuropsychiatr.* (2018) 30:275–96. doi: 10.1017/neu.2017.3
24. Gracie DJ, Guthrie EA, Hamlin PJ, Ford AC. Bi-directionality of brain-gut interactions in patients with inflammatory bowel disease. *Gastroenterology.* (2018) 154:1635.e–46.e. doi: 10.1053/j.gastro.2018.01.027
25. Knowles SR, Graff LA, Wilding H, Hewitt C, Keefer L, Mikocka-Walus A. Quality of life in inflammatory bowel disease: a systematic review and meta-analyses-part I. *Inflamm Bowel Dis.* (2018) 24:742–51. doi: 10.1093/ibd/izx100
26. Alrubaiy L, Rikaby I, Dodds P, Hutchings HA, Williams JG. Systematic review of health-related quality of life measures for inflammatory bowel disease. *J Crohns colitis.* (2015) 9:284–92. doi: 10.1093/ecco-jcc/jjv002
27. Laguette V, Apostolidis T, Dany L, Bellon N, Grimaud JC, Lagouanelle-Simeoni MC. Quality of life and time perspective in inflammatory bowel disease patients. *Qual Life Res.* (2013) 22:2721–36. doi: 10.1007/s11136-013-0399-4
28. Conti C, Rosa I, Zito L, Grossi L, Efthymakis K, Neri M, et al. Influence of the COVID-19 outbreak on disease activity and quality of life in inflammatory bowel disease patients. *Front psychiatry.* (2021) 12:664088. doi: 10.3389/fpsyt.2021.664088
29. Trindade IA, Ferreira NB. COVID-19 pandemic's effects on disease and psychological outcomes of people with inflammatory bowel disease in Portugal: a preliminary research. *Inflamm Bowel Dis.* (2021) 27:1224–9. doi: 10.1093/ibd/izaa261
30. Magro F, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. part 1: definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch dis orders. *J Crohns Colitis.* (2017) 11:649–70. doi: 10.1093/ecco-jcc/jjx008
31. Gomollon F, Dignass A, Annese V, Tilg H, Van Assche G, Lindsay JO, et al. European evidence-based consensus on the diagnosis and management of Crohn's disease 2016: part 1: diagnosis and medical management. *J Crohns Colitis.* (2017) 11:3–25. doi: 10.1093/ecco-jcc/jjw168
32. Spitzer RL, Kroenke K, Williams JBW, Löwe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med.* (2006) 166:1092–7. doi: 10.1001/archinte.166.10.1092
33. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med.* (2001) 16:606–13. doi: 10.1046/j.1525-1497.2001.016009606.x
34. Guyatt G, Mitchell A, Irvine EJ, Singer J, Williams N, Goodacre R, et al. A new measure of health status for clinical trials in inflammatory bowel disease. *Gastroenterology.* (1989) 96:804–10. doi: 10.1016/0016-5085(89)90905-0
35. Nass BYS, Dibbets P, Markus CR. Impact of the COVID-19 pandemic on inflammatory bowel disease: the role of emotional stress and social isolation. *Stress Health.* (2021). doi: 10.1002/smi.3080 [Epub ahead of print].
36. Al-Rahimi JS, Nass NM, Hassoubah SA, Wazqar DY, Alamoudi SA. Levels and predictors of fear and health anxiety during the current outbreak of COVID-19 in immunocompromised and chronic disease patients in Saudi Arabia: A cross-sectional correlational study. *PLoS One.* (2021) 16:e0250554. doi: 10.1371/journal.pone.0250554
37. van den Brink G, Stapersma L, Bom AS, Rizopolous D, van der Woude CJ, Stuyt RJL, et al. Effect of cognitive behavioral therapy on clinical disease course in adolescents and young adults with inflammatory bowel disease and subclinical anxiety and/or depression: results of a randomized trial. *Inflamm Bowel Dis.* (2019) 25:1945–56. doi: 10.1093/ibd/izz073
38. Wynne B, McHugh L, Gao W, Keegan D, Byrne K, Rowan C, et al. Acceptance and commitment therapy reduces psychological stress in patients with inflammatory bowel diseases. *Gastroenterology.* (2019) 156:935.e–45.e. doi: 10.1053/j.gastro.2018.11.030

39. Gracie DJ, Hamlin PJ, Ford AC. The influence of the brain-gut axis in inflammatory bowel disease and possible implications for treatment. *Lancet Gastroenterol Hepatol.* (2019) 4:632–42. doi: 10.1016/S2468-1253(19)30089-5
40. Mikocka-Walus A, Pittet V, Rossel JB, von Känel R. Symptoms of depression and anxiety are independently associated with clinical recurrence of inflammatory bowel disease. *Clin Gastroenterol Hepatol.* (2016) 14:829.e–35.e. doi: 10.1016/j.cgh.2015.12.045
41. Tabibian A, Tabibian JH, Beckman LJ, Raffals LL, Papadakis KA, Kane SV. Predictors of health-related quality of life and adherence in Crohn's disease and ulcerative colitis: implications for clinical management. *Dig Dis Sci.* (2015) 60:1366–74. doi: 10.1007/s10620-014-3471-1
42. Danese S, Cecconi M, Spinelli A. Management of IBD during the COVID-19 outbreak: resetting clinical priorities. *Nat Rev Gastroenterol Hepatol.* (2020) 17:253–5. doi: 10.1038/s41575-020-0294-8
43. Stone ML, Feng M, Forster EM. COVID-19 pandemic increased anxiety among patients with inflammatory bowel disease: a patient survey in a tertiary referral center. *Dig Dis Sci.* (2021):1–6. doi: 10.1007/s10620-021-07095-y [Epub ahead of print].
44. Krammer F. SARS-CoV-2 vaccines in development. *Nature.* (2020) 586:516–27. doi: 10.1038/s41586-020-2798-3
45. Hadi YB, Thakkar S, Shah-Khan SM, Hutson W, Sarwari A, Singh S. COVID-19 vaccination is safe and effective in patients with inflammatory bowel disease: analysis of a large multi-institutional research network in the United States. *Gastroenterology.* (2021) 161:1336.e–9.e. doi: 10.1053/j.gastro.2021.06.014
46. Khan N, Mahmud N. Effectiveness of SARS-CoV-2 vaccination in a veterans affairs cohort of patients with inflammatory bowel disease with diverse exposure to immunosuppressive medications. *Gastroenterology.* (2021) 161:827–36. doi: 10.1053/j.gastro.2021.05.044
47. Alexander JL, Moran GW, Gaya DR, Raine T, Hart A, Kennedy NA, et al. 2 vaccination for patients with inflammatory bowel disease: a British society of gastroenterology inflammatory bowel disease section and IBD clinical research group position statement. *lancet Gastroenterol Hepatol.* (2021) 6:218–24. doi: 10.1016/S2468-1253(21)00024-8
48. Siegel CA, Melmed GY, McGovern DP, Rai V, Krammer F, Rubin DT, et al. 2 vaccination for patients with inflammatory bowel diseases: recommendations from an international consensus meeting. *Gut.* (2021) 70:635–40. doi: 10.1136/gutjnl-2020-324000
49. Lopez Bernal J, Andrews N, Gower C, Gallagher E, Simmons R, Thelwall S, et al. Effectiveness of covid-19 vaccines against the B.1.617.2 (Delta) variant. *N Engl J Med.* (2021) 385:585–94.

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REVIEW
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Endoscopic Surveillance in Inflammatory Bowel Diseases: Selecting a Suitable Technology

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In the treat-to-target era, endoscopy has become the backbone of the assessment of remission, defined as mucosal healing, in inflammatory bowel disease (IBD) patients. Current recommendations indicate that endoscopic procedures should be performed with high-definition white-light endoscopy (HD-WLE), as it guarantees the best possible visualization of the mucosa. With respect to endoscopic surveillance, the preventive strategy for dysplasia and colorectal cancer (CRC) in long-standing IBD, is the use of dye-chromoendoscopy (DCE), which enhances the mucosal pattern of the colonic walls. DCE has been established as the gold standard for dysplasia detection and is at present incorporated in all international guidelines. Over the past years, novel technologies, such as high-definition endoscopic imaging, and optical and digital enhancement tools have revolutionized the quality and level of fine details of vascular and mucosal patterns. These endoscopic images have the ambition to reflect histological changes for suspected neoplastic lesions and inflammation or healing and are emerging as potential alternatives to DCE. Indeed, the comparison of DCE with high-definition imaging is an open issue that deserves further investigation. We aimed to examine and summarize the technical aspects and the current evidence on endoscopic technologies with a specific focus on the surveillance in IBD patients.

Keywords: endoscopy, colorectal cancer, surveillance, chromoendoscopy, dysplasia, inflammatory bowel disease

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INTRODUCTION

Over the past years, the role of endoscopy in inflammatory bowel diseases (IBD) has been established in the diagnosis, monitoring, and surveillance (1, 2). The endoscopic evaluation allows to confirm the diagnosis of IBD, and the assessment of the extent and severity of the disease (1, 2). However, in the treat-to-target era, endoscopy is not a mere imaging technique any longer, but it has become the backbone of the assessment of remission. The endoscopic remission defined as mucosal healing (MH) has been proven to predict better long-term outcomes and has been incorporated as the principal target in the management of IBD (3, 4). Data on the prevention of bowel damage in

the long-term, in patients achieving MH, are largely available both for Ulcerative Colitis (UC) and Crohn's Disease (CD) (3, 4), and have been endorsed by meta-analyses (5, 6).

As concerns technical aspects, current recommendations indicate that endoscopic procedures should be performed with high-definition white-light endoscopy (HD-WLE), as it guarantees the optimal visualization of the mucosa (1, 2). On the other hand, with respect to endoscopic surveillance as a preventive strategy for dysplasia and colorectal cancer (CRC), the use of dye-chromoendoscopy (DCE), which enhances the mucosal pattern through spraying topical dyes on the colonic walls, has been established as the gold standard for dysplasia detection (7–9).

Patients with long-standing (more than 8 years from the onset of symptoms) both extensive UC and colonic CD are at an increased risk of developing CRC compared with the general population, with a cumulative risk of 2% after 10 years, 8% at 20 years, and 18% at 30 years of disease (10). Among the established clinical factors that affect the cancer risk in IBD patients, the duration and extent of disease, chronic uncontrolled inflammatory activity, a concomitant diagnosis of primary sclerosing cholangitis (PSC), and a family history of CRC are included (11). On the other hand, due to improved therapies and increased integration of surveillance programs in the clinical practice, a decreasing risk of CRC in IBD patients has been observed in the recent years (12). Hence, an optimal surveillance colonoscopy is a crucial prevention, based on the detection of early colonic dysplastic lesions. Colonic precancerous lesions in patients with IBD are frequently flat and elusive, and during the colonoscopy, they can be easily missed especially when hidden behind folds, when there are regenerative patterns, scars, pseudopolyps, or actively inflamed mucosa.

As stated by the Surveillance for Colorectal Endoscopic Neoplasia Detection and Management in Inflammatory Bowel Disease Patients (SCENIC) consensus, HD-WLE is considered superior over standard definition WLE, and DCE should be preferred over WLE in the surveillance setting of IBD patients (8). According to SCENIC recommendations, whenever DCE cannot be performed, for instance due to disease activity or sub-optimal bowel preparation, random quadrant biopsies every 10 cm in the colon are advised (8). Recent novel technologies, such as optical and digital enhancement tools, have revolutionized the quality and level of fine details of vascular and mucosal patterns. These endoscopic images have the ambition to reflect histological changes in suspected neoplastic lesions and inflammation or healing, and are emerging as potential alternatives to DCE in the field of surveillance programs. The optimal endoscopic technique to detect dysplasia in IBD is still a matter of debate. In this review, we aim to examine and summarize the technical aspects and the current evidence on endoscopic technologies with a specific focus on the surveillance, in terms of dysplasia detection and characterization, in IBD patients.

METHODS

PubMed/MEDLINE databases were searched up to December 2021 to identify relevant studies investigating the accuracy of endoscopic techniques in dysplasia detection and characterization in IBD patients. The following text words and corresponding Medical Subject Heading/Entree terms were used: "surveillance," "dye chromoendoscopy," "virtual chromoendoscopy," and "endocytoscopy," individually and in combination with "dysplasia," "inflammatory bowel disease(s)," "IBD," "ulcerative colitis," and "Crohn's disease." No publication date restrictions were applied. Articles were included in this review based on their relevance, and additional publications were identified through their reference lists.

DYE-CHROMOENDOSCOPY

The European Society of Gastrointestinal Endoscopy (ESGE) and the SCENIC consensus recommend the routine use of 0.1% methylene blue or 0.1–0.5% indigo carmine pancolonic chromoendoscopy with targeted biopsies for neoplasia surveillance in patients with long-standing IBD. Indigo carmine is a non-absorbed dye, while methylene blue is an absorbed dye, which can be applied using different concentrations depending on whether the purpose is detection (lower concentrations) or characterization of lesions (higher concentrations). The dye solution can be distributed with a catheter spray or a pump jet. All guidelines indicate that DCE with targeted biopsies should be favored over random biopsies (1, 2, 8, 9). **Figure 1** shows the typical appearance of colonic mucosa after the application of methylene blue.

As demonstrated by a recent observational study, the most frequent causes of an unsuccessful DCE were a poor bowel preparation, active inflammation, and/or the presence of pseudopolyps (13) (**Figure 1**). Moreover, the non-adherence to a clear liquid diet the day before DCE compromised the successful completeness of DCE, compared with previous identified risk factors for inadequate bowel preparation (i.e., older age, history of diabetes mellitus, timing of split dose, narcotic use, and constipation), that were not associated with the ability of performing DCE (13).

DCE by appropriately trained operators, in all cases of quiescent disease and satisfactory bowel preparation, is preferred over the traditional non-targeted four-quadrant biopsies (9).

A properly trained operator is experienced in inspection, advanced imaging, and characterization of colonic lesions. Favorably, she/he is also trained in therapeutic endoscopic resection techniques such as endoscopic mucosal resection (EMR) and endoscopic submucosal dissections (ESD).

Few data are available on the characteristics of a complete training in DCE, and currently, there is no quality index to establish the expertise of endoscopists in performing DCE. However, according to the European Society of Gastrointestinal Endoscopy (ESGE), it is suggested that the endoscopist, in order to achieve the proper competence, should attend an onsite training of at least a week with an expert operator in optical

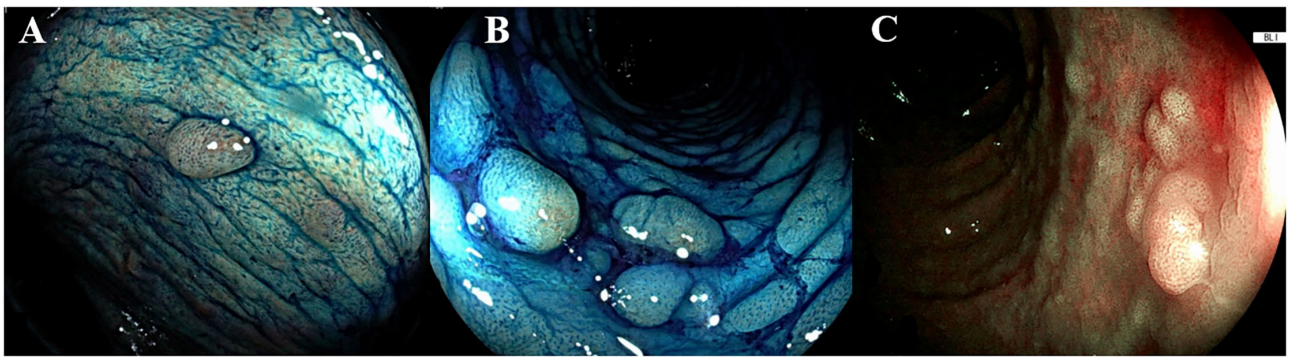

FIGURE 1 | Endoscopic appearance of dye-chromoendoscopy and virtual chromoendoscopy. **(A,B)** The dye is segmentally applied to the colon every 20 to 30 cm with rotational movements of the endoscope, after spraying the dye, once the excess liquid has been suctioned, the mucosa is carefully examined. The colic mucosa presents a regular pattern, the presence of inflammatory pseudo-polyps is enhanced (pit pattern Kudo I-II). **(C)** A sporadic flat lesion with adenomatous appearance (pit pattern Kudo IIIS) in a UC patient, characterized through the blue laser image (BLI, Fujifilm, Japan). According to Kudo classification type I pits appear roundish; type II pits appear as stellar or papillary; type III-s pits are small roundish, tubular pits and type III-L are larger roundish and tubular; type IV pits appear as branch-like or gyrus-like while type V pits are non-structured. The normal mucosa, hyperplastic lesions and inflammatory polyps are comprehended in type I and II, whereas the pit pattern of the classes III-V is considered neoplastic.

diagnosis of IBD (14). The indicated number for adequate self-learning is at least 20 DCE surveillance procedures in IBD patients (14).

As concerns the performance of DCE, this technique has been shown to improve dysplasia detection by 4-fold, with less biopsies (15–18). In the first randomized controlled study by Kiesslich et al., as compared with WLE random biopsy sampling, DCE showed a significant 3.2-fold increase in the total detected neoplastic lesions in UC patients (16). Data from the prospective study by Picco et al. showed that DCE with indigo carmine in the surveillance of long-standing UC was superior in terms of dysplasia detection in comparison to WLE (21.3 vs. 9.3%, $p = 0.007$) (17). In a recent trial where IBD patients were randomized either to HD-DCE with indigo carmine or to HD-WLE ($n = 152$ and $n = 153$, respectively), dysplastic lesions were significantly more frequently detected in the HD-DCE arm (17 versus 7, respectively; $p = 0.032$) (18).

The SCENIC meta-analyses, which included eight studies, confirmed that DCE detected a significantly higher number of dysplastic lesions compared with standard-definition WLE (SD-WLE) [relative risk (RR) = 1.8, 95% confidence interval (CI): 1.2–2.6; and absolute risk increase = 6%, 95% CI: 3%−9%] (8).

Table 1 summarizes the available studies on DCE, WLE, and virtual chromoendoscopy (VCE) in the field of surveillance in IBD.

Necessarily, chromoendoscopy considerably increases the duration of colonoscopy (mean of 11 min, range 9–12 min) (8).

It appears clear that most of the available randomized clinical trials (RCTs) compared DCE with SD-WLE rather than with HD-WLE. Indeed, HD-WLE has been demonstrated to be not inferior to DCE for the detection of colonic dysplasia in a recent RCT, where HD-WLE alone was sufficient for detecting dysplasia, adenocarcinoma, or all neoplastic lesions (19). The comparison of DCE with high-definition imaging is an open issue that deserves further investigation.

VIRTUAL CHROMOENDOSCOPY

The currently available international guidelines instruct on the use of virtual chromoendoscopy (VCE) as a suitable alternative, due to insufficient evidence to recommend it as preferred method (9, 25). Initial studies for dysplasia detection in IBD have explored VCE. The technologies available in the market include the optical diagnosis narrow band imaging (NBI) (Olympus, Japan) that uses only wavelengths absorbed by hemoglobin for maximizing the contrast; the optical enhancement iSCAN (iSCAN, Pentax, Japan) that digitally adds blue color to relatively dark areas; the blue laser image (BLI) (Fujifilm, Japan) that can be used for evaluating both the microvessels and the mucosa; the linked color imaging (LCI) (Fujifilm, Japan) that expands the color range between red and white, enhancing slight mucosal differences in conditions of inflammation and cancer; and the flexible imaging color enhancement (FICE) (Fujinon) that selectively emphasizes certain ranges of wavelength. Through a digital post-processing of the endoscopic images, the VCE systems enhance the details of tissue surface and yearn to reflect histology with increased accuracy.

Among the available studies, a multicenter prospective study that randomized 66 patients in the DCE arm and 65 patients in the NBI arm, revealed no significant difference in neoplasia detection between the two techniques (20). In details, the mean number of neoplastic lesions per colonoscopy were 0.47 and 0.32, respectively (20). The dysplasia detection rate did not significantly differ between the two groups (21.2% with DCE, 21.5% with NBI; $p = 0{\cdot}964$). However, in the DCE group, an average of additional 7 min to procedure time was reported (20). Two previous studies examining HD-NBI have reported the non-inferiority of HD-VCE over HD-DCE (21, 22).

Iacucci et al. compared HD-WLE with HD-iSCAN and HD-DCE, in a randomized non-inferiority trial ($n = 270$), and found no significant difference in the detection rates of neoplastic

TABLE 1 | Studies investigating dysplasia detection rate in IBD with different endoscopic technologies.

Reference	Year	Study design	*N*	Type of endoscopy	Investigated outcome
Kiesslich et al. (16)	2003	Randomized clinical trial	263	DCE vs. WLE	Dysplasia detection rates
Picco et al. (17)	2013	Prospective, non-randomized	75	DCE vs. WLE	Dysplasia detection rates and interobserver variability in the detection of dysplastic lesions
Alexandersson et al. (18)	2020	Randomized clinical trial	305	HD-DCE vs. HD-WLE	Dysplasia detection rates
Iacucci et al. (19)	2018	Randomized clinical trial	270	iSCAN vs. DCE vs. HD-WLE	Detection of colonic lesions
Bisschops et al. (20)	2018	Randomized clinical trial	131	NBI vs. DCE	Performance of DCE and VCE for the detection of neoplastic lesions
Efthymiou et al. (21)	2013	Prospective, non-randomized	44	NBI vs. DCE	Diagnostic yield of each modality for dysplastic lesions
Pellisé et al. (22)	2011	Prospective, randomized, crossover study	60	NBI vs. DCE	Detection of colonic lesions
Iannone et al. (23)	2017	Meta-analysis	1,500	DCE vs. SD-WLE/HD-WLE/NBI	Dysplasia detection rates
Kandiah et al. (24)	2021	Randomized clinical trial	188	iSCAN vs. HD-WLE	Dysplasia detection rates

IBD, inflammatory bowel disease; DCE, dye-chromoendoscopy; WLE, wight light endoscopy; HD, high definition; NBI, narrow bad imaging.

lesions between the techniques (19). **Table 1** elucidates the characteristics of the above-mentioned studies.

A recent meta-analysis including 10 RCTs confirmed that DCE was able to identify more dysplastic lesions as compared with SD-WLE (RR = 2.12; 95% CI: 1.15–3.91). However, no statistically significant difference was observed between DCE and HD-WLE (RR = 1.42; 95% CI: 0.80–2.52) or NBI (RR = 1.05; 95% CI: 0.64–1.71) (23).

Data from the newly published VIRTUOSO trial, enrolling 188 IBD patients, which were randomized either to VCE (iSCAN, $n = 94$) or HD-WLE ($n = 94$), reported no significant difference between the two techniques for neoplasia detection (24). The dysplasia detection rates were assessed as 24.2 and 14.9% for HD-WLE and VCE, respectively, with no statistical significance ($p = 0.14$) (24). The authors observed similar withdrawal times in both arms of the study (median of 24 vs. 25.5 min for HD-WLE and VCE, respectively) (24).

Finally, no published data about either BLI or LCI to detect colonic lesion in patients with IBD are available yet.

CHARACTERIZATION OF COLONIC LESIONS

Since the SCENIC consensus, the term "dysplasia-associated lesion or mass" has been abandoned, with the recommendation to distinguish dysplasia as invisible or visible. In accordance with the knowledge development in endoscopy, the modified Paris classification was introduced to describe the morphology of the lesion and the border as regular or irregular, with or without ulceration (26). According to the Paris classification, colonic lesions are divided into polypoid and non-polypoid (if it protrudes <2.5 mm into the lumen). The polypoid lesions are then classified as pedunculated (with a stalk) or sessile (with a large base). Within the non-polypoid lesions, a distinction can be made between superficial elevated, flat, and depressed. Once morphology has been described, the operator inspects the surface pit pattern of the lesion, which can be described through Kudo's pit pattern classification. This classification comprises six categories (I, II, IIIS, IIIL, IV, and V) (27). The neoplastic Kudo pit pattern IIIS-IIIL-IV-V was found to be a significant predictor of dysplasia in patients with IBD (19). Further studies reported the same association, thereby making the neoplastic Kudo pit pattern (i.e., IIIS-IIIL-IV-V) one of the four main predictors of colonic dysplasia (28). In **Figure 1**, an example of the characterization of colonic lesions according to Kudo's pit pattern is shown.

It is known that the Kudo's pit pattern classification without magnification might have some limitations, especially in those IBD patients with a regenerative hyperplastic or villous mucosal appearance (29). Indeed, it has been demonstrated that in UC patients, on adding magnification, the pit pattern classification well-correlated with the histopathological diagnosis of low-grade and high-grade dysplasia and *in situ* carcinoma (30). Moreover, magnifying endoscopy allows to distinguish the margins of lesions from the surrounding mucosa and the differentiation between deep and superficial submucosal invasion in very suspicious lesions (31).

Lately, a new classification for IBD lesions has been introduced, which is the Frankfurt Advanced Chromoendoscopic IBD LEsions (FACILE) classification (32). This was developed by international experts and fully validated. The FACILE classification completely abandons the Kudo's pit pattern (32). Four characteristics, predicting neoplastic histology are incorporated in the FACILE classification: the morphology (i.e., nonpolypoid or polypoid), the irregular surface and vascular, and any sign of inflammation within the studied lesion (32). Furthermore, the authors demonstrated that trainees without endoscopic expertise could significantly ameliorate their ability in lesions' characterization with FACILE, with a sensitivity and an accuracy of 80% and 77%, respectively, after training ($p < 0.001$) (32).

Endocytoscopy, based on the principle of contact light microscopy providing real-time ultra-magnified images, has been more extensively investigated in the assessment of inflammation, but appears as a promising technology also in the field of

colorectal lesions, especially in their characterization (33, 34). Endocytoscopy has been described in the setting of IBD-associated dysplasia in several case reports, thereby suggesting its future applicability (33, 34). It appears reasonable to expect that the use of endocytoscopy in IBD might potentially close the gap between endoscopic pit pattern diagnosis and histologic assessment.

CONCLUSIONS AND FUTURE PERSPECTIVES

This review elucidates the current evidence on the performance and accuracy of the available endoscopic techniques and technologies in the field of CRC surveillance in IBD. It is known that DCE in comparison to SD-WLE improves the dysplasia detection by 4-fold, without the need of the four-quadrant random biopsies per each colonic tract (15–18).

As mentioned above, the published RCTs compared DCE with SD-WLE rather than with HD-WLE. The evaluation of the accuracy of DCE compared with high-definition endoscopy will be certainly addressed by dedicated studies in the immediate future. Importantly, so far, the non-inferiority of HD-WLE to DCE has been already reported, and based on these data, HD-WLE alone can be considered appropriate and satisfactory as concerns the detection of dysplasia (19).

An important matter of debate is the registered difficulty of adopting DCE into routine clinical practice. This might be due to the absence of properly trained and expert endoscopists and the low confidence of the operators in interpreting the DCE images. However, the mainly addressed cause is undoubtedly the additional time needed to perform a good quality DCE procedure. When comparing the usual colonoscopy with multiple non-targeted biopsies, chromoendoscopy considerably increases the duration of colonoscopy, with a mean of 11 min longer duration (range 9–12 min) (8).

This key issue represents the rational of the latter studies investigating VCE as an alternative method for CRC surveillance in IBD patients. If further multicenter studies and RCTs confirm the non-inferiority of HD-VCE over HD-DCE, and eventually its superiority in terms of time consumption and patients' tolerance, it might turn into an alternative procedure used by experienced IBD endoscopists in the coming years.

Moreover, surveillance with random biopsies results in extremely low dysplasia detection rates (24, 35). Indeed, the dysplasia detection of random four-quadrant biopsies ranges from 0/924 to 1/6,751 (24, 35).

Nevertheless, we agree with the scientific position that random biopsies should still have a role, always in association with DCE, in all IBD patients with a personal history of neoplasia/dysplasia, concomitant PSC, and/or a tubular appearance of the colon during colonoscopy (36, 37). Still, more data are needed on the true utility of random biopsies in these categories of IBD patients.

Lastly, artificial intelligence (AI)-based detection systems and computer-assisted diagnosis (CAD) systems are under increasing employment and development in endoscopy including IBD endoscopy (38). In this respect, the very first cases of AI used for the detection of dysplasia in patients with IBD have been recently reported (39). The application of AI in IBD endoscopy represents an appealing field for the future research.

Patients with IBD are at increased risk of developing colonic dysplasia and CRC (10). Endoscopic surveillance is demonstrated to significantly reduce CRC development and CRC-associated death, and increases the detection of early-stage CRC in IBD patients ($p < 0.01$) (40). As far as we are concerned, the best strategy to reduce this risk involves both the best quality surveillance colonoscopy method and an optimal medical control of disease activity through targeted therapies.

AUTHOR CONTRIBUTIONS

GR is the guarantor of the article, conceived the subject of the article, contributed to the critical interpretation, and supervised the project. AD and RG performed the research. AD wrote the manuscript. MA, TT, FF, and AR critically reviewed the content of the article. All authors approved the final version of the manuscript.

REFERENCES

1. Maaser C, Sturm A, Vavricka SR, Kucharzik T, Fiorino G, Annese V, et al. ECCO-ESGAR guideline for diagnostic assessment in IBD part 1: initial diagnosis, monitoring of known IBD, detection of complications. *J Crohns Colitis*. (2019) 13:144–64. doi: 10.1093/ecco-jcc/jjy113
2. Magro F, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, et al. European Crohn's and Colitis Organisation [ECCO]. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. *J Crohns Colitis*. (2017) 11:649–70. doi: 10.1093/ecco-jcc/jjx008
3. Ungaro RC, Yzet C, Bossuyt P, Baert FJ, Vanasek T, D'Haens GR, et al. Deep remission at 1 year prevents progression of early Crohn's disease. *Gastroenterology*. (2020) 159:139–47. doi: 10.1053/j.gastro.2020.03.039
4. Colombel JF, Rutgeerts P, Reinisch W, Esser D, Wang Y, Lang Y, et al. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. *Gastroenterology*. (2011) 141:1194–201. doi: 10.1053/j.gastro.2011.06.054
5. Colombel JF, Rutgeerts P, Reinisch W, Esser D, Wang Y, Lang Y, et al. Systematic review with meta-analysis: mucosal healing is associated with improved long-term outcomes in Crohn's disease. *Aliment Pharmacol Ther*. (2016) 43:317–33. doi: 10.1111/apt.13475
6. Shah SC, Colombel JF, Sands BE, Narula N. Mucosal healing is associated with improved long-term outcomes of patients with ulcerative colitis: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. (2016) 14:1245–55.e8. doi: 10.1016/j.cgh.2016.01.015
7. Kiesslich R, von Bergh M, Hahn M, Hermann G, Jung M. Chromoendoscopy with indigo carmine improves the detection of adenomatous and non-adenomatous lesions in the colon. *Endoscopy*. (2001) 33:1001–6. doi: 10.1055/s-2001-18932
8. Laine L, Kaltenbach T, Barkun A, McQuaid KR, Subramanian V, Soetikno R. SCENIC international consensus statement on surveillance and management

of dysplasia in inflammatory bowel disease. *Gastroenterology*. (2015) 148:639–51.e28. doi: 10.1053/j.gastro.2015.01.031
9. Bisschops R, East JE, Hassan C, Hazewinkel Y, Kamiński MF, Neumann H, et al. Advanced imaging for detection and differentiation of colorectal neoplasia: European Society of Gastrointestinal Endoscopy (ESGE) Guideline - Update 2019. *Endoscopy*. (2019) 51:1155–79. doi: 10.1055/a-1031-7657
10. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut*. (2001) 48:526–35 doi: 10.1136/gut.48.4.526
11. Herrinton LJ, Liu L, Levin TR, Allison JE, Lewis JD, Velayos F. Incidence and mortality of colorectal adenocarcinoma in persons with inflammatory bowel disease from 1998 to 2010. *Gastroenterology*. (2012) 143:382–89. doi: 10.1053/j.gastro.2012.04.054
12. Jess T, Simonsen J, Jørgensen KT, Pedersen BV, Nielsen NM, Frisch M. Decreasing risk of colorectal cancer in patients with inflammatory bowel disease over 30 years. *Gastroenterology*. (2012) 143:375–81.e1. doi: 10.1053/j.gastro.2012.04.016
13. Megna B, Weiss J, Ley D, Saha S, Pfau P, Grimes I, et al. Clear liquid diet before bowel preparation predicts successful chromoendoscopy in patients with inflammatory bowel disease. *Gastrointest Endosc*. (2019) 89:373–9.e2. doi: 10.1016/j.gie.2018.09.039
14. Dekker E, Houwen BBSL, Puig I, Bustamante-Balén M, Coron E, Dobru DE, et al. Curriculum for optical diagnosis training in Europe: European Society of Gastrointestinal Endoscopy (ESGE) Position Statement. *Endoscopy*. (2020) 52:899–923. doi: 10.1055/a-1231-5123
15. Hurlstone DP, Sanders DS, Lobo AJ, McAlindon ME, Cross SS. Indigo carmine-assisted high magnification chromoscopic colonoscopy for the detection and characterization of intraepithelial neoplasia in ulcerative colitis: a prospective evaluation. *Endoscopy*. (2005) 37:1186–92. doi: 10.1055/s-2005-921032
16. Kiesslich R, Fritsch J, Holtmann M, Koehler HH, Stolte M, Kanzler S, et al. Methylene blue-aided chromoendoscopy for the detection of intraepithelial neoplasia and colon cancer in ulcerative colitis. *Gastroenterology*. (2003) 124:880–8. doi: 10.1053/gast.2003.50146
17. Picco MF, Pasha S, Leighton JA, Bruining D, Loftus EV Jr, Thomas CS, et al. Procedure time and the determination of polypoid abnormalities with experience: implementation of a chromoendoscopy program for surveillance colonoscopy for ulcerative colitis. *Inflamm Bowel Dis*. (2013) 19:1913–20. doi: 10.1097/MIB.0b013e3182902aba
18. Alexandersson B, Hamad Y, Andreasson A, Rubio CA, Ando Y, Tanaka K, et al. High-definition chromoendoscopy superior to high-definition white-light endoscopy in surveillance of inflammatory bowel diseases in a randomized trial. *Clin Gastroenterol Hepatol*. (2020) 18:2101–7. doi: 10.1016/j.cgh.2020.04.049
19. Iacucci M, Kaplan GG, Panaccione R, Akinola O, Lethebe BC, Lowerison M, et al. A randomized trial comparing high-definition colonoscopy alone with high-definition dye spraying and electronic virtual chromoendoscopy for detection of colonic neoplastic lesions during IBD surveillance colonoscopy. *Am J Gastroenterol*. (2018) 113:225–34. doi: 10.1038/ajg.2017.417
20. Bisschops R, Bessissow T, Joseph JA, Baert F, Ferrante M, Ballet V, et al. Chromoendoscopy versus narrow band imaging in UC: a prospective randomised controlled trial. *Gut*. (2018) 67:1087–94. doi: 10.1136/gutjnl-2016-313213
21. Efthymiou M, Allen PB, Taylor AC, Desmond PV, Jayasakera C, De Cruz P, et al. Chromoendoscopy versus narrow band imaging for colonic surveillance in inflammatory bowel disease. *Inflamm Bowel Dis*. (2013) 19:2132–8. doi: 10.1097/MIB.0b013e31829637b9
22. Pellisé M, López-Cerón M, Rodríguez de Miguel C, Jimeno M, Zabalza M, Ricart E, et al. Narrow-band imaging as an alternative to chromoendoscopy for the detection of dysplasia in long-standing inflammatory bowel disease: a prospective, randomized, crossover study. *Gastrointest Endosc*. (2011) 74:840–48. doi: 10.1016/j.gie.2011.05.013
23. Iannone A, Ruospo M, Wong G, Principi M, Barone M, Strippoli GFM, et al. Chromoendoscopy for surveillance in ulcerative colitis and Crohn's disease: a systematic review of randomized trials. *Clin Gastroenterol Hepatol*. (2017) 15:1684–97.e11. doi: 10.1016/j.cgh.2016.11.021
24. Kandiah K, Subramaniam S, Thayalasekaran S, Chedgy FJ, Longcroft-Wheaton G, Fogg C, et al. Multicentre randomised controlled trial on virtual chromoendoscopy in the detection of neoplasia during colitis surveillance high-definition colonoscopy (the VIRTUOSO trial). *Gut*. (2021) 70:1684–90. doi: 10.1136/gutjnl-2020-320980
25. Murthy SK, Feuerstein JD, Nguyen GC, Velayos FS. AGA clinical practice update on endoscopic surveillance and management of colorectal dysplasia in inflammatory bowel diseases: expert review. *Gastroenterology*. (2021) 161:1043–51.e4. doi: 10.1053/j.gastro.2021.05.063
26. Sawaki A, Nakamura T, Suzuki T, Hara K, Kato T, Kato T, et al. A two-step method for marking polypectomy sites in the colon and rectum. *Gastrointest Endosc*. (2003) 57:735–37. doi: 10.1067/mge.2003.174
27. Kudo S, Hirota S, Nakajima T, Hosobe S, Kusaka H, Kobayashi T, et al. Colorectal tumours and pit pattern. *J Clin Pathol*. (1994) 47:880–85. doi: 10.1136/jcp.47.10.880
28. Carballal S, Maisterra S, Lopez-Serrano A, Gimeno-García AZ, Vera MI, Marín-Garbriel JC, et al. Real-life chromoendoscopy for neoplasia detection and characterisation in long-standing IBD. *Gut*. (2018) 67:70–8. doi: 10.1136/gutjnl-2016-312332
29. Carballal S, Maisterra S, López-Serrano A, Gimeno-García AZ, Vera MI, Marín-Garbriel JC, et al. Clinical usefulness of narrow band imaging magnifying colonoscopy for assessing ulcerative colitis-associated cancer/dysplasia. *Endosc Int Open*. (2016) 4:E1183–87. doi: 10.1055/s-0042-116488
30. Kawasaki K, Nakamura S, Esaki M, Kurahara K, Eizuka M, Nuki Y, et al. Clinical usefulness of magnifying colonoscopy for the diagnosis of ulcerative colitis-associated neoplasia. *Dig Endosc*. (2019) 31(Suppl. 1):36–42. doi: 10.1111/den.13382
31. Sugimoto S, Naganuma M, Iwao Y, Matsuoka K, Shimoda M, Mikami S, et al. Endoscopic morphologic features of ulcerative colitis-associated dysplasia classified according to the SCENIC consensus statement. *Gastrointest Endosc*. (2017) 85:639–46.e2. doi: 10.1016/j.gie.2016.11.013
32. Iacucci M, McQuaid K, Gui XS, Iwao Y, Lethebe BC, Lowerison M, et al. A multimodal (FACILE) classification for optical diagnosis of inflammatory bowel disease associated neoplasia. *Endoscopy*. (2019) 51:133–41. doi: 10.1055/a-0757-7759
33. Fukunaga S, Kusaba Y, Tsuruta O. Use of endocytoscopy for ulcerative colitis surveillance: a case study. *Gastroenterology*. (2020) 158:e1–2. doi: 10.1053/j.gastro.2019.11.018
34. Misawa M, Kudo SE, Ogata N. Two cases of colitis-associated neoplasia observed with endocytoscopy. *Dig Endosc*. (2019) 31(Suppl. 1):43–4. doi: 10.1111/den.13325
35. Bopanna S, Roy M, Das P, Dattagupta S, Sreenivas V, Mouli VP, et al. Role of random biopsies in surveillance of dysplasia in ulcerative colitis patients with high risk of colorectal cancer. *Intest Res*. (2016) 14:264–9. doi: 10.5217/ir.2016.14.3.264
36. Moussata D, Allez M, Cazals-Hatem D, Treton X, Laharie D, Reimund JM, et al. Are random biopsies still useful for the detection of neoplasia in patients with IBD undergoing surveillance colonoscopy with chromoendoscopy? *Gut*. (2018) 67:616–24. doi: 10.1136/gutjnl-2016-311892
37. Hu AB, Burke KE, Kochar B, Ananthakrishnan AN. Yield of random biopsies during colonoscopies in inflammatory bowel disease patients undergoing dysplasia surveillance. *Inflamm Bowel Dis*. (2021) 27:779–86. doi: 10.1093/ibd/izaa205
38. Misawa M, Kudo SE, Mori Y, Hotta K, Ohtsuka K, Matsuda T, et al. Development of a computer-aided detection system for colonoscopy and a publicly accessible large colonoscopy video database (with video). *Gastrointest Endosc*. (2021) 93:960–7.e3. doi: 10.1016/j.gie.2020.07.060
39. Maeda Y, Kudo SE, Ogata N, Misawa M, Mori Y, Mori K, Ohtsuka K. Can artificial intelligence help to detect dysplasia in patients with ulcerative colitis? *Endoscopy*. (2021) 53:E273–4. doi: 10.1055/a-1261-2944
40. Bye WA, Ma C, Nguyen TM, Parker CE, Jairath V, East JE. Strategies for detecting colorectal cancer in patients with inflammatory bowel disease: a cochrane systematic review and meta-analysis. *Am J Gastroenterol*. (2018) 113:1801–9. doi: 10.1038/s41395-018-0354-7

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The Role and Function of Mucins and Its Relationship to Inflammatory Bowel Disease

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Mucus is present throughout the gastrointestinal tract and is essential for regulating gut microbiota homeostasis and preventing disease by protecting the gastrointestinal barrier from microorganisms, pathogens and toxins or other irritants. Mucin (MUC)-2 is a secreted protein produced by epithelial goblet cells as the main component of mucus. Defects in the gastrointestinal tract, such as inflammation and ulcers, cause damage to the mucus barrier, which can worsen mucus quality and reduce mucus production. Therefore, we would like to review the characteristics of MUC2 and its role in intestinal disorders and highlight the importance of further studies. We also investigated whether the role of MUC2 differs between children and adults, ulcerative colitis (UC) and Crohn's disease (CD).

Keywords: mucins, mucus, inflammatory bowel diseases, ulcerative colitis, Crohn's disease

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INTRODUCTION

Inflammatory bowel disease (IBD) is a digestive disorder in which chronic inflammation repeats exacerbation and improvement. The two main types of IBD are ulcerative colitis (UC) and Crohn's disease (CD). IBD is a multifactorial disease, with both genetic and environmental factors contributing to the disease. In particular, loss of intestinal epithelium completeness plays a very important role in IBD (1, 2). Dysfunction of the innate immune system due to abnormal signal transduction caused by pathogens, excessive leakage of bacterial antigens into the mucosa, or inappropriate immune responses due to changes in the composition of the intestinal microflora induce an inflammatory response that progressively degrades the intestinal epithelium. This allows more antigen to leak and worsens inflammation, further damaging the barrier, so the integrity of the mucosal barrier is a key factor in determining the status of IBD (3, 4).

Given the role of the mucus layer, there is growing interest in it because loss of intestinal barrier integrity can cause or exacerbate the progression of inflammatory diseases of the gastrointestinal tract, such as IBD. Although the prevalence is continuously increasing, the cause and treatment are still difficult to identify (5). In particular, IBD onset during childhood and adolescence affects more than 70,000 children in the United States, accounting for 25% of all patients with IBD cases (6–11). About 20–30% of pediatric patients with IBD require surgery within 10 years of diagnosis, so they are more severe than adults, and many receive additional surgical intervention in consideration of recurrence and complications (12–16).

Early diagnosis and treatment of pediatric patients with IBD is very important because children and adolescents are often accompanied by symptoms such as growth retardation and depression due to the characteristics of growth and puberty. In addition, since it requires active treatment from

the early stage of diagnosis, high medical costs cannot be ignored. Therefore, to identify differences between adult and pediatric patients with IBD and to enable them to be applied to treatment and prevention, this review highlights the characteristics of MUC2 mucin and its role in IBD, factors affecting MUC2, and differences between IBD in adults and children.

STRUCTURE AND CLASSIFICATION OF MUCINS

From MUC1 to MUC22, up to 22 distinct mucin genes have been identified in the sequence of discovery. They are expressed according to tissues and cell types and they are broadly categorized into two types: secretory and membrane related (17–23). MUC2, MUC5AC, MUC5B, and MUC6 are gel-forming secretory mucins found on the mucosal surface. However, MUC1, MUC3, MUC4, MUC13, and MUC17 are membrane-associated mucins found in the apical membranes of epithelial cells (**Figure 1**). MUC1, MUC2, MUC3, and MUC4 are the predominant mucus detected in colorectum, with MUC2 being produced specifically in goblet cells (21, 24, 25). MUC2 exhibits similar structural and physicochemical properties to other secreted mucins expressed in the gastric and respiratory glandular epithelium, such as MUC5AC, MUC5B, and MUC6. MUC2 monomer contains over 5,000 amino acids and is rich in proline, serine, and threonine (19, 26, 27). MUC2 consists of five distinct regions, including an N-terminal domain comprising von Willebrand D4 domain and CysD domain, a small PTS domain, another CysD domain, a large PTS domain, and a C-terminal part containing von Willebrand D4-C domains, and a cystine knot domain (28).

MUC2 BIOSYNTHESIS, SECRETION AND REGULATION

MUC2 is expressed early in fetal development and is detected in the colon at 12 weeks of gestation and is expressed by individual cells that are goblet cell precursors (29). And most of these cells are localized in the crypt region (30). In the normal colon, MUC2 is expressed in goblet cells (31). Several bioactive factors that enhance mucin secretion regulate mucin synthesis. According to numerous research, mucin expression is regulated by transcriptional or epigenetic regulation (18, 32, 33). MUC2 transcription is regulated by signal transduction pathways, which target transcription factors binding to specific regions of the promoter. Activation of nuclear factor (NF)-κB, a transcription factor, is common during inflammation of the gastrointestinal tract, and the promoter of MUC2 contains an NF-κB binding site. Several pathways activate NF-κB, and the representative pathways are the Ras-mitogen-activated protein kinase (MAPK) pathway through lipopolysaccharide (LPS) and the phosphoinositide 3-kinase (PI3K)/Akt pathway by tumor necrosis factor (TNF)-α. Interleukin (IL)-4 and IL-13 also activate NF-κB through MAPK and upregulate MUC2 transcription (34–36). In contrast, the neuropeptide hormone vasoactive intestinal peptide upregulates MUC2 transcription by activating the

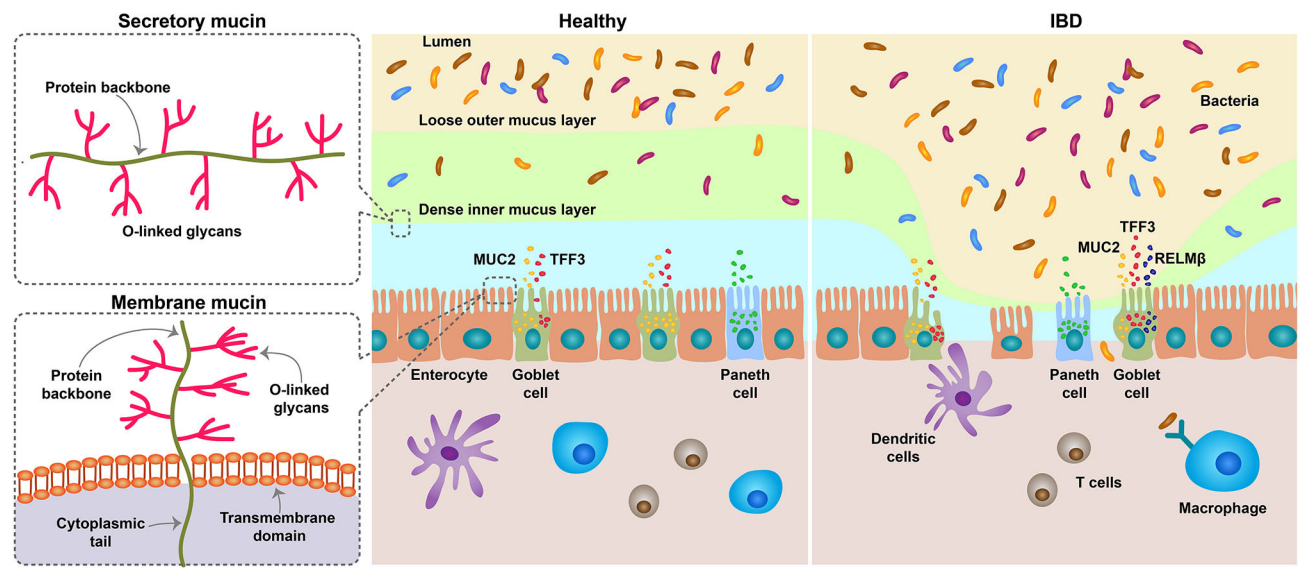

FIGURE 1 | Schematic diagram depicting the role of MUC2 in IBD. The mucous membrane consists of two layers, a loose outer layer, which is permeable to bacteria, and a tightly attached inner layer, which cannot penetrate. Mucins are produced by goblet cells of the intestinal epithelium and are divided into secretory and membranous types. Secreted mucins are gel-forming mucus, composed mostly of MUC2, and provide viscoelasticity. Although the degradation and synthesis of mucin are generally in equilibrium, the number of goblet cells is reduced in the IBD state, and thus the secretion of mucin is also reduced. It also increases the number of immature goblet cells that produce incomplete mucus. When the mucous membrane breaks down, the bacteria in the lumen penetrate inside and cause inflammation. MUC, Mucin; IBD, inflammatory bowel disease.

transcription factor cAMP response element-binding protein (CREB)/cyclic AMP-dependent transcription factor (ATF)-1, whereas prostaglandin PGE2 also induces MUC2 transcription by activating CREB/ATF1 (37). A recent study reported that the transcription factor SAM pointed a domain-containing Ets (SPDEF) can upregulate MUC2 production by stimulating the differentiation of secretory progenitors into Paneth and goblet cells (38). Epigenetic studies have revealed that MUC2 expression can be downregulated by methylation of CpG islands in specific regions of the MUC2 promoter. Additionally, we reported that MUC2 gene expression is regulated by epigenetic mechanisms of DNA methylation and histone modification of specific regions of the MUC2 promoter (39, 40).

INTESTINAL MUCUS LAYER

Absorption of nutrition is an important function of the small intestine, and the mucous system facilitates this by lubricating and protecting against endogenous enzymes and microorganisms. Mucus entirely covers the surface of the epithelium (20). The intestinal mucus layer consists of a dense mesh-like network of MUC2 secreted by goblet cells, which serve as host defense against endogenous and exogenous irritants and microbial adhesion and invasion but permit the transport of nutrients. The mucus layer is primarily composed of MUC2, but also contains other goblet cell products, such as trefoil factor (TFF)-3, resistin-like molecule (RELM)-β, and Fc fragment of IgG binding protein (Fcgbp) (41–44). The mucous membrane has two mucus layers, which are densely and firmly attached to the interior and loosely attached to the exterior compared to the interior. The thickness of the inner and outer mucus layers was similar in the stomach and jejunum, increased significantly in the ileum, and was thickest in the colon. The thickness of the entire mucus layer exhibited a similar trend. The thickness of the mucus layer is maintained by a balance of synthesis, secretion, and degradation, which is regulated by glycosidases, proteases, and other enzymes (45, 46) (**Figure 1**).

MUCUS BARRIER ALTERATIONS IN IBD

MUC2 Alterations

MUC2 plays a significant role in the pathogenesis of IBD, and deficiency of MUC2 mucin can change the composition of the mucus, which can affect the pathogenesis of IBD. UC is a disease of the inner colonic mucous layer, and the host's immune response to the microbiome increases with increased exposure to bacteria.

UC was observed to be thinner and more discontinuous in mucus than CD, and less MUC2 was excreted during the active phase of UC. The decreased secretion of MUC2 may be attributed to the translational or post-translational modifications, and it is also associated with a decrease in goblet cells (47, 48). Moreover, patients with UC have fewer goblet cells and decreased MUC2 synthesis and secretion, especially at the onset of severe disease, enabling direct interaction between the colonic microbiota and the epithelial barrier (49). Patients with UC in the active phase showed low levels of goblet cell differentiation factors HATH1 and kruppel-like factor (KLF)-4 (50). The results of genome-wide association studies (GWAS) showed that IBD pathogenesis was associated with mutations in the MUC genes, including MUC3 and MUC19, and variants of MUC2 have also been detected in IBD cases (49, 51–53).

CD affects all parts of the intestine but is most common in the small intestine. CD, as opposed to UC, is characterized by increased mucus production and discontinuous inflammation. Mucus in the small intestine serves as a diffusion barrier, and mucus secretion occurs primarily in the crypt entrance. Genetic associations are more evident in CD than in UC, involving genes, such as nucleotide binding oligomerization domain containing (NOD)-2, autophagy related 16 like 1 (ATG16L1), immunity related GTPase M (IRGM), and X-box binding protein (XBP)-1 associated with autophagy, unfolded protein responses, and bacterial detection and defense (54–56).

The failure to restrict bacterial access to the epithelium despite increased levels of mucus production in CD patients indicates that mucus level and secondary structural modifications are critical for mucus barrier function. The viscoelastic function of mucus depends on proper glycosylation, sulfation, and sialylation (4, 49). People and mice with IBD have higher levels of sulfide, a product of sulfate-reducing bacteria, which reduces the disulfide bonds between the mucus and breaks down the mucus network (57, 58). Even in the UC cohort, glycosylation and sulfation defects indicated that the mucus was deformed and unable to act effectively as a barrier (57, 59, 60). Because of the GWAS, a mutation in the core 1 beta-3-galactosyltransferase (β3GalT)-specific molecular chaperone (Cosmc), a chaperone of the T-synthase glycosyltransferase responsible for O-glycan synthesis of mucin protein, was associated with IBD (58, 61). Indeed, Muc2 knockout mice developed spontaneous colitis after 5 weeks of age and exhibited increased susceptibility to DSS-induced colitis. Additionally, mice lacking core 1-derived O-glycans lose mucus complexity and exhibit rapid spontaneous colitis (59). Thus, the mucus barrier acts as a means to prevent or limit the contact of epithelial cells with bacteria and antigens, implying that MUC plays an important role in mucus barrier formation and alleviation of colitis such as IBD.

MUC2 Related Alterations

Glycosylation

The glycosylation degree of mucus is essential for its mucosal protective role, and the MUC2 protein core is generally proteolytic resistant because of glycosylation. Bacteria have exoglycosidase, which releases monosaccharide residues and uses them as an energy source. Under normal conditions, the mucus broken down by bacteria is balanced with the new mucus production. However, altered glycosylation can affect this balance, causing glycans to be shortened and digested faster, exposing the protein core and thereby breaking down the mucus barrier faster. Mucus glycosylation defects reduce the mucus layer, placing germs and hosts closer together (59, 62, 63). The glycosylation pattern of MUC2 correlates with the degree of inflammation and disease course, with CD overproduction and abnormal mucin glycosylation, whereas in UC, alterations

TABLE 1 | Characteristics of MUC and TFF expression in adults and pediatric IBD patients.

	Adult	Pediatric
CD	↑Mucus thickness or no change	↓MUC2, TFF2, TFF3 (inflamed TI)
	↓MUC2, MUC3, MUC4, MUC5B, MUC7	↑MUC2, TFF2, TFF3 (non-inflamed TI)>healthy control
	MUC5AC and MUC6 present	↑MUC1, TFF1 (during remission)
	Goblet cell depletion	MUC5AC and TFF1 present
		Goblet cell depletion (TI)
		↓MUC2, TFF2 adjusted for goblet cell density (inflamed)
		↑MUC2, TFF2 adjusted for goblet cell density (non-inflamed)
UC	↓Mucus thickness	TFF1, TFF3, MUC1 not significant
	↓Glycosylation and sulphation	↓TFF2 or not significant (inflamed TI)
	↑Sialylation	↓MUC2 (inflamed TI)
	↓MUC2, MUC9, MUC20	MUC5AC and TFF1 present
	↑MUC1, MUC16	Goblet cell depletion (ascending colon)
	MUC5AC present	
	Goblet cell depletion	

in MUC2 O-glycosylation in active phase recovered during remission (57, 64).

Tight Junction

Since tight junction (TJ) is interdependent with the mucus barrier, the loss of one decreases the other. Mice deficient in Claudin 7, hepatocyte nuclear factor 4 alpha (Hnf4a), and Muc2 develop spontaneous colitis (35, 65, 66). Muc2−/−mice show increased epithelial barrier permeability and mucus defects, and the claudin gene expression is unregulated (66). Also, in Hnf4a−/−mice, the number of goblet cells and mucus decreased (67). This interdependence may result from dysregulation of the signals regulating both mucus and TJ, thereby promoting the inflammatory response that sustains IBD (68).

Differences Between Adult and Pediatric Patients

We reviewed a number of papers to identify differences in MUC2 expression in UC and CD as well as in adult and pediatric patients. We summarized the MUC and TFF expression characteristics of adult and pediatric IBD patients in **Table 1** based on the results of many researchers (**Table 1**) (11, 24, 69).

A paper by Bankole et al. (69) published in 2021 systematically reviewed the correlation between adult UC patients and MUC. Among them, according to the contents related to MUC2, when they reviewed the papers reported for 30 years under similar conditions, the decreased and unchanged MUC2 mRNA expression in UC patients were the same in three cases and increased in one case. On the other hand, the protein expression level was increased in five cases, unchanged in two cases, and increased in one case. They explained that the failure to reproduce in all cases was due to differences in mucin expression assessment.

While the correlation between UC and the mucous layer is relatively well-established, it remains controversial in CD. According to the results of the initial study, the thickness of the mucus layer increased in adult CD patients (70), but a recent study showed that it was not significantly different from that of the normal control group (71). However, according to a meta-analysis, total mucin levels were reduced by 34% in adult CD patients (72), and MUC2 expression was observed to be reduced in CD in the ileum similar to UC (73). Grondin et al. (21) suggested that although it is clear that changes in mucin expression and function play a unique role in IBD, characterizing a distinct mucin expression profile is difficult because there is considerable heterogeneity.

Shaoul et al. (30) and Hensel et al. (11), who investigated the expression of MUC and TFF in pediatric IBD patients, showed similar results. In pediatric CD patients with acute inflammation, significantly lower mRNA levels of MUC2, TFF2 and TFF3 were observed in the mucosa of the TI, but higher mRNA levels than healthy controls in tissues without inflammation. Also, MUC1 and TFF1 showed high levels, especially during clinical remission. In UC, the expression level of MUC2 decreased in acute inflammatory conditions, but TFF2 and 3 did not change significantly. In addition, MUC5AC and TFF1, which were not seen in the normal state, were found in the tissues of CD and UC patients, the same as in adults. These results can be seen as a compensatory recovery mechanism for healing and regeneration of mucosal barriers due to tissue damage caused by inflammation.

Alterations in MUC2 amount and quality, and changes in intestinal trefoil factor expression during inflammation, may upregulate MUC5AC and TFF1 expression to compensate for the impaired barrier and repair functions. MUC5AC and TFF1 are not expressed in normal colonic tissue. MUC5AC was sporadically expressed in goblet cells expressing MUC2 in both UC and CD. This pattern was not limited to a specific region of the colon and was not affected by disease severity or local inflammation. It has also been observed in colonic biopsy specimens of autologous restrictive colitis and solitary rectal ulcer syndrome, but unrestricted to IBD. Some of the goblet cells expressing MUC5AC showed expression of TFF1. This change in expression of MUC5AC and TFF1 was suggested to be a non-specific repair function to compensate for the impairment of colonic barrier function (30).

CONCLUSIONS

Mucin acts as an innate host defense mechanism by forming a mucus layer that protects the host from pathogenic microflora invasion and is vital for intestinal microflora formation. Defects in the mucosal barrier result in abnormal bacterial symbiosis and defects in the host's innate and adaptive immune responses, resulting in intestinal inflammatory responses and damage (**Figure 1**). Therefore, in order to maintain the integrity of the mucosal barrier, the quantity and quality of mucus are

also important, and MUC, which is a component of mucus, is inevitably important.

Mucin genes were identified from MUC1 to MUC22 in the order of discovery, and are largely divided into secretory and membrane-related types. Among them, MUC1, 2, 3 and 4 are mucins detected in the colorectal, and MUC2 is the main mucin produced specifically in goblet cells. MUC2 is expressed in colon goblet cells of UC and CD patients as well as healthy individuals. When the colon is depleted of goblet cells due to IBD and other inflammatory conditions, the expression of MUC2 is also reduced and remains in a relatively immature state. This mucus loses its barrier function and exposes the mucous membrane to inflammatory substances. The factors that cause IBD and inflammatory disease are very diverse, but when the integrity of the mucus barrier is lost, it can be a key factor in exacerbating the disease state.

When reviewing the results of various researchers, there are not many studies targeting pediatric IBD patients and many contradictory results have been reported in adult IBD patients, so it is difficult to clearly conclude that the pattern between adults and children is the same. However, at least according to our investigation, it can be suggested that MUC2 expression is decreased and goblet cell decrease is the same in both adults and children in CD and UC in acute inflammatory state. In addition, in the tissues of CD or UC patients, the opposite expression levels may be observed depending on the state of the tissue with or without inflammation or the degree of remission of inflammation, and these results can be considered as a compensatory repair mechanism for mucosal barrier healing and regeneration.

When IBD occurs, pediatric patients need active treatment with biological agents from the beginning, unlike adult patients. As such, pediatric patients have different symptoms or treatment methods from adult patients, but the level of MUC expression is similar. Most of the experimental systems show reproducible results, but for patients, some studies have weak correlations. This is because the factors involved are very diverse and complex. In addition, MUC expression patterns may vary depending on the presence or absence of inflammation and the degree of inflammation in the tissue sample.

Overall, the complexity and heterogeneity of both MUC and TFF makes it difficult to decipher their diverse roles in the pathophysiological process as either a cause or a consequence of IBD. Although many researchers analyzed the results of IBD patient samples, many were excluded when they attempted to draw conclusions by comparing them to each other under similar conditions. Although some studies have documented detailed conditions, many studies have been conducted under relatively broad conditions. In the case of pediatric IBD patients, the number of studies is very limited, regardless of study comparison conditions. This is probably because the patient's age is very young or the sample volume is small, making it very difficult to obtain sufficient samples for the study. To overcome these problems, it is necessary to analyze the sample by subdividing it under more various conditions, and accordingly, it is believed that more consistent results can be derived. In addition, if the criteria for comparative conditions are established and shared, clinical results will be much richer and comparative analysis will be possible for each researcher under the conditions they want. More clinical findings and further studies in pediatric IBD and other inflammatory diseases continue to be needed in the future to determine immune function and potential interaction partners.

AUTHOR CONTRIBUTIONS

YK and HP contributed in the acquisition, analysis and interpretation of data, and drafting of the initial manuscript. B-HC contributed in the acquisition, analysis and interpretation of data, and critical revision for important intellectual content. BK contributed in the conception of the study, acquisition, analysis and interpretation of data, and critical revision for important intellectual content. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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REFERENCES

1. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet.* (2007) 369:16s27–40. doi: 10.1016/S0140-6736(07)60750-8
2. Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature.* (2011) 474:298–306. doi: 10.1038/nature10208
3. Capaldo CT, Nusrat A. Cytokine regulation of tight junctions. *Biochim Biophys Acta.* (2009) 1788:864–71. doi: 10.1016/j.bbamem.2008.08.027
4. Smirnova MG, Birchall JP, Pearson JP. TNF-alpha in the regulation of MUC5AC secretion: some aspects of cytokine-induced mucin hypersecretion on the *in vitro* model. *Cytokine.* (2000) 12:1732–6. doi: 10.1006/cyto.2000.0763
5. Kaplan GG. The global burden of IBD: from 2015 to 2025. *Nat Rev Gastroenterol Hepatol.* (2015) 12:720–7. doi: 10.1038/nrgastro.2015.150
6. Silverberg MS, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol.* (2005) 19(Suppl. A): 5A−36A. doi: 10.1155/2005/269076
7. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology.* (2012) 142:46–54 e42; quiz e30. doi: 10.1053/j.gastro.2011.10.001
8. Taupin D, Podolsky DK. Trefoil factors: initiators of mucosal healing. *Nat Rev Mol Cell Biol.* (2003) 4:721–32. doi: 10.1038/nrm1203

9. Aamann L, Vestergaard EM, Gronbaek H. Trefoil factors in inflammatory bowel disease. *World J Gastroenterol.* (2014) 20:3223–30. doi: 10.3748/wjg.v20.i12.3223
10. Dignass AU, Lynch-Devaney K, Podolsky DK. Hepatocyte growth factor/scatter factor modulates intestinal epithelial cell proliferation and migration. *Biochem Biophys Res Commun.* (1994) 202:701–9. doi: 10.1006/bbrc.1994.1987
11. Hensel KO, Boland V, Postberg J, Zilbauer M, Heuschkel R, Vogel S, et al. Differential expression of mucosal trefoil factors and mucins in pediatric inflammatory bowel diseases. *Sci Rep.* (2014) 4:7343. doi: 10.1038/srep07343
12. Gronbaek H, Vestergaard EM, Hey H, Nielsen JN, Nexo E. Serum trefoil factors in patients with inflammatory bowel disease. *Digestion.* (2006) 74:33–9. doi: 10.1159/000096591
13. Tran CP, Cook GA, Yeomans ND, Thim L, Giraud AS. Trefoil peptide TFF2 (spasmolytic polypeptide) potently accelerates healing and reduces inflammation in a rat model of colitis. *Gut.* (1999) 44:636–42. doi: 10.1136/gut.44.5.636
14. Wright NA, Poulsom R, Stamp G, Van Noorden S, Sarraf C, Elia G, et al. Trefoil peptide gene expression in gastrointestinal epithelial cells in inflammatory bowel disease. *Gastroenterology.* (1993) 104:12–20. doi: 10.1016/0016-5085(93)90830-6
15. Farrell JJ, Taupin D, Koh TJ, Chen D, Zhao CM, Podolsky DK, et al. TFF2/SP-deficient mice show decreased gastric proliferation, increased acid secretion, and increased susceptibility to NSAID injury. *J Clin Invest.* (2002) 109:193–204. doi: 10.1172/JCI0212529
16. Longman RJ, Douthwaite J, Sylvester PA, Poulsom R, Corfield AP, Thomas MG, et al. Coordinated localisation of mucins and trefoil peptides in the ulcer associated cell lineage and the gastrointestinal mucosa. *Gut.* (2000) 47:792–800. doi: 10.1136/gut.47.6.792
17. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer.* (2004) 4:45–60. doi: 10.1038/nrc1251
18. Andrianifahanana M, Moniaux N, Batra SK. Regulation of mucin expression: mechanistic aspects and implications for cancer and inflammatory diseases. *Biochim Biophys Acta.* (2006) 1765:189–222. doi: 10.1016/j.bbcan.2006.01.002
19. Gum JR, Byrd JC, Hicks JW, Toribara NW, Lamport DT, Kim YS. Molecular cloning of human intestinal mucin cDNAs. Sequence analysis and evidence for genetic polymorphism. *J Biol Chem.* (1989) 264:6480–7. doi: 10.1016/S0021-9258(18)83373-7
20. Johansson ME. Mucus layers in inflammatory bowel disease. *Inflamm Bowel Dis.* (2014) 20:2124–31. doi: 10.1097/MIB.0000000000000117
21. Grondin JA, Kwon YH, Far PM, Haq S, Khan WI. Mucins in intestinal mucosal defense and inflammation: learning from clinical and experimental studies. *Front Immunol.* (2020) 11:2054. doi: 10.3389/fimmu.2020.02054
22. Norman PJ, Norberg SJ, Guethlein LA, Nemat-Gorgani N, Royce T, Wroblewski EE, et al. Sequences of 95 human MHC haplotypes reveal extreme coding variation in genes other than highly polymorphic HLA class I and II. *Genome Res.* (2017) 27:813–23. doi: 10.1101/gr.213538.116
23. Hoorens PR, Rinaldi M, Li RW, Goddeeris B, Claerebout E, Vercruysse J, et al. Genome wide analysis of the bovine mucin genes and their gastrointestinal transcription profile. *BMC Genomics.* (2011) 12:140. doi: 10.1186/1471-2164-12-140
24. Gum JR Jr, Hicks JW, Toribara NW, Siddiki B, Kim YS. Molecular cloning of human intestinal mucin (MUC2) cDNA Identification of the amino terminus and overall sequence similarity to prepro-von Willebrand factor. *J Biol Chem.* (1994) 269:2440–6. doi: 10.1016/S0021-9258(17)41965-X
25. Tytgat KM, Buller HA, Opdam FJ, Kim YS, Einerhand AW, Dekker J. Biosynthesis of human colonic mucin: Muc2 is the prominent secretory mucin. *Gastroenterology.* (1994) 107:1352–63. doi: 10.1016/0016-5085(94)90537-1
26. Godl K, Johansson ME, Lidell ME, Morgelin M, Karlsson H, Olson FJ, et al. The N terminus of the MUC2 mucin forms trimers that are held together within a trypsin-resistant core fragment. *J Biol Chem.* (2002) 277:47248–56. doi: 10.1074/jbc.M208483200
27. Lidell ME, Johansson ME, Morgelin M, Asker N, Gum JR Jr, Kim YS, et al. The recombinant C-terminus of the human MUC2 mucin forms dimers in Chinese-hamster ovary cells and heterodimers with full-length MUC2 in LS 174T cells. *Biochem J.* (2003) 372(Pt 2):335–45. doi: 10.1042/bj20030003
28. Ambort D, van der Post S, Johansson ME, Mackenzie J, Thomsson E, Krengel U, et al. Function of the CysD domain of the gel-forming MUC2 mucin. *Biochem J.* (2011) 436:61–70. doi: 10.1042/BJ20102066
29. Clamp JR, Fraser G, Read AE. Study of the carbohydrate content of mucus glycoproteins from normal and diseased colons. *Clin Sci.* (1981) 61:229–34. doi: 10.1042/cs0610229
30. Shaoul R, Okada Y, Cutz E, Marcon MA. Colonic expression of MUC2, MUC5AC, and TFF1 in inflammatory bowel disease in children. *J Pediatr Gastroenterol Nutr.* (2004) 38:488–93. doi: 10.1097/00005176-200405000-00006
31. Tytgat KM, Opdam FJ, Einerhand AW, Buller HA, Dekker J. MUC2 is the prominent colonic mucin expressed in ulcerative colitis. *Gut.* (1996) 38:554–63. doi: 10.1136/gut.38.4.554
32. Theodoropoulos G, Carraway KL. Molecular signaling in the regulation of mucins. *J Cell Biochem.* (2007) 102:1103–16. doi: 10.1002/jcb.21539
33. Thai P, Loukoianov A, Wachi S, Wu R. Regulation of airway mucin gene expression. *Annu Rev Physiol.* (2008) 70:405–29. doi: 10.1146/annurev.physiol.70.113006.100441
34. Li JD, Feng W, Gallup M, Kim JH, Gum J, Kim Y, et al. Activation of NF-kappaB via a Src-dependent Ras-MAPK-pp90rsk pathway is required for Pseudomonas aeruginosa-induced mucin overproduction in epithelial cells. *Proc Natl Acad Sci USA.* (1998) 95:5718–23. doi: 10.1073/pnas.95.10.5718
35. Ahn DH, Crawley SC, Hokari R, Kato S, Yang SC Li JD, Kim YS. TNF-alpha activates MUC2 transcription via NF-kappaB but inhibits via JNK activation. *Cell Physiol Biochem.* (2005) 15:29–40. doi: 10.1159/000083636
36. Iwashita J, Sato Y, Sugaya H, Takahashi N, Sasaki H, Abe T. mRNA of MUC2 is stimulated by IL-4, IL-13 or TNF-alpha through a mitogen-activated protein kinase pathway in human colon cancer cells. *Immunol Cell Biol.* (2003) 81:275–82. doi: 10.1046/j.1440-1711.2003.t01-1-01163.x
37. Hokari R, Lee H, Crawley SC, Yang SC, Gum JR. Jr., Miura S, Kim YS. Vasoactive intestinal peptide upregulates MUC2 intestinal mucin via CREB/ATF1. *Am J Physiol Gastrointest Liver Physiol.* (2005) 289:G949–59. doi: 10.1152/ajpgi.00142.2005
38. Gregorieff A, Stange DE, Kujala P, Begthel H, van den Born M, Korving J, et al. The ets-domain transcription factor Spdef promotes maturation of goblet and paneth cells in the intestinal epithelium. *Gastroenterology.* (2009) 137:1333–45 e1331-1333. doi: 10.1053/j.gastro.2009.06.044
39. Okudaira K, Kakar S, Cun L, Choi E, Wu Decamillis R, Miura S, et al. MUC2 gene promoter methylation in mucinous and non-mucinous colorectal cancer tissues. *Int J Oncol.* (2010) 36:765–75. doi: 10.3892/ijo_00000552
40. Yamada N, Hamada T, Goto M, Tsutsumida H, Higashi M, Nomoto M, et al. MUC2 expression is regulated by histone H3 modification and DNA methylation in pancreatic cancer. *Int J Cancer.* (2006) 119:1850–7. doi: 10.1002/ijc.22047
41. Lievin-Le Moal V, Servin AL. The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. *Clin Microbiol Rev.* (2006) 19:315–37. doi: 10.1128/CMR.19.2.315-337.2006
42. Dharmani P, Srivastava V, Kissoon-Singh V, Chadee K. Role of intestinal mucins in innate host defense mechanisms against pathogens. *J Innate Immun.* (2009) 1:123–35. doi: 10.1159/000163037
43. McGuckin MA, Eri R, Simms LA, Florin TH, Radford-Smith G. Intestinal barrier dysfunction in inflammatory bowel diseases. *Inflamm Bowel Dis.* (2009) 15:100–13. doi: 10.1002/ibd.20539
44. Deplancke B, Gaskins HR. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. *Am J Clin Nutr.* (2001) 73:1131S—41S. doi: 10.1093/ajcn/73.6.1131S
45. Atuma C, Strugala V, Allen A, Holm L. The adherent gastrointestinal mucus gel layer: thickness and physical state *in vivo*. *Am J Physiol Gastrointest Liver Physiol.* (2001) 280:G922–9. doi: 10.1152/ajpgi.2001.280.5.G922
46. Johansson ME, Thomsson KA, Hansson GC. Proteomic analyses of the two mucus layers of the colon barrier reveal that their main component, the Muc2 mucin, is strongly bound to the Fcgbp protein. *J Proteome Res.* (2009) 8:3549–57. doi: 10.1021/pr9002504
47. Grootjans J, Hundscheid IH, Lenaerts K, Boonen B, Renes IB, Verheyen FK, et al. Ischaemia-induced mucus barrier loss and bacterial penetration are rapidly counteracted by increased goblet cell secretory activity in human and rat colon. *Gut.* (2013) 62:250–8. doi: 10.1136/gutjnl-2011-301956

48. Gustafsson JK, Navabi N, Rodriguez-Pineiro AM, Alomran AH, Premaratne P, Fernandez HR, et al. Dynamic changes in mucus thickness and ion secretion during Citrobacter rodentium infection and clearance. *PLoS ONE.* (2013) 8:e84430. doi: 10.1371/journal.pone.0084430
49. Sun J, Shen X, Li Y, Guo Z, Zhu W, Zuo L, et al. Therapeutic potential to modify the mucus barrier in inflammatory bowel disease. *Nutrients*. (2016) 8:44. doi: 10.3390/nu8010044
50. Gersemann M, Becker S, Kubler I, Koslowski M, Wang G, Herrlinger KR, et al. Differences in goblet cell differentiation between Crohn's disease and ulcerative colitis. *Differentiation.* (2009) 77:84–94. doi: 10.1016/j.diff.2008.09.008
51. McCole DF. IBD candidate genes and intestinal barrier regulation. *Inflamm Bowel Dis*. (2014) 20:1829–49. doi: 10.1097/MIB.0000000000000090
52. Kyo K, Muto T, Nagawa H, Lathrop GM, Nakamura Y. Associations of distinct variants of the intestinal mucin gene MUC3A with ulcerative colitis and Crohn's disease. *J Hum Genet*. (2001) 46:5–20. doi: 10.1007/s100380170118
53. Visschedijk MC, Alberts R, Mucha S, Deelen P, de Jong DJ, Pierik M, et al. Pooled resequencing of 122 ulcerative colitis genes in a large dutch cohort suggests population-specific associations of rare variants in MUC2. *PLoS ONE*. (2016) 11:e0159609. doi: 10.1371/journal.pone.0159609
54. Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK, et al. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature.* (2008) 456:259–63. doi: 10.1038/nature07416
55. Liu B, Gulati AS, Cantillana V, Henry SC, Schmidt EA, Daniell X, et al. Irgm1-deficient mice exhibit Paneth cell abnormalities and increased susceptibility to acute intestinal inflammation. *Am J Physiol Gastrointest Liver Physiol*. (2013) 305:G573–84. doi: 10.1152/ajpgi.00071.2013
56. Wehkamp J, Harder J, Weichenthal M, Schwab M, Schaffeler E, Schlee M, et al. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. *Gut.* (2004) 53:1658–64. doi: 10.1136/gut.2003.032805
57. Larsson JM, Karlsson H, Crespo JG, Johansson ME, Eklund L, Sjovall H, et al. Altered O-glycosylation profile of MUC2 mucin occurs in active ulcerative colitis and is associated with increased inflammation. *Inflamm Bowel Dis.* (2011) 17:2299–307. doi: 10.1002/ibd.21625
58. Chang D, Gao F, Slavney A, Ma L, Waldman YY, Sams AJ, et al. Accounting for eXentricities: analysis of the X chromosome in GWAS reveals X-linked genes implicated in autoimmune diseases. *PLoS ONE.* (2014) 9:e113684. doi: 10.1371/journal.pone.0113684
59. Fu J, Wei B, Wen T, Johansson ME, Liu X, Bradford E, et al. Loss of intestinal core 1-derived O-glycans causes spontaneous colitis in mice. *J Clin Invest.* (2011) 121:1657–66. doi: 10.1172/JCI45538
60. Raouf AH, Tsai HH, Parker N, Hoffman J, Walker RJ, Rhodes JM. Sulphation of colonic and rectal mucin in inflammatory bowel disease: reduced sulphation of rectal mucus in ulcerative colitis. *Clin Sci.* (1992) 83:623–6. doi: 10.1042/cs0830623
61. Kudelka MR, Hinrichs BH, Darby T, Moreno CS, Nishio H, Cutler CE, et al. Cosmc is an X-linked inflammatory bowel disease risk gene that spatially regulates gut microbiota and contributes to sex-specific risk. *Proc Natl Acad Sci USA*. (2016) 113:14787–92. doi: 10.1073/pnas.1612158114
62. Johansson ME, Gustafsson JK, Holmen-Larsson J, Jabbar KS, Xia L, Xu H, et al. Bacteria penetrate the normally impenetrable inner colon mucus layer in both murine colitis models and patients with ulcerative colitis. *Gut.* (2014) 63:281–91. doi: 10.1136/gutjnl-2012-303207
63. An G, Wei B, Xia B, McDaniel JM, Ju T, Cummings RD, et al. Increased susceptibility to colitis and colorectal tumors in mice lacking core 3-derived O-glycans. *J Exp Med.* (2007) 204:1417–29. doi: 10.1084/jem.20061929
64. Dorofeyev AE, Vasilenko IV, Rassokhina OA, Kondratiuk RB. Mucosal barrier in ulcerative colitis and Crohn's disease. *Gastroenterol Res Pract.* (2013) 2013:431231. doi: 10.1155/2013/431231
65. Tanaka H, Takechi M, Kiyonari H, Shioi G, Tamura A, Tsukita S. Intestinal deletion of Claudin-7 enhances paracellular organic solute flux and initiates colonic inflammation in mice. *Gut.* (2015) 64:1529–38. doi: 10.1136/gutjnl-2014-308419
66. Lu P, Burger-van Paassen N, van der Sluis M, Witte-Bouma J, Kerckaert JP, van Goudoever JB, et al. Colonic gene expression patterns of mucin Muc2 knockout mice reveal various phases in colitis development. *Inflamm Bowel Dis.* (2011) 17:2047–57. doi: 10.1002/ibd.21592
67. Darsigny M, Babeu JP, Dupuis AA, Furth EE, Seidman EG, Levy E, et al. Loss of hepatocyte-nuclear-factor-4alpha affects colonic ion transport and causes chronic inflammation resembling inflammatory bowel disease in mice. *PLoS ONE.* (2009) 4:e7609. doi: 10.1371/journal.pone.0007609
68. Capaldo CT, Powell DN, Kalman D. Layered defense: how mucus and tight junctions seal the intestinal barrier. *J Mol Med.* (2017) 95:927–34. doi: 10.1007/s00109-017-1557-x
69. Bankole E, Read E, Curtis MA, Neves JF, Garnett JA. The relationship between mucins and ulcerative colitis: a systematic review. *J Clin Med.* (2021) 10:1935. doi: 10.3390/jcm10091935
70. Pullan RD, Thomas GA, Rhodes M, Newcombe RG, Williams GT, Allen A, et al. Thickness of adherent mucus gel on colonic mucosa in humans and its relevance to colitis. *Gut.* (1994) 35:353–9. doi: 10.1136/gut.35.3.353
71. Strugala V, Dettmar PW, Pearson JP. Thickness and continuity of the adherent colonic mucus barrier in active and quiescent ulcerative colitis and Crohn's disease. *Int J Clin Pract.* (2008) 62:762–9. doi: 10.1111/j.1742-1241.2007.01665.x
72. Niv Y. Mucin gene expression in the intestine of ulcerative colitis patients: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol.* (2016) 28:1241–5. doi: 10.1097/MEG.0000000000000707
73. Buisine MP, Desreumaux P, Leteurtre E, Copin MC, Colombel JF, Porchet N, et al. Mucin gene expression in intestinal epithelial cells in Crohn's disease. *Gut.* (2001) 49:544–51. doi: 10.1136/gut.49.4.544

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REVIEW
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Management of Non-response and Loss of Response to Anti-tumor Necrosis Factor Therapy in Inflammatory Bowel Disease

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Anti-tumor necrosis factor (anti-TNF) therapy has been successfully used as first-line biologic treatment for moderate-to-severe inflammatory bowel disease (IBD), in both "step-up" and "top-down" approaches, and has become a cornerstone of IBD management. However, in a proportion of patients the effectiveness of anti-TNF therapy is sub-optimal. Either patients do not achieve adequate initial response (primary non-response) or they lose response after initial success (loss of response). Therapeutic drug monitoring determines drug serum concentrations and the presence of anti-drug antibodies (ADAbs) and can help guide treatment optimization to improve patient outcomes. For patients with low drug concentrations who are ADAb-negative or display low levels of ADAbs, dose escalation is recommended. Should response remain unchanged following dose optimization the question whether to switch within class (anti-TNF) or out of class (different mechanism of action) arises. If ADAb levels are high and the patient has previously benefited from anti-TNF therapy, then switching within class is a viable option as ADAbs are molecule specific. Addition of an immunomodulator may lead to a decrease in ADAbs and a regaining of response in a proportion of patients. If a patient does not achieve a robust therapeutic response with an initial anti-TNF despite adequate drug levels, then switching out of class is appropriate. In conjunction with the guidance above, other factors including patient preference, age, comorbidities, disease phenotype, extra-intestinal manifestations, and treatment costs need to be factored into the treatment decision. In this review we discuss current evidence in this field and provide guidance on therapeutic decision-making in clinical situations.

Keywords: anti-TNF, loss of response, primary non-response, switch out of class, switch within class, therapeutic drug monitoring

INTRODUCTION

Inflammatory bowel disease (IBD), broadly comprising Crohn's disease (CD) and ulcerative colitis (UC), is a lifelong, debilitating condition necessitating a tailored and cost-effective approach to its management. Overarching therapeutic goals are to eliminate symptoms, avoid disease complications and optimize the patient's quality of life (QoL) (1–5). By reaching certain therapeutic targets (the "treat-to-target" approach) it is believed that the chances of achieving these therapeutic goals are markedly improved. Recently, these therapeutic targets have evolved beyond symptomatic control to the normalization of objective markers of inflammation and endoscopic healing with the aim of modifying the disease course (5, 6).

There are two main strategies for the management of IBD. The "step up" approach is used for patients with mild-to-moderate disease without poor prognosis factors starting with conventional therapies (e.g., 5-ASA, azathioprine, methotrexate) before moving on to newer and more expensive biologic or small molecule treatments, all of which have specific side effects that need to be taken into account when choosing a therapy (7). An accelerated version of the step-up approach involves moving quickly upwards through traditional therapies, driven by predefined time-points for therapeutic evaluation with prespecified criteria for therapeutic targets. If these are not reached one goes quickly to the next level of therapy with the aim of avoiding prolonged periods of under-treatment, but still following the step-up approach (8). The "top down" approach has been proposed for patients with severe disease and a high risk for disease-related complications. It uses the most potent treatments available, including biologics and immunomodulators in combination, earlier in the disease course with the aim of inducing remission and maintaining corticosteroid-free remission (7, 9–11). Over the past two decades, anti-tumor necrosis factor (anti-TNF) therapy has been successfully used as first-line biologic treatment to treat moderate-to-severe IBD in both "step up" and "top down" approaches. In addition, the more recent introduction of vedolizumab, ustekinumab, and tofacitinib provide alternative first-line treatment options, although their use may be limited by regulatory and reimbursement constraints in some countries (1–4, 12–14). A summary of available treatments for IBD is shown in **Figure 1**.

The use of anti-TNFs has been shown to improve clinical symptoms, promote endoscopic healing, improve QoL and reduce hospitalizations and surgeries in patients with IBD (15, 16), benefits that can be increased by use early in the disease course, at least in CD (10, 15, 17). While anti-TNFs usually follow initial treatment in a step-up approach, in some patients with moderate-to-severe IBD and prognostic factors of unfavorable outcome (i.e., young age at diagnosis, perianal disease, penetrating disease in CD, and extensive disease) early anti-TNF and immunomodulator combination therapy may be beneficial (18–23).

Unfortunately, failure of anti-TNF therapy can occur and questions that naturally arise are whether regaining response with the current drug or drug class is possible and/or what the patient should be treated with next. This review explores the management of treatment options for IBD patients with a primary non-response (PNR) or loss of response (LOR) to anti-TNF therapy.

PROBLEM OF NON-RESPONSE AND LOSS OF RESPONSE TO ANTI-TUMOR NECROSIS FACTORS

Primary Non-response

While there is no consensus definition of PNR it has been suggested to mean the failure to achieve a clinical response within 14 weeks of initiating treatment (1–4, 13). It has been reported that PNR to anti-TNFs occurs in 10–40% of patients with IBD (24–26). Primary non-response to anti-TNFs may be caused by a number of pharmacokinetic (drug concentrations) or pharmacodynamic (mechanistic) factors (6). Pharmacokinetic PNR is due to increased drug clearance, which may be immune mediated or non-immune mediated. It has also been shown that a proportion of administered anti-TNF is lost from the intestines of UC patients with active disease and that PNR is associated with the highest levels of anti-TNF observed in the feces (27). In contrast, pharmacodynamic PNR occurs when active disease persists despite therapeutic biologic drug levels, which implies that the binding of the drug to TNF is blocked or the presence of a non-inflammatory complication such as stenosis, abscess or a superimposed infection that has not been recognized; or that the underlying disease pathophysiology is primarily driven by inflammatory mediators other than TNF. Low albumin levels have been consistently associated with low infliximab levels and correlate with diminished clinical response, particularly in the setting of severe IBD such as in acute UC (28, 29).

Loss of Response

Loss of response refers to those situations where patients respond to initial treatment with anti-TNFs but then subsequently and progressively lose this response. It has been reported that up to 50% of patients experience LOR over time and that the annual rate is ∼5–20% (30–33). The wide range of frequencies reported for LOR between studies can be explained by the differing definitions that have been used. These include those based on a worsening of symptoms, the need for dose escalation, an increased level of inflammation, stopping the drug, as well as differences depending on which anti-TNF agent is being studied (30). Loss of response to anti-TNFs may be related to low trough serum drug concentrations and/or the potential presence of anti-drug antibodies (ADAbs), which result in suboptimal drug concentrations (34) or a reduction in TNF-binding capacity (35). However, in some cases, other mechanisms such as the disease transitioning to other cytokine pathways are thought to cause LOR (12, 34).

Clinical Assessment

In patients with a suspicion of PNR or LOR to anti-TNF therapy, guidelines suggest detailed assessment to determine the possible cause as this will guide therapeutic management

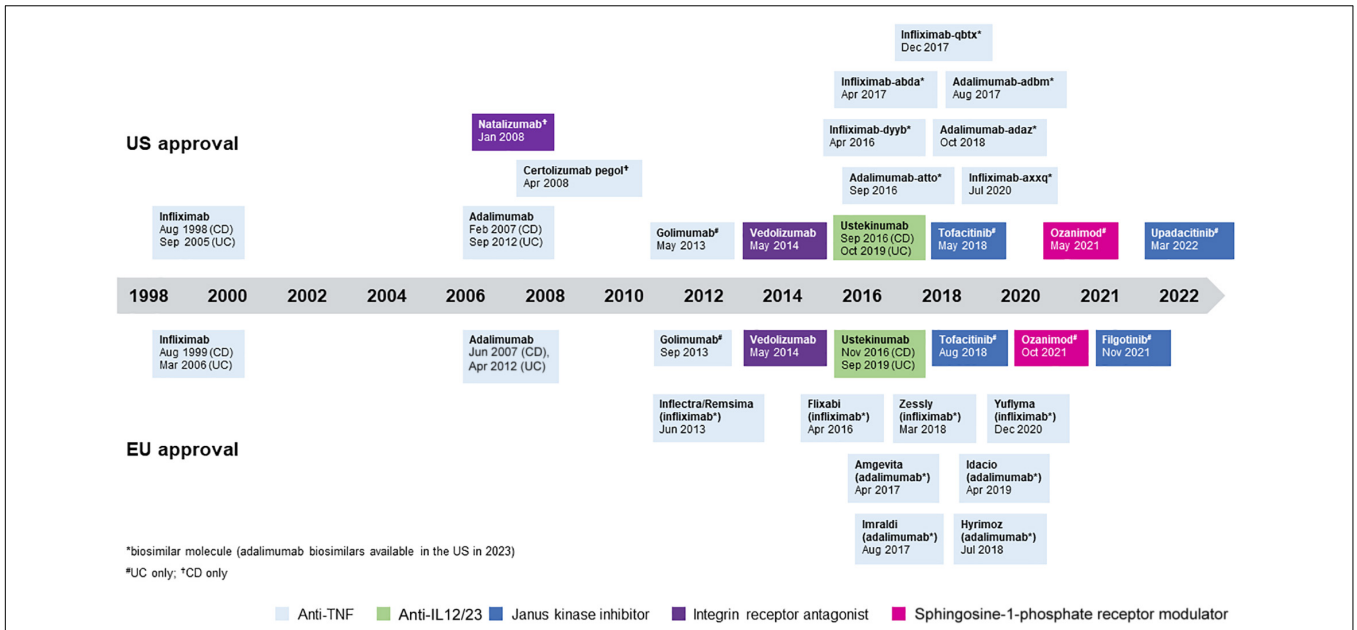

FIGURE 1 | Approved treatments for inflammatory bowel disease (IBD). All treatments are approved for Crohn's disease (CD) and ulcerative colitis (UC) unless otherwise specified.

options (1–4, 13). The first step is to determine whether the increase in symptoms is caused by a true increase in IBD activity or something else. Alternative causes for an increase in symptoms that should be ruled out include gastrointestinal infections, irritable bowel disease, bacterial overgrowth, and bile acid malabsorption (the latter being typically seen in patients with CD that have extensive ileal disease or have undergone ileal resection). The second step is to assess the level of disease-associated inflammatory activity present. A summary of various tools that can be used to assess inflammation is shown in **Table 1**.

OPTIONS FOR THE THERAPEUTIC MANAGEMENT OF NON-RESPONSE TO ANTI-TUMOR NECROSIS FACTORS

Given the still limited number of available therapies for IBD in 2021, early optimization of a patient's current treatment and maintenance of clinical response/remission is important to avoid a rapid progression through therapeutic options. A key factor in this is assessment of adherence to treatment as this remains a critical factor in achieving and sustaining remission in IBD (36, 37). Patient-related factors that have been shown to be associated with poorer adherence to treatment include male sex, shorter IBD duration, and clinic non-attendances, Conversely, patients' preferences have been shown to be important to consider to optimize adherence (36, 38).

Therapeutic drug monitoring (TDM) to determine drug trough serum concentrations and anti-drug antibodies (ADAbs) can help guide treatment optimization, improve outcomes of patients receiving anti-TNFs, and enhance cost efficiency (39–42). Treatment decisions where TDM may offer guidance include dose escalation, de-escalation or stopping, adding an immunomodulator, or switching to an alternative anti-TNF agent (switch within class) or a drug with a different mechanism of action (switch out of class). Such decisions can be made empirically but studies have shown that the use of TDM as a support for decision making is more cost-effective and provides better outcomes (43). An algorithm to guide the optimization of IBD therapy using TDM is shown in **Figure 2** (42).

TDM can be either reactive (occurs in response to treatment failure to guide therapy) or proactive [occurs at prescheduled time-points irrespective of disease activity to prevent LOR (39, 43)]. As this review discusses management options following failure of first-line anti-TNF, TDM here refers primarily to the reactive version.

Optimizing Current Therapy in Primary Non-responders

TDM is recommended for patients suspected of experiencing PNR to anti-TNFs (41, 44). However, the results of TDM need to be reviewed alongside other factors to ensure that the patient is having a true PNR and that drug levels are not low due to other causes, including poor adherence. For primary non-responders to anti-TNF therapy with low drug concentrations and who are ADAb negative or low ADAb positive (as defined by the method used), dose escalation is recommended in an attempt to optimize symptom and inflammation management (**Figure 2**; 1–4, 12, 13).

Optimizing Current Therapy Following Loss of Response

TDM is also recommended for patients suspected of having LOR to anti-TNFs (41, 44), as treatment optimization can be

TABLE 1 | Available tools for assessing the level of disease-associated inflammatory activity.

Tool	Additional notes
Blood inflammatory markers (153, 154)	• Serum CRP and albumin can be used as parallel measures of disease severity/inflammation • CRP can be used as a prognostic marker for the effectiveness of therapy • ESR is a marker for inflammation but can be influenced by factors such as pregnancy, older age and anemia and is not widely used currently
Fecal biomarkers (1–4, 13, 155)	• Fecal calprotectin is a useful biomarker to assess the degree of mucosal inflammation • Fecal calprotectin is correlated with endoscopic inflammatory scores • Fecal calprotectin should be used in the management of patients with IBD
Endoscopy (156)	• "Gold standard" for assessing the response to treatment in patients with UC and CD
Histology (157)	• Endoscopic biopsies or resection specimens

Cross-sectional imaging (39, 158–162)	• MRI and computed tomography have a high sensitivity and specificity for assessing CD activity and can be used to monitor response to treatment
	• Bowel ultrasonography is increasingly being used in clinical practice
	  • Good correlation between bowel ultrasound findings and CD activity and location, as well as endoscopic remission
	  • Accurate method for assessing transmural healing, correlating well with time-consuming and costly MRI
	  • Convincing support for the use of ultrasonography as a monitoring tool for UC

CD, Crohn's disease; CRP, C-reactive protein; MRI, magnetic resonance imaging; UC, ulcerative colitis. ESR, erythrocyte sedimentation rate.

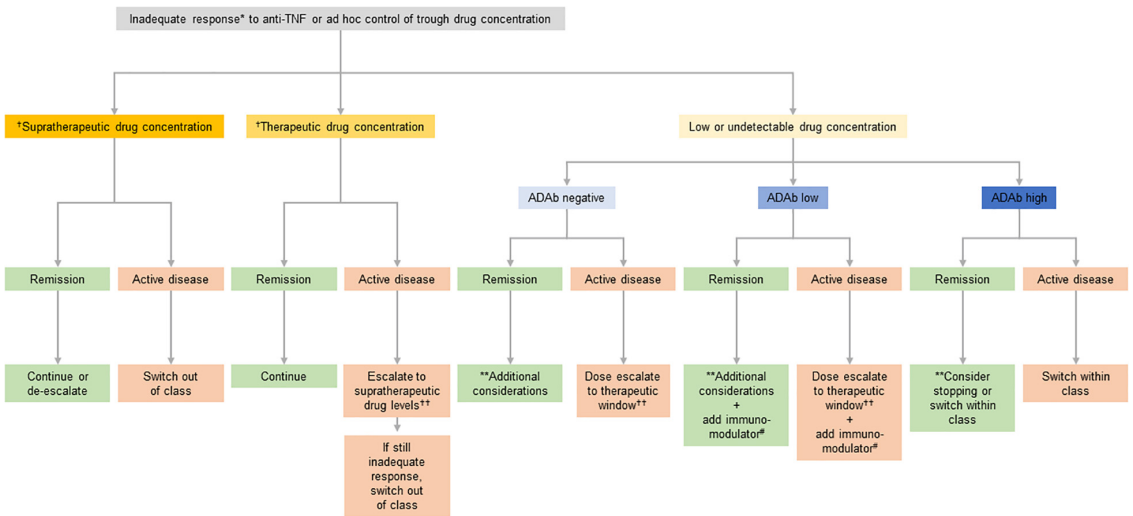

FIGURE 2 | Suggested clinical therapeutic drug monitoring (TDM)-based algorithm for optimizing anti-tumor necrosis factor (anti-TNF) therapy. *If disease activity is defined by symptoms confirm inflammatory activity and/or rule out potential non-inflammatory causes. Potential non-inflammatory causes of increased symptoms include fibrotic stricture, gastrointestinal infection, irritable bowel syndrome, bacterial overgrowth, bile salt diarrhea, colorectal cancer, and andamyloidosis. **This situation may be interpreted either as: (A) the patient being in remission despite not having any relevant anti-TNF activity (low/undetectable drug concentration) and thus it may be stopped; or (B) the patient is in the first step toward a potential relapse according to the multi-step hypothesis suggesting that the first step toward a relapse is a decline in drug concentration, the second step an increase in subclinical inflammation, and the final step a clinical relapse, and thus the drug concentration should be brought back to the therapeutic window. Deciding on which of the two is most likely involves taking several aspects into account including the patient's disease history, comorbidities, and concomitant medications. †See **Table 2** for suggested supratherapeutic and therapeutic drug concentrations. ††Both increase in dose (at standard doses) and increase in frequency are appropriate but maintaining the dose interval saves on nurse/infusion-related resources. #Immunomodulator defined as azathioprine or methotrexate.

guided by TDM in a similar way as in the event of PNR (**Figure 2**). Dose escalation may reduce or even reverse the loss of therapeutic response to anti-TNF therapy (37, 45–47). Billioud et al. (45) reported that while one fifth of patients with CD experience a LOR after initiation of adalimumab therapy, dose escalation resulted in response recovery in the majority of patients. Similarly, adalimumab dose escalation enabled recovery of response in nearly half of patients with UC that had experienced LOR (47). In patients with CD with LOR to a standard infliximab dose, shortening the dosing interval from 8 to 6 weeks was at least as effective as doubling the dose (46). On balance, published data suggest that there is no increased risk of infections or other complications with increased doses or serum concentrations of anti-TNFs (47–53).

A number of studies based on small numbers of patients suggest that the addition of an immunomodulator can reverse ADAb formation and LOR, with some studies reporting that response could be regained in over half of all patients treated

with anti-TNFs (54–59). The effects of immunomodulation can impact outcomes as early as 4 weeks after the addition of the immunomodulator (56), but on occasion they can take 2–3 months to achieve the full therapeutic effect. A course of steroids used as a bridge until the immunomodulator becomes effective may be an option for these patients.

Switching Within Class to Another Anti-tumor Necrosis Factor

The use of TDM is helpful in the decision to switch within class to another anti-TNF. If the patient has developed ADAbs, and has previously benefited from an anti-TNF, then using another anti-TNF is a viable option as antibodies are specific for a given therapeutic molecule (a biosimilar is considered as the same anti-TNF in this specific context). This can be an effective alternative treatment strategy for patients with PNR or LOR if they have subtherapeutic drug concentrations and high levels of ADAbs. Based on published data and the authors' clinical experience, **Table 2** proposes levels of anti-TNFs that can be used to make clinical decisions at various clinical situations. Of note, some occurrences of PNR within 14 weeks from start of treatment may actually be a rapid LOR. **Supplementary Table 1** provides a summary of selected relevant studies. Overall, available data suggest that switching within class to another anti-TNF following LOR is a viable strategy for a sub-group of patients and that TDM may help identify these patients (60). In addition, some small studies have reported clinical effectiveness following the use of a third, and even fourth, anti-TNF in some patients with CD following failure of two or more previous anti-TNFs (61–63). However, with the arrival of agents with alternative mechanisms of action, this option is not commonly used and may be reserved for certain patients with extra-intestinal manifestations (EIMs).

Switching Out of Class to an Agent With a Different Mechanism of Action

If a patient does not achieve an adequate therapeutic response with an anti-TNF agent and has therapeutic or supratherapeutic drug levels (**Table 3**), then selecting an agent from a different treatment class is an appropriate approach. Treatments with alternative mechanisms of action, such as vedolizumab, ustekinumab, and tofacitinib (the latter has only been approved for UC), may be considered (**Supplementary Table 2**; 1–4, 12, 13).

The degree of efficacy following switching appears to vary by treatment type and previous therapy received. Singh et al. (64) reported that patients with PNR to anti-TNF agents were less likely to respond to second-line non-TNF biologics, as compared with patients who discontinued therapy due to intolerance. In addition, patients with PNR were less likely to respond to second-line ustekinumab than patients with LOR, but there was no difference between patients treated with vedolizumab. These findings may be attributed to the pharmacokinetics and pharmacodynamics of anti-TNFs in patients with PNR.

Some data suggest that biologic-naïve patients respond better to therapy than anti-TNF experienced patients. For example, *post-hoc* analyses of efficacy data from the GEMINI 2 and GEMINI 3 studies reported rates of response and remission to be numerically higher in patients with CD receiving vedolizumab as a first biologic than in patients who had previously experienced an inadequate response with anti-TNFs (65); clinical efficacy of vedolizumab appeared similar between the different types and number of anti-TNFs previously used. A meta-analysis based upon the CERTIFI and UNITI-1 clinical trials demonstrated that use of ustekinumab resulted in significantly higher responses than placebo in patients with LOR to anti-TNFs, those who had previously received $\geq$ 2 anti-TNFs, and in intolerant patients, but not in the case of PNR (66). Similar data have been published for patients with UC. A retrospective, observational cohort study of 722 patients with UC showed that vedolizumab-treated patients were more likely to achieve deep clinical remission than those treated with anti-TNFs and that this response was blunted by prior exposure to anti-TNFs (67). For ustekinumab, while an extensive literature review of clinical trials and real-world evidence noted that the efficacy of ustekinumab appears to be blunted by increased use of anti-TNF agents (68), an analysis of data from 95 UC patients from the ENEIDA registry found that number of previous biologic treatments did not affect the response to ustekinumab (69). Finally, exposure to anti-TNFs does not seem to affect the response to tofacitinib (70). Recently, ozanimod has been approved for the treatment of UC. Data from the phase III trial indicated that while treatment effect sizes for ozanimod were not different between anti-TNF naïve and experienced patients, rates of clinical response and clinical remission tended to favor the anti-TNF naïve group, mirroring what has been observed with vedolizumab and ustekinumab (71–73). Thus, while switching out of class can be an effective strategy for some patients, the reason for switching and the patient's treatment history needs to be considered.

Prior immunogenicity to anti-TNFs does not appear to confer an increased risk of immunogenicity to ustekinumab or vedolizumab (74). The efficacy profiles of non-anti-TNF biologics may also influence treatment choice given that some may additionally treat EIMs of IBD. For example, while ustekinumab may be selected to treat UC or CD, it has also demonstrated efficacy in the treatment of paradoxical psoriasiform skin drug reactions and cutaneous manifestations of IBD (75).

It should also be borne in mind that PNR to anti-TNFs may be representative of a very sick patient who is thus less likely to respond to any biologic that is prescribed.

IMPORTANT CONSIDERATIONS FOR THE PHYSICIAN IN CASE OF NON-RESPONSE TO ANTI-TUMOR NECROSIS FACTORS

Understanding different features that contribute to the efficacy of a certain drug may help to predict the therapeutic response in patients with IBD, thus providing the potential for personalized medicine (76, 77). Factors that are important to consider in this context are patient characteristics, comorbidities,

TABLE 2 | Proposed target levels of anti-tumor necrosis factors (anti-TNFs) for clinical decision making based on published data and expert opinion.

Clinical time point	Infliximab	Adalimumab	Golimumab
After induction (week 14)	4–15 μg/mL (163)	N/D	N/D
During remission (therapeutic)	4–8 μg/mL (164–167)	5–10 μg/mL (163, 165–167)	1.4–4 μg/mL* (168–171)
To treat flare or before discontinuing due to loss of response (supratherapeutic)	>10 μg/mL (163)	>12 μg/mL (163)	N/D
For fistula healing	>12 μg/mL (172)	>14 μg/mL	N/A

*N/A, not applicable; N/D, no consistent data; TDM, therapeutic dose monitoring; *Assay dependent.*

TABLE 3 | Potential factors affecting biologic drug levels/drug clearance (27, 173, 174).

Anti-drug antibody/drug complex formation
Concomitant treatment with immunomodulators
Leakage/loss to gut lumen
Inflammatory burden and drug consumption
CRP levels
TNF-α levels
FcRn (Brambell receptor) rescue system
Albumin levels
Body weight
Male gender

CRP, C-reactive protein; FcRn, neonatal Fc receptor; TNF, tumor necrosis factor.

disease phenotype, EIMs, the patient's preferences, results from biomarker analyses, and treatment costs.

Patient Characteristics

Patient-related factors, such as smoking and obesity, may increase the risk of LOR to anti-TNFs, suggesting the need for dose-escalation and alternative therapeutic approaches, such as possible lifestyle changes (34, 78, 79). Kennedy et al. (34) reported the need for dose intensification during induction for at-risk individuals (e.g., patients with obesity and regular smokers) and iterative dose adjustment to achieve target drug concentrations greater than those currently recommended had the potential to improve the durability and effectiveness of anti-TNF therapy in these patients.

The clinical effectiveness of anti-TNF therapy does not seem to differ between older and younger patients [≥60 vs. <60 years (80, 81)]. However, it has been reported that elderly patients had a higher risk of treatment failure with an initial anti-TNF agent compared with younger individuals (81, 82). Furthermore, the risk of serious adverse events and/or serious infections were significantly higher in those ≥60 years, which could be linked to potential comorbidities present (80).

For patients who are, or aim to become, pregnant, available guidelines suggest that all anti-TNFs are safe but could be discontinued at the start of the third trimester in patients with inactive disease (12). For patients with active disease or a high risk of relapse it is recommended to continue this treatment throughout pregnancy. Of note, the potential long-term effects of anti-TNFs on the unborn child throughout pregnancy are still unknown. However, as more data are being collected that are reassuring regarding long-term safety, experts in the field advocate an increasingly lower threshold for maintaining remission-protective treatment throughout pregnancy (83, 84). Although vedolizumab and ustekinumab are recommended to be used with caution, data to suggest that these agents are equally safe as anti-TNF agents are accumulating (85, 86). A recent analysis of 1,490 pregnancies among women with IBD across multiple centers in the US showed that biologic, thiopurine or combination therapy during pregnancy was not associated with increased maternal or fetal outcomes during the first year of life (85). Tofacitinib and ozanimod are thus far contraindicated in pregnancy; patients planning a pregnancy should not start either agent if alternative options are available. However, the data on tofacitinib are continually evolving and as such the decision to continue tofacitinib during pregnancy should be made in discussion with maternal-fetal medicine experts and following full explanation of uncertainty with the patient.

Comorbidities

While the presence of comorbidities did not increase the risk of malignancies with anti-TNF use, the presence of cardiovascular disease was independently associated with the occurrence of serious infections (80) and no differences in the clinical effectiveness of anti-TNFs between patients with and without comorbidity with IBD were reported. Thus, patients with cardiovascular disease deemed to be at increased risk of infection may require additional assessment including an overview of the patient's vaccination status prior to the use of anti-TNFs (see below). In patients with heart disease, such as congestive heart failure and rhythm disturbances, use of anti-TNFs may lead to worsening of cardiac function and alternative agents should be considered.

Patients with IBD may also develop serious infections due to the disease itself or its treatment, including biologic therapies. Increased susceptibility to infections with anti-TNFs, such as tuberculosis, prompts that physicians should try to detect and treat any latent infections and consider the overall risk of opportunistic infections prior to anti-TNF therapy (87, 88); of note, screening does not completely eliminate risk of infection. While the use of vaccinations is country dependent, guidance on opportunistic infections has recently been published by ECCO (89). All patient candidates for treatment with immunomodulators and/or targeted therapies or who are already receiving a targeted therapy should have their vaccine history checked and be provided with influenza and pneumococcal vaccines. While hepatitis B vaccination is usually performed in newborns, immunization status should be assessed and

vaccination provided, where seronegative. Patients should be vaccinated for herpes zoster; while the old vaccine had to be administered at least 3 months prior to the initiation of anti-TNFs, the new inactivated vaccine, which is now readily available in many countries, can be given at any time and should therefore be recommended. Availability of the human papillomavirus vaccine varies by country, but should be used, where possible. The use of varicella vaccine should also be considered in those patients without any history of varicella (89). Vaccination for SARS-CoV-2 should also be recommended and this can be administered at any time (90). A recent report suggests that the vaccine response could be blunted by the use of anti-TNFs (91). However, other data suggest that IBD patients become seropositive after two doses of vaccine despite being under treatment with biologics (92) and that anti-TNFs could provide a protective effect against the disease (93). Taken together, booster doses are most likely beneficial for the patients with a blunted SARS-CoV-2 vaccine response, such as those under potent immunomodulatory/targeted therapy including IBD patients (94), and is recommended by local health authorities.

There are conflicting data on the safety of anti-TNFs in patients with active cancer or a history of cancer. In some patients the use of anti-TNFs may be an option in discussion with an experienced oncologist (95–97).

Disease Phenotype

Some treatments may not be suitable for every CD or UC phenotype suggesting the need to select the management approach (e.g., biologics, immunomodulators, steroids and/or surgery) that best targets and addresses the structural complications of the specific patient (15, 98, 99). Importantly, multidisciplinary teams may be needed to support and implement appropriate therapeutic decisions (100).

Available guidelines recommend the use of infliximab for the induction and maintenance of remission in complex perianal fistulae in patients with CD (4, 12). Of note, fistula healing may be more likely in patients with higher infliximab trough levels, suggesting the need for personalized dosing in this setting (4). Adalimumab may also be used to manage complex perianal fistulae (4, 12). There is insufficient evidence regarding the effect of adding immunomodulators to anti-TNFs on fistula healing. In addition, there is currently insufficient evidence to recommend the use of vedolizumab for fistula healing in patients with CD (4, 12, 101). A recent meta-analysis including 198 patients from four studies demonstrated that use of vedolizumab led to the healing of perianal fistulas in approximately one third of patients (102). Finally, recent evidence suggests that ustekinumab may be effective against fistulas (103, 104).

For patients with acute severe UC, guidelines recommend the use of infliximab (1, 2), although no guidance is available regarding the routine use of intensive compared with standard infliximab dosing (1). There are indications that an accelerated dosing regimen could be beneficial (105, 106), however data are scarce and weak in this area thus far.

Extra-Intestinal Manifestations

Up to 50% of patients with IBD experience EIMs (most commonly affecting the joints, skin, hepatobiliary tract, and eyes), which may parallel luminal disease activity or have an independent course (15, 107–109). For EIMs that are typically independent of intestinal disease activity choosing a more systemic therapy such as an anti-TNF, ustekinumab, or tofacitinib is preferred (15), although ustekinumab has not been shown to be effective in the management of axial arthropathies (110). In general, anti-TNFs appear to provide good response rates for cutaneous manifestations, arthritis, and ocular EIMs (100, 109). However, although data are sparse, ustekinumab may be preferred for some (but not all) cutaneous conditions, such as psoriasis or paradoxical psoriasiform drug reactions. Data remain both limited and conflicting for the use of vedolizumab for EIMs, with some suggesting an improvement in EIMs with treatment (111–113), while others suggest an increase in both the development and worsening of EIMs during treatment (114, 115).

Patient Preference

Denesh et al. (116) recently reported that most patients with IBD prefer oral treatments. However, those patients who have already experienced biologic agents have a high level of acceptance for both subcutaneous and intravenous forms of medication (116). While oral formulations remain limited to the JAK inhibitors in IBD with regards to targeted therapies, subcutaneous and intravenous formulations of anti-TNFs, and subsequent anti-IL12/23s and integrin receptor antagonists allow additional patient choice which may support both patient empowerment and compliance (117–119). Of note, while many physicians think that patients prefer subcutaneous treatments over intravenous administration, this is not true for all patients (117). Some patients prefer IV administration with reasons given varying from less frequent dosing, convenience, the chance for interaction with hospital staff, and reassurance with medical presence (120).

Biomarkers

Clinicians currently lack a valid tool that can predict an individual patient's response to treatment and support both initial and subsequent therapeutic choices (76). Several candidate genetic, immunological, pharmacokinetic, and microbial biomarkers have been tested but due to low sensitivity and specificity, low practical feasibility and high costs associated with the suggested procedures, they are difficult to use in clinical practice. However, gene expression profiling, molecular imaging, and the microbiome have potential as future predictive factors of therapeutic efficacy (121).

Genetics may play a part in the therapeutic response given genetic risk alleles appear to predict PNR and durable response to anti-TNF therapy in patients with CD (122–124). A genome-wide association study by Sazonovs et al. reported a significant association between allelic variation in the HLA-DQA1 gene (HLA-DQA1*05 allele) and the development of ADAbs against anti-TNF agents. Thus, HLA-DQ1A*05 may serve as a useful

biomarker of immunogenicity risk and testing for this variant might help physicians to decide whether they should receive anti-TNFs in combination with immunomodulator therapy (124). In addition, pharmacogenetic testing has the potential to support improved patient stratification, optimize treatment selection/dose, and to minimize harm caused by adverse drug reactions (125). Arijs et al. (126) reported a 100% accurate predictive gene signature for (non) response to infliximab in patients with Crohn's colitis, although no such a predictive gene set could be identified for those with Crohn's ileitis. Finally, Lee et al. (123) showed that the presence of a gene expression signature associated with $CD8^+$ T cells was significantly associated with an increased risk of LOR in patients with CD.

The relationship between the gut microbiota and drugs used in the treatment of IBD may prove to be a source of future biomarkers (127). Aden et al. (128) suggest that metabolic network reconstruction and assessment of metabolic profiles of fecal samples could be used to identify patients with IBD likely to achieve clinical remission following anti-TNF therapy. Other studies suggest that low levels of *Faecalibacterium prausnitzii* and *Bacteroides* in the gut may predict relapse after discontinuation of anti-TNF therapy (129), and differences in gut microbiome may be able to differentiate between responders and non-responders (130–132).

While biomarkers predictive of efficacy constitute a promising area of research, their use is currently not recommended in clinical practice.

Cost

Cost may also play a role in a physician's choice of treatment in IBD (4), motivating the use of dose optimization or switching within class instead of switching out of class when no other factors influence treatment choice (**Figure 2**). Biologic drugs are associated with a high cost (133, 134) which may limit access and result in non-optimized initiation and duration of therapy (135). Due to the chronic nature of IBD and associated high clinical, economic and societal burden, an efficacious, yet cost-effective, approach to its long-term management needs to be considered (136–139). Clinical trials, analytical models and systematic reviews have consistently found TDM-guided strategies for the treatment of IBD to be cost-saving or cost-effective compared with standard treatment without TDM (140–144). The introduction of less costly biosimilar anti-TNF drugs has also been associated with significant cost reductions and has expanded access to biologics in countries, including low-income countries (145–147). The safety and effectiveness of biosimilars within IBD have been established in an increasing body of evidence since the introduction of the first infliximab biosimilar in 2013 (12, 148, 149). As such, anti-TNF biosimilars are strongly recommended as first-line therapy by regulatory authorities. The increasing availability of subcutaneous forms of biologics, such as infliximab (CT-P13), adalimumab, ustekinumab and vedolizumab, are also expected to affect cost considerations (150–152), and the relationship between cost and subcutaneous administration should be clarified.

CONCLUSION

Several factors need to be considered when deciding upon the best treatment following PNR or LOR to anti-TNF therapy. Here we have presented evidence and experience-based decision-making factors that may help clinicians when deciding to switch within class or to switch out of class to a treatment with a different mechanism of action. Prior to switching treatment, it is critical to understand the reason as to why a patient is not responding, since this can affect management decisions and treatment choices. Switching within class should be considered in those patients with LOR due to high levels of ADAbs and/or where dose escalation has failed. The addition of an immunomodulator may also be considered, if ADAb-levels are low. Switching out of class appears to be an appropriate strategy in true PNR and those patients with a LOR with adequate serum trough drug levels. However, there is no consensus on the standardization of cut-off values for anti-TNF serum concentrations and some patients who are within a "therapeutic window" may still benefit from increased dosing. Treatment decisions also need to incorporate factors that may favor switching within, or out of, class including patient characteristics, disease phenotypes, comorbidities, EIMs, patient preference, and cost. Hopefully the guidance contained within this review will assist physicians in making informed treatment choices resulting in optimal long-term outcomes for their patients.

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All authors made substantial contributions to the concept and design, analysis and interpretation of data, drafting of the manuscript or revising it critically for important intellectual content, and provided final approval of the manuscript.

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REFERENCES

1. Feuerstein JD, Isaacs KL, Schneider Y, Siddique SM, Falck-Ytter Y, Singh S, et al. Aga clinical practice guidelines on the management of moderate to severe ulcerative colitis. *Gastroenterology.* (2020) 158:1450–61. doi: 10.1053/j.gastro.2020.01.006
2. Harbord M, Eliakim R, Bettenworth D, Karmiris K, Katsanos K, Kopylov U, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 2: current management. *J Crohns Colitis.* (2017) 11:769–84. doi: 10.1093/ecco-jcc/jjx009
3. Terdiman JP, Gruss CB, Heidelbaugh JJ, Sultan S, Falck-Ytter YT, Practice AGAIC, et al. American gastroenterological association institute guideline on the use of thiopurines, methotrexate, and anti-Tnf-alpha biologic drugs for the induction and maintenance of remission in inflammatory Crohn's Disease. *Gastroenterology.* (2013) 145:1459–63. doi: 10.1053/j.gastro.2013.10.047
4. Torres J, Bonovas S, Doherty G, Kucharzik T, Gisbert JP, Raine T, et al. Ecco guidelines on therapeutics in Crohn's Disease: medical treatment. *J Crohns Colitis.* (2020) 14:4–22. doi: 10.1093/ecco-jcc/jjz180
5. Turner D, Ricciuto A, Lewis A, D'Amico F, Dhaliwal J, Griffiths AM, et al. Stride-Ii: an update on the selecting therapeutic targets in inflammatory bowel disease (Stride) initiative of the international organization for the study of Ibd (Ioibd): determining therapeutic goals for treat-to-target strategies in Ibd. *Gastroenterology.* (2021) 160:1570–83. doi: 10.1053/j.gastro.2020.12.031
6. Sparrow MP, Papamichael K, Ward MG, Riviere P, Laharie D, Paul S, et al. Therapeutic drug monitoring of biologics during induction to prevent primary non-response. *J Crohns Colitis.* (2020) 14:542–56. doi: 10.1093/ecco-jcc/jjz162
7. Tsui JJ, Huynh HQ. Is top-down therapy a more effective alternative to conventional step-up therapy for crohn's disease? *Ann Gastroenterol.* (2018) 31:413–24. doi: 10.20524/aog.2018.0253
8. Ordas I, Feagan BG, Sandborn WJ. Early use of immunosuppressives or tnf antagonists for the treatment of Crohn's Disease: time for a change. *Gut.* (2011) 60:1754–63. doi: 10.1136/gutjnl-2011-300934
9. D'Haens GR. Top-down therapy for Ibd: rationale and requisite evidence. *Nat Rev Gastroenterol Hepatol.* (2010) 7:86–92. doi: 10.1038/nrgastro.2009.222
10. Berg DR, Colombel JF, Ungaro R. The role of early biologic therapy in inflammatory bowel disease. *Inflamm Bowel Dis.* (2019) 25:1896–905. doi: 10.1093/ibd/izz059
11. Salahudeen MS. A review of current evidence allied to step-up and top-down medication therapy in inflammatory bowel disease. *Drugs Today.* (2019) 55:385–405. doi: 10.1358/dot.2019.55.6.2969816
12. Lamb CA, Kennedy NA, Raine T, Hendy PA, Smith PJ, Limdi JK, et al. British society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut.* (2019) 68:s1–106. doi: 10.1136/gutjnl-2019-318484
13. Magro F, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. part 1: definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. *J Crohns Colitis.* (2017) 11:649–70. doi: 10.1093/ecco-jcc/jjx008
14. Sandborn WJ, Feagan BG, Hanauer SB, Lichtenstein GR. The guide to guidelines in ulcerative colitis: interpretation and appropriate use in clinical practice. *Gastroenterol Hepatol* (2021) 17:3–13.
15. Chang S, Hudesman D. First-line biologics or small molecules in inflammatory bowel disease: a practical guide for the clinician. *Curr Gastroenterol Rep.* (2020) 22:7. doi: 10.1007/s11894-020-0745-y
16. Hossain A, Lordal M, Olsson AE, Storlahls A, Aleman S, Eberhardson M, et al. Sustained clinical benefit, improved quality of life, and reduced intestinal surgery from maintenance infliximab treatment in inflammatory bowel disease. *Scand J Gastroenterol.* (2020) 55:178–83. doi: 10.1080/00365521.2020.1722738
17. Frei R, Fournier N, Zeitz J, Scharl M, Morell B, Greuter T, et al. Early initiation of anti-tnf is associated with favourable long-term outcome in crohn's disease: 10-year-follow-up data from the swiss ibd cohort study. *J Crohns Colitis.* (2019) 13:1292–301. doi: 10.1093/ecco-jcc/jjz057
18. Beaugerie L, Seksik P, Nion-Larmurier I, Gendre JP, Cosnes J. Predictors of crohn's disease. *Gastroenterology.* (2006) 130:650–6. doi: 10.1053/j.gastro.2005.12.019
19. D'Haens G, Baert F, van Assche G, Caenepeel P, Vergauwe P, Tuynman H, et al. Early combined immunosuppression or conventional management in patients with newly diagnosed crohn's disease: an open randomised trial. *Lancet.* (2008) 371:660–7. doi: 10.1016/S0140-6736(08)60304-9
20. Dias CC, Rodrigues PP, da Costa-Pereira A, Magro F. Clinical prognostic factors for disabling crohn's disease: a systematic review and meta-analysis. *World J Gastroenterol.* (2013) 19:3866–71. doi: 10.3748/wjg.v19.i24.3866
21. Loly C, Belaiche J, Louis E. Predictors of severe crohn's disease. *Scand J Gastroenterol.* (2008) 43:948–54. doi: 10.1080/00365520801957149
22. Reinisch W, Reinink AR, Higgins PD. Factors associated with poor outcomes in adults with newly diagnosed ulcerative colitis. *Clin Gastroenterol Hepatol.* (2015) 13:635–42. doi: 10.1016/j.cgh.2014.03.037
23. Torres J, Caprioli F, Katsanos KH, Lobaton T, Micic D, Zeroncio M, et al. Predicting outcomes to optimize disease management in inflammatory bowel diseases. *J Crohns Colitis.* (2016) 10:1385–94. doi: 10.1093/ecco-jcc/jjw116
24. Kassouri L, Amiot A, Kirchgesner J, Treton X, Allez M, Bouhnik Y, et al. The outcome of crohn's disease patients refractory to anti-tnf and either vedolizumab or ustekinumab. *Dig Liver Dis.* (2020) 52:1148–55. doi: 10.1016/j.dld.2020.07.031
25. Panaccione R, Ghosh S. Optimal use of biologics in the management of crohn's disease. *Therap Adv Gastroenterol.* (2010) 3:179–89. doi: 10.1177/1756283X09357579
26. Sabino J, Verstockt B, Vermeire S, Ferrante M. New biologics and small molecules in inflammatory bowel disease: an update. *Therap Adv Gastroenterol.* (2019) 12:1756284819853208. doi: 10.1177/1756284819853208
27. Brandse JF, van den Brink GR, Wildenberg ME, van der Kleij D, Rispens T, Jansen JM, et al. Loss of infliximab into feces is associated with lack of response to therapy in patients with severe ulcerative colitis. *Gastroenterology.* (2015) 149:350–5e2. doi: 10.1053/j.gastro.2015.04.016
28. Fasanmade AA, Adedokun OJ, Ford J, Hernandez D, Johanns J, Hu C, et al. Population pharmacokinetic analysis of infliximab in patients with ulcerative colitis. *Eur J Clin Pharmacol.* (2009) 65:1211–28. doi: 10.1007/s00228-009-0718-4
29. Syal G, Robbins L, Kashani A, Bonthala N, Feldman E, Fleshner P, et al. Hypoalbuminemia and bandemia predict failure of infliximab rescue therapy in acute severe ulcerative colitis. *Dig Dis Sci.* (2021) 66:199–205. doi: 10.1007/s10620-020-06177-7
30. Ben-Horin S, Chowers Y. Review article: loss of response to anti-tnf treatments in crohn's disease. *Aliment Pharmacol Ther.* (2011) 33:987–95. doi: 10.1111/j.1365-2036.2011.04612.x
31. Fine S, Papamichael K, Cheifetz AS. Etiology and management of lack or loss of response to anti-tumor necrosis factor therapy in patients with inflammatory bowel disease. *Gastroenterol Hepatol.* (2019) 15:656–65.
32. Gisbert JP, Panes J. Loss of response and requirement of infliximab dose intensification in crohn's disease: a review. *Am J Gastroenterol.* (2009) 104:760–7. doi: 10.1038/ajg.2008.88
33. Roda G, Jharap B, Neeraj N, Colombel JF. Loss of response to anti-tnfs: definition, epidemiology, and management. *Clin Transl Gastroenterol.* (2016) 7:e135. doi: 10.1038/ctg.2015.63
34. Kennedy NA, Heap GA, Green HD, Hamilton B, Bewshea C, Walker GJ, et al. Predictors of anti-Tnf treatment failure in anti-Tnf-naive patients with active luminal crohn's disease: a prospective, multicentre, cohort study. *Lancet Gastroenterol Hepatol.* (2019) 4:341–53. doi: 10.1016/S2468-1253(19)30012-3
35. Vande Casteele N, Khanna R, Levesque BG, Stitt L, Zou GY, Singh S, et al. The relationship between infliximab concentrations, antibodies to infliximab and disease activity in crohn's disease. *Gut.* (2015) 64:1539–45. doi: 10.1136/gutjnl-2014-307883
36. Tripathi K, Dong J, Mishkin BF, Feuerstein JD. Patient preference and adherence to aminosalicylates for the treatment of ulcerative colitis. *Clin Exp Gastroenterol.* (2021) 14:343–51. doi: 10.2147/CEG.S237653
37. Khan S, Rupniewska E, Neighbors M, Singer D, Chiarappa J, Obando C. Real-world evidence on adherence, persistence, switching and dose escalation with biologics in adult inflammatory bowel disease in the united states: a

systematic review. *J Clin Pharm Ther.* (2019) 44:495–507. doi: 10.1111/jcpt.12830
38. Haar GS, Vasudevan A, Curtain CM, van Langenberg DR. Assessing adherence to infusion-based biologic therapies in patients with inflammatory bowel disease. *Res Social Adm Pharm.* (2021) 17:1420–5. doi: 10.1016/j.sapharm.2020.10.011
39. Argollo M, Kotze PG, Kakkadasam P, D'Haens G. Optimizing biologic therapy in ibd: how essential is therapeutic drug monitoring? *Nat Rev Gastroenterol Hepatol.* (2020) 17:702–10. doi: 10.1038/s41575-020-0352-2
40. Dreesen E, Bossuyt P, Mulleman D, Gils A, Pascual-Salcedo D. Practical recommendations for the use of therapeutic drug monitoring of biopharmaceuticals in inflammatory diseases. *Clin Pharmacol.* (2017) 9:101–11. doi: 10.2147/CPAA.S138414
41. Mitrev N, Vande Casteele N, Seow CH, Andrews JM, Connor SJ, Moore GT, et al. Review article: consensus statements on therapeutic drug monitoring of anti-tumour necrosis factor therapy in inflammatory bowel diseases. *Aliment Pharmacol Ther.* (2017) 46:1037–53. doi: 10.1111/apt.14368
42. Papamichael K, Vande Casteele N, Ferrante M, Gils A, Cheifetz AS. Therapeutic drug monitoring during induction of anti-tumor necrosis factor therapy in inflammatory bowel disease: defining a therapeutic drug window. *Inflamm Bowel Dis.* (2017) 23:1510–5. doi: 10.1097/MIB.0000000000001231
43. Papamichael K, Cheifetz AS. Therapeutic drug monitoring in patients on biologics: lessons from gastroenterology. *Curr Opin Rheumatol.* (2020) 32:371–9. doi: 10.1097/BOR.0000000000000713
44. Papamichael K, Cheifetz AS, Melmed GY, Irving PM, Vande Casteele N, Kozuch PL, et al. Appropriate therapeutic drug monitoring of biologic agents for patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol.* (2019) 17:1655–68e3. doi: 10.1016/j.cgh.2019.03.037
45. Billioud V, Sandborn WJ, Peyrin-Biroulet L. Loss of response and need for adalimumab dose intensification in crohn's disease: a systematic review. *Am J Gastroenterol.* (2011) 106:674–84. doi: 10.1038/ajg.2011.60
46. Kopylov U, Mantzaris GJ, Katsanos KH, Reenaers C, Ellul P, Rahier JF, et al. The efficacy of shortening the dosing interval to once every six weeks in crohn's patients losing response to maintenance dose of infliximab. *Aliment Pharmacol Ther.* (2011) 33:349–57. doi: 10.1111/j.1365-2036.2010.04523.x
47. Taxonera C, Iglesias E, Munoz F, Calvo M, Barreiro-de Acosta M, Busquets D, et al. Adalimumab maintenance treatment in ulcerative colitis: outcomes by prior anti-Tnf use and efficacy of dose escalation. *Dig Dis Sci.* (2017) 62:481–90. doi: 10.1007/s10620-016-4398-5
48. Taxonera C, Olivares D, Mendoza JL, Diaz-Rubio M, Rey E. Need for infliximab dose intensification in crohn's disease and ulcerative colitis. *World J Gastroenterol.* (2014) 20:9170–7. doi: 10.3748/wjg.v20.i27.9170
49. Dumitrescu G, Amiot A, Seksik P, Baudry C, Stefanescu C, Gagniere C, et al. The outcome of infliximab dose doubling in 157 patients with ulcerative colitis after loss of response to infliximab. *Aliment Pharmacol Ther.* (2015) 42:1192–9. doi: 10.1111/apt.13393
50. Hendler SA, Cohen BL, Colombel JF, Sands BE, Mayer L, Agarwal S. High-dose infliximab therapy in crohn's disease: clinical experience, safety, and efficacy. *J Crohns Colitis.* (2015) 9:266–75. doi: 10.1093/ecco-jcc/jju026
51. Landemaine A, Petitcollin A, Brochard C, Miard C, Dewitte M, Le Balc'h E, et al. Cumulative exposure to infliximab, but not trough concentrations, correlates with rate of infection. *Clin Gastroenterol Hepatol.* (2021) 19:288–95e4. doi: 10.1016/j.cgh.2020.03.018
52. Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* (2005) 353:2462–76. doi: 10.1056/NEJMoa050516
53. Taxonera C, Barreiro-de Acosta M, Calvo M, Saro C, Bastida G, Martin-Arranz MD, et al. Infliximab dose escalation as an effective strategy for managing secondary loss of response in ulcerative colitis. *Dig Dis Sci.* (2015) 60:3075–84. doi: 10.1007/s10620-015-3735-4
54. Ben-Horin S, Waterman M, Kopylov U, Yavzori M, Picard O, Fudim E, et al. Addition of an immunomodulator to infliximab therapy eliminates antidrug antibodies in serum and restores clinical response of patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol.* (2013) 11:444–7. doi: 10.1016/j.cgh.2012.10.020
55. Bond A, Clark K, Gregg B, Collins P, Dibb M, Probert C, et al. Pth-049 successful reversal of high titre antibodies to infliximab and adalimumab with the addition of immunomodulator therapy. *Gut.* (2016) 65:A243.
56. Macaluso FS, Sapienza C, Ventimiglia M, Renna S, Rizzuto G, Orlando R, et al. The addition of an immunosuppressant after loss of response to anti-tnfalpha monotherapy in inflammatory bowel disease: a 2-year study. *Inflamm Bowel Dis.* (2018) 24:394–401. doi: 10.1093/ibd/izx010
57. Strik AS, van den Brink GR, Ponsioen C, Mathot R, Lowenberg M, D'Haens GR. Suppression of anti-drug antibodies to infliximab or adalimumab with the addition of an immunomodulator in patients with inflammatory bowel disease. *Aliment Pharmacol Ther.* (2017) 45:1128–34. doi: 10.1111/apt.13994
58. Ungar B, Kopylov U, Engel T, Yavzori M, Fudim E, Picard O, et al. Addition of an immunomodulator can reverse antibody formation and loss of response in patients treated with adalimumab. *Aliment Pharmacol Ther.* (2017) 45:276–82. doi: 10.1111/apt.13862
59. Roblin X, Williet N, Boschetti G, Phelip JM, Del Tedesco E, Berger AE, et al. Addition of azathioprine to the switch of anti-Tnf in patients with Ibd in clinical relapse with undetectable anti-Tnf trough levels and antidrug antibodies: a prospective randomised trial. *Gut.* (2020) 69:1206–12. doi: 10.1136/gutjnl-2019-319758
60. Gisbert JP, Marin AC, McNicholl AG, Chaparro M. Systematic review with meta-analysis: the efficacy of a second anti-Tnf in patients with inflammatory bowel disease whose previous anti-tnf treatment has failed. *Aliment Pharmacol Ther.* (2015) 41:613–23. doi: 10.1111/apt.13083
61. Allez M, Vermeire S, Mozziconacci N, Michetti P, Laharie D, Louis E, et al. The efficacy and safety of a third anti-tnf monoclonal antibody in crohn's disease after failure of two other anti-tnf antibodies. *Aliment Pharmacol Ther.* (2010) 31:92–101. doi: 10.1111/j.1365-2036.2009.04130.x
62. de Silva PS, Nguyen DD, Sauk J, Korzenik J, Yajnik V, Ananthakrishnan AN. Long-term outcome of a third anti-tnf monoclonal antibody after the failure of two prior anti-tnfs in inflammatory bowel disease. *Aliment Pharmacol Ther.* (2012) 36:459–66. doi: 10.1111/j.1365-2036.2012.05214.x
63. Russi L, Scharl M, Rogler G, Biedermann L. The efficacy and safety of golimumab as third- or fourth-line anti-tnf therapy in patients with refractory crohn's disease: a case series. *Inflamm Intest Dis.* (2017) 2:131–8. doi: 10.1159/000481400
64. Singh S, George J, Boland BS, Vande Casteele N, Sandborn WJ. Primary non-response to tumor necrosis factor antagonists is associated with inferior response to second-line biologics in patients with inflammatory bowel diseases: a systematic review and meta-analysis. *J Crohns Colitis.* (2018) 12:635–43. doi: 10.1093/ecco-jcc/jjy004
65. Sands BE, Sandborn WJ, Van Assche G, Lukas M, Xu J, James A, et al. Vedolizumab as induction and maintenance therapy for crohn's disease in patients naive to or who have failed tumor necrosis factor antagonist therapy. *Inflamm Bowel Dis.* (2017) 23:97–106. doi: 10.1097/MIB.0000000000000979
66. Kawalec P, Mocko P, Malinowska-Lipien I, Brzostek T. Efficacy and safety of ustekinumab in the induction therapy of tnf-alpha-refractory crohn's disease patients: a systematic review and meta-analysis. *J Comp Eff Res.* (2017) 6:601–12. doi: 10.2217/cer-2017-0022
67. Lukin D, Faleck D, Xu R, Zhang Y, Weiss A, Aniwan S, et al. Comparative safety and effectiveness of vedolizumab to tumor necrosis factor antagonist therapy for ulcerative colitis. *Clin Gastroenterol Hepatol.* (2020) 20:126–35. doi: 10.1016/j.cgh.2020.10.003
68. Gutierrez A, Rodriguez-Lago I. How to optimize treatment with ustekinumab in inflammatory bowel disease: lessons learned from clinical trials and real-world data. *Front Med.* (2021) 8:640813. doi: 10.3389/fmed.2021.640813
69. Chaparro M, Garre A, Iborra M, Sierra M, Barreiro-de Acosta M, Fernandez-Clotet A, et al. Effectiveness and safety of ustekinumab in ulcerative colitis: real-world evidence from the eneida registry. *J Crohns Colitis.* (2021) 15:1846–51. doi: 10.1093/ecco-jcc/jjab070
70. Sandborn WJ, Su C, Sands BE, D'Haens GR, Vermeire S, Schreiber S, et al. Tofacitinib as induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* (2017) 376:1723–36. doi: 10.1056/NEJMoa1606910
71. Danese S, Feagan B, Hanauer S, Jovanovic I, Ghosh S, Petersen AK, et al. Ozanimod efficacy, safety, and histology in patients with moderate-to-severe ulcerative colitis during maintenance in the phase 3 true north study. *Am J Gastroenterol.* (2020) 115:030.
72. Sandborn W, D'Haens G, Wolf D, Hanauer S, Jovanovic I, Ghosh S, et al. Ozanimod efficacy, safety, and histology in patients with moderate-to-severe ulcerative colitis during induction in the phase 3 true north study. *Am J Gastroenterol.* (2020) 115:025.

73. Sandborn WJ, Feagan BG, D'Haens G, Wolf DC, Jovanovic I, Hanauer SB, et al. Ozanimod as induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* (2021) 385:1280–91. doi: 10.1056/NEJMoa2033617
74. Costable NJ, Borman ZA, Ji J, Dubinsky MC, Ungaro RC. Prior immunogenicity to anti-tnf biologics is not associated with increased anti-drug antibodies to vedolizumab or ustekinumab. *Dig Dis Sci.* (2021) [Online ahead of print]. doi: 10.1007/s10620-021-07046-7
75. Wu J, Smogorzewski J. Ustekinumab for the treatment of paradoxical skin reactions and cutaneous manifestations of inflammatory bowel diseases. *Dermatol Ther.* (2021) 34:e14883. doi: 10.1111/dth.14883
76. Privitera G, Pugliese D, Rapaccini GL, Gasbarrini A, Armuzzi A, Guidi L. Predictors and early markers of response to biological therapies in inflammatory bowel diseases. *J Clin Med.* (2021) 10:10040853. doi: 10.3390/jcm10040853
77. Volk N, Siegel CA. Defining failure of medical therapy for inflammatory bowel disease. *Inflamm Bowel Dis.* (2019) 25:74–7. doi: 10.1093/ibd/izy238
78. Danese S, Fiorino G, Reinisch W. Review article: causative factors and the clinical management of patients with crohn's disease who lose response to anti-tnf-alpha therapy. *Aliment Pharmacol Ther.* (2011) 34:1–10. doi: 10.1111/j.1365-2036.2011.04679.x
79. Singh S, Facciorusso A, Singh AG, Vande Casteele N, Zarrinpar A, Prokop LJ, et al. Obesity and response to anti-tumor necrosis factor-alpha agents in patients with select immune-mediated inflammatory diseases: a systematic review and meta-analysis. *PLoS One.* (2018) 13:e0195123. doi: 10.1371/journal.pone.0195123
80. Asscher VER, van der Vliet Q, van der Aalst K, van der Aalst A, Brand EC, van der Meulen-de Jong AE, et al. Anti-tumor necrosis factor therapy in patients with inflammatory bowel disease; comorbidity, not patient age, is a predictor of severe adverse events. *Int J Colorectal Dis.* (2020) 35:2331–8. doi: 10.1007/s00384-020-03716-6
81. de Jong ME, Smits LJT, van Ruijven B, den Broeder N, Russel M, Romkens TEH, et al. Increased discontinuation rates of anti-tnf therapy in elderly inflammatory bowel disease patients. *J Crohns Colitis.* (2020) 14:888–95. doi: 10.1093/ecco-jcc/jjaa012
82. Cottone M, Kohn A, Daperno M, Armuzzi A, Guidi L, D'Inca R, et al. Advanced age is an independent risk factor for severe infections and mortality in patients given anti-tumor necrosis factor therapy for inflammatory bowel disease. *Clin Gastroenterol Hepatol.* (2011) 9:30–5. doi: 10.1016/j.cgh.2010.09.026
83. Duricova D, Dvorakova E, Hradsky O, Mitrova K, Durilova M, Kozeluhova J, et al. Safety of anti-tnf-alpha therapy during pregnancy on long-term outcome of exposed children: a controlled, multicenter observation. *Inflamm Bowel Dis.* (2019) 25:789–96. doi: 10.1093/ibd/izy294
84. Mahadevan U. Overview of pregnancy in patients with inflammatory bowel disease. *Gastroenterol Hepatol.* (2021) 17:73–5.
85. Mahadevan U, Long MD, Kane SV, Roy A, Dubinsky MC, Sands BE, et al. Pregnancy and neonatal outcomes after fetal exposure to biologics and thiopurines among women with inflammatory bowel disease. *Gastroenterology.* (2021) 160:1131–9. doi: 10.1053/j.gastro.2020.11.038
86. Wils P, Seksik P, Stefanescu C, Nancey S, Allez M, Pineton de Chambrun G, et al. Safety of ustekinumab or vedolizumab in pregnant inflammatory bowel disease patients: a multicentre cohort study. *Aliment Pharmacol Ther.* (2021) 53:460–70. doi: 10.1111/apt.16192
87. Murdaca G, Negrini S, Pellecchio M, Greco M, Schiavi C, Giusti F, et al. Update upon the infection risk in patients receiving tnf alpha inhibitors. *Expert Opin Drug Saf.* (2019) 18:219–29. doi: 10.1080/14740338.2019.1577817
88. Theis VS, Rhodes JM. Review article: minimizing tuberculosis during anti-tumour necrosis factor-alpha treatment of inflammatory bowel disease. *Aliment Pharmacol Ther.* (2008) 27:19–30. doi: 10.1111/j.1365-2036.2007.03553.x
89. Kucharzik T, Ellul P, Greuter T, Rahier JF, Verstockt B, Abreu C, et al. Ecco guidelines on the prevention, diagnosis, and management of infections in inflammatory bowel disease. *J Crohns Colitis.* (2021) 15:879–913. doi: 10.1093/ecco-jcc/jjab052
90. Neurath MF. Covid-19: biologic and immunosuppressive therapy in gastroenterology and hepatology. *Nat Rev Gastroenterol Hepatol.* (2021) 18:705–15. doi: 10.1038/s41575-021-00480-y
91. Kennedy NA, Goodhand JR, Bewshea C, Nice R, Chee D, Lin S, et al. Anti-Sars-Cov-2 antibody responses are attenuated in patients with ibd treated with infliximab. *Gut.* (2021) 70:865–75. doi: 10.1136/gutjnl-2021-324388
92. Wong SY, Dixon R, Martinez Pazos V, Gnjatic S, Colombel JF, Cadwell K, et al. Serologic response to messenger Rna coronavirus disease 2019 vaccines in inflammatory bowel disease patients receiving biologic therapies. *Gastroenterology.* (2021) 161:715–8e4. doi: 10.1053/j.gastro.2021.04.025
93. Ungaro RC, Brenner EJ, Agrawal M, Zhang X, Kappelman MD, Colombel JF, et al. Impact of medications on Covid-19 outcomes in inflammatory bowel disease: analysis of over 6,000 patients from an international registry. *Gastroenterology.* (2021) 162:316.e–9.e. doi: 10.1053/j.gastro.2021.09.011
94. Bar-On YM, Goldberg Y, Mandel M, Bodenheimer O, Freedman L, Kalkstein N, et al. Protection of Bnt162b2 vaccine booster against Covid-19 in Israel. *N Engl J Med.* (2021) 385:1393–400. doi: 10.1056/NEJMoa2114255
95. Chupin A, Perduca V, Meyer A, Bellanger C, Carbonnel F, Dong C. Systematic review with meta-analysis: comparative risk of lymphoma with anti-tumour necrosis factor agents and/or thiopurines in patients with inflammatory bowel disease. *Aliment Pharmacol Ther.* (2020) 52:1289–97. doi: 10.1111/apt.16050
96. Dahmus J, Rosario M, Clarke K. Risk of lymphoma associated with anti-tnf therapy in patients with inflammatory bowel disease: implications for therapy. *Clin Exp Gastroenterol.* (2020) 13:339–50. doi: 10.2147/CEG.S237646
97. Sebastian S, Neilaj S. Practical guidance for the management of inflammatory bowel disease in patients with cancer. which treatment? *Therap Adv Gastroenterol.* (2019) 12:1756284818817293. doi: 10.1177/1756284818817293
98. Ding NS, Hart A, De Cruz P. Systematic review: predicting and optimising response to anti-tnf therapy in crohn's disease - algorithm for practical management. *Aliment Pharmacol Ther.* (2016) 43:30–51. doi: 10.1111/apt.13445
99. Dubinsky MC, Mei L, Friedman M, Dhere T, Haritunians T, Hakonarson H, et al. Genome wide association (Gwa) predictors of anti-tnfalpha therapeutic responsiveness in pediatric inflammatory bowel disease. *Inflamm Bowel Dis.* (2010) 16:1357–66. doi: 10.1002/ibd.21174
100. Harbord M, Annese V, Vavricka SR, Allez M, Barreiro-de Acosta M, Boberg KM, et al. The first European evidence-based consensus on extra-intestinal manifestations in inflammatory bowel disease. *J Crohns Colitis.* (2016) 10:239–54. doi: 10.1093/ecco-jcc/jjv213
101. Singh S, Proctor D, Scott FI, Falck-Ytter Y, Feuerstein JD. Aga technical review on the medical management of moderate to severe luminal and perianal fistulizing crohn's disease. *Gastroenterology.* (2021) 160:2512–56e9. doi: 10.1053/j.gastro.2021.04.023
102. Ayoub F, Odenwald M, Micic D, Dalal SR, Pekow J, Cohen RD, et al. Vedolizumab for perianal fistulizing crohn's disease: systematic review and meta-analysis. *Intest Res.* (2022) 20:240–50. doi: 10.5217/ir.2021.00091
103. Attauabi M, Burisch J, Seidelin JB. Efficacy of ustekinumab for active perianal fistulizing crohn disease: a double-center cohort study. *Inflamm Bowel Dis.* (2021) 27:e37–8. doi: 10.1093/ibd/izaa297
104. Godoy Brewer GM, Salem G, Afzal MA, Limketkai BN, Haq Z, Tajamal M, et al. Ustekinumab Is effective for perianal fistulising crohn's disease: a real-world experience and systematic review with meta-analysis. *BMJ Open Gastroenterol.* (2021) 8:702. doi: 10.1136/bmjgast-2021-000702
105. Choy MC, Seah D, Faleck DM, Shah SC, Chao CY, An YK, et al. Systematic review and meta-analysis: optimal salvage therapy in acute severe ulcerative colitis. *Inflamm Bowel Dis.* (2019) 25:1169–86. doi: 10.1093/ibd/izy383
106. Gibson DJ, Doherty J, McNally M, Campion J, Keegan D, Keogh A, et al. Comparison of medium to long-term outcomes of acute severe ulcerative colitis patients receiving accelerated and standard infliximab induction. *Frontline Gastroenterol.* (2020) 11:441–7. doi: 10.1136/flgastro-2019-101335
107. Greuter T, Rieder F, Kucharzik T, Peyrin-Biroulet L, Schoepfer AM, Rubin DT, et al. Emerging treatment options for extraintestinal manifestations in Ibd. *Gut.* (2021) 70:796–802. doi: 10.1136/gutjnl-2020-322129
108. Hanzel J, Ma C, Casteele NV, Khanna R, Jairath V, Feagan BG. Vedolizumab and extraintestinal manifestations in inflammatory bowel disease. *Drugs.* (2021) 81:333–47. doi: 10.1007/s40265-020-01460-3
109. Hedin CRH, Vavricka SR, Stagg AJ, Schoepfer A, Raine T, Puig L, et al. The pathogenesis of extraintestinal manifestations: implications for Ibd research,

diagnosis, and therapy. *J Crohns Colitis.* (2019) 13:541–54. doi: 10.1093/ecco-jcc/jjy191
110. Deodhar A, Gensler LS, Sieper J, Clark M, Calderon C, Wang Y, et al. Three multicenter, randomized, double-blind, placebo-controlled studies evaluating the efficacy and safety of ustekinumab in axial spondyloarthritis. *Arthritis Rheumatol.* (2019) 71:258–70. doi: 10.1002/art.40728
111. Feagan BG, Sandborn WJ, Colombel JF, Byrne SO, Khalid JM, Kempf C, et al. Incidence of arthritis/arthralgia in inflammatory bowel disease with long-term vedolizumab treatment: post hoc analyses of the gemini trials. *J Crohns Colitis.* (2019) 13:50–7. doi: 10.1093/ecco-jcc/jjy125
112. Fleisher M, Marsal J, Lee SD, Frado LE, Parian A, Korelitz BI, et al. Effects of vedolizumab therapy on extraintestinal manifestations in inflammatory bowel disease. *Dig Dis Sci.* (2018) 63:825–33. doi: 10.1007/s10620-018-4971-1
113. Tadbiri S, Peyrin-Biroulet L, Serrero M, Filippi J, Pariente B, Roblin X, et al. Impact of vedolizumab therapy on extra-intestinal manifestations in patients with inflammatory bowel disease: a multicentre cohort study nested in the observ-ibd cohort. *Aliment Pharmacol Ther.* (2018) 47:485–93. doi: 10.1111/apt.14419
114. Dubinsky MC, Cross RK, Sandborn WJ, Long M, Song X, Shi N, et al. Extraintestinal manifestations in vedolizumab and anti-tnf-treated patients with inflammatory bowel disease. *Inflamm Bowel Dis.* (2018) 24:1876–82. doi: 10.1093/ibd/izy065
115. Ramos GP, Dimopoulos C, McDonald NM, Janssens LP, Hung KW, Proctor D, et al. The impact of vedolizumab on pre-existing extraintestinal manifestations of inflammatory bowel disease: a multicenter study. *Inflamm Bowel Dis.* (2021) 27:1270–6. doi: 10.1093/ibd/izaa293
116. Denesh D, Carbonell J, Kane JS, Gracie D, Selinger CP. Patients with Inflammatory Bowel Disease (Ibd) prefer oral tablets over other modes of medicine administration. *Expert Rev Gastroenterol Hepatol.* (2021) 15:1091–6. doi: 10.1080/17474124.2021.1898944
117. Allen PB, Lindsay H, Tham TC. How do patients with inflammatory bowel disease want their biological therapy administered? *BMC Gastroenterol.* (2010) 10:1. doi: 10.1186/1471-230X-10-1
118. Bell CF, Lau M, Lee M, Poulos C. Insights into the choice between intravenous infusion and subcutaneous injection: physician and patient characteristics driving treatment in Sle. *Clin Rheumatol.* (2021) 40:581–90. doi: 10.1007/s10067-020-05226-w
119. Liu J, Sylwestrzak G, Ruggieri AP, DeVries A. Intravenous versus subcutaneous anti-tnf-alpha agents for crohn's disease: a comparison of effectiveness and safety. *J Manag Care Spec Pharm.* (2015) 21:559–66. doi: 10.18553/jmcp.2015.21.7.559
120. Overton PM, Shalet N, Somers F, Allen JA. Patient preferences for subcutaneous versus intravenous administration of treatment for chronic immune system disorders: a systematic review. *Patient Prefer Adherence.* (2021) 15:811–34. doi: 10.2147/PPA.S303279
121. Flamant M, Roblin X. Inflammatory bowel disease: towards a personalized medicine. *Therap Adv Gastroenterol.* (2018) 11:1756283X17745029. doi: 10.1177/1756283X17745029
122. Barber GE, Yajnik V, Khalili H, Giallourakis C, Garber J, Xavier R, et al. Genetic markers predict primary non-response and durable response to anti-tnf biologic therapies in crohn's disease. *Am J Gastroenterol.* (2016) 111:1816–22. doi: 10.1038/ajg.2016.408
123. Lee JC, Lyons PA, McKinney EF, Sowerby JM, Carr EJ, Bredin F, et al. Gene expression profiling of cd8+ t cells predicts prognosis in patients with crohn disease and ulcerative colitis. *J Clin Invest.* (2011) 121:4170–9. doi: 10.1172/JCI59255
124. Sazonovs A, Kennedy NA, Moutsianas L, Heap GA, Rice DL, Reppell M, et al. Hla-Dqa1*05 carriage associated with development of anti-drug antibodies to infliximab and adalimumab in patients with crohn's disease. *Gastroenterology.* (2020) 158:189–99. doi: 10.1053/j.gastro.2019.09.041
125. Voskuil MD, Bangma A, Weersma RK, Festen EAM. Predicting (Side) effects for patients with inflammatory bowel disease: the promise of pharmacogenetics. *World J Gastroenterol.* (2019) 25:2539–48. doi: 10.3748/wjg.v25.i21.2539
126. Arijs I, Quintens R, Van Lommel L, Van Steen K, De Hertogh G, Lemaire K, et al. Predictive value of epithelial gene expression profiles for response to infliximab in crohn's disease. *Inflamm Bowel Dis.* (2010) 16:2090–8. doi: 10.1002/ibd.21301
127. Franzin M, Stefancic K, Lucafo M, Decorti G, Stocco G. Microbiota and drug response in inflammatory bowel disease. *Pathogens.* (2021) 10:10020211. doi: 10.3390/pathogens10020211
128. Aden K, Rehman A, Waschina S, Pan WH, Walker A, Lucio M, et al. Metabolic functions of gut microbes associate with efficacy of tumor necrosis factor antagonists in patients with inflammatory bowel diseases. *Gastroenterology.* (2019) 157:1279–92e11. doi: 10.1053/j.gastro.2019.07.025
129. Rajca S, Grondin V, Louis E, Vernier-Massouille G, Grimaud JC, Bouhnik Y, et al. Alterations in the intestinal microbiome (dysbiosis) as a predictor of relapse after infliximab withdrawal in crohn's disease. *Inflamm Bowel Dis.* (2014) 20:978–86. doi: 10.1097/MIB.0000000000000036
130. Shaw KA, Bertha M, Hofmekler T, Chopra P, Vatanen T, Srivatsa A, et al. Dysbiosis, inflammation, and response to treatment: a longitudinal study of pediatric subjects with newly diagnosed inflammatory bowel disease. *Genome Med.* (2016) 8:75. doi: 10.1186/s13073-016-0331-y
131. Busquets D, Oliver L, Amoedo J, Ramio-Pujol S, Malagon M, Serrano M, et al. Raid prediction: pilot study of fecal microbial signature with capacity to predict response to anti-tnf treatment. *Inflamm Bowel Dis.* (2021) 27:S63–6. doi: 10.1093/ibd/izab273
132. Ventin-Holmberg R, Eberl A, Saqib S, Korpela K, Virtanen S, Sipponen T, et al. Bacterial and fungal profiles as markers of infliximab drug response in inflammatory bowel disease. *J Crohns Colitis.* (2021) 15:1019–31. doi: 10.1093/ecco-jcc/jjaa252
133. Gottlieb S. *Don't Give up on Biosimilars—Congress Can Give Them a Boost*. New York City: Wall Street Journal (2019). Available line at: www.wsj.com/articles/dont-give-up-on-biosimilarscongress-can-give-them-a-boost-11566755042 (accessed July 19, 2021)
134. IQVIA. *Global, European and Belgian Pharmaceutical Market Trends*. (2019). Available online at:https://www.cib-pharma.be/uploads/global-european-and-belgian-pharmaceutical-market-trends-2019-final1552840137.pdf (accessed July 19, 2021)
135. Rezk MF, Pieper B. Unlocking the value of anti-tnf biosimilars: reducing disease burden and improving outcomes in chronic immune-mediated inflammatory diseases: a narrative review. *Adv Ther.* (2020) 37:3732–45. doi: 10.1007/s12325-020-01437-4
136. Armuzzi A, Bouhnik Y, Cummings F, Bettey M, Pieper B, Kang T. Enhancing treatment success in inflammatory bowel disease: optimising the use of anti-tnf agents and utilising their biosimilars in clinical practice. *Dig Liver Dis.* (2020) 52:1259–65. doi: 10.1016/j.dld.2020.06.008
137. Kim H, Alten R, Avedano L, Dignass A, Gomollon F, Greveson K, et al. The future of biosimilars: maximizing benefits across immune-mediated inflammatory diseases. *Drugs.* (2020) 80:99–113. doi: 10.1007/s40265-020-01256-5
138. Park SH, Park JC, Lukas M, Kolar M, Loftus EV. Biosimilars: concept, current status, and future perspectives in inflammatory bowel diseases. *Intest Res.* (2020) 18:34–44. doi: 10.5217/ir.2019.09147
139. Reinisch W, Gecse K, Halfvarson J, Irving PM, Jahnsen J, Peyrin-Biroulet L, et al. Clinical practice of adalimumab and infliximab biosimilar treatment in adult patients with crohn's disease. *Inflamm Bowel Dis.* (2021) 27:106–22. doi: 10.1093/ibd/izaa078
140. Guidi L, Pugliese D, Panici Tonucci T, Berrino A, Tolusso B, Basile M, et al. Therapeutic drug monitoring is more cost-effective than a clinically based approach in the management of loss of response to infliximab in inflammatory bowel disease: an observational multicentre study. *J Crohns Colitis.* (2018) 12:1079–88. doi: 10.1093/ecco-jcc/jjy076
141. Martelli L, Olivera P, Roblin X, Attar A, Peyrin-Biroulet L. Cost-effectiveness of drug monitoring of anti-tnf therapy in inflammatory bowel disease and rheumatoid arthritis: a systematic review. *J Gastroenterol.* (2017) 52:19–25. doi: 10.1007/s00535-016-1266-1
142. Steenholdt C, Brynskov J, Thomsen OO, Munck LK, Fallingborg J, Christensen LA, et al. Individualised therapy is more cost-effective than dose intensification in patients with crohn's disease who lose response to

anti-tnf treatment: a randomised, controlled trial. *Gut.* (2014) 63:919–27. doi: 10.1136/gutjnl-2013-305279
143. Velayos FS, Kahn JG, Sandborn WJ, Feagan BG. A test-based strategy is more cost effective than empiric dose escalation for patients with crohn's disease who lose responsiveness to infliximab. *Clin Gastroenterol Hepatol.* (2013) 11:654–66. doi: 10.1016/j.cgh.2012.12.035
144. Yao J, Jiang X, You JHS. A systematic review on cost-effectiveness analyses of therapeutic drug monitoring for patients with inflammatory bowel disease: from immunosuppressive to anti-tnf therapy. *Inflamm Bowel Dis.* (2021) 27:275–82. doi: 10.1093/ibd/izaa073
145. Di Giuseppe D, Frisell T, Ernestam S, Forsblad-D'Elia H, Lindqvist E, Lindstrom U, et al. Uptake of rheumatology biosimilars in the absence of forced switching. *Expert Opin Biol Ther.* (2018) 18:499–504. doi: 10.1080/14712598.2018.1458089
146. Pentek M, Lakatos PL, Oorsprong T, Gulacsi L, Pavlova M, Groot W, et al. Access to biologicals in crohn's disease in ten European countries. *World J Gastroenterol.* (2017) 23:6294–305. doi: 10.3748/wjg.v23.i34.6294
147. Razanskaite V, Bettey M, Downey L, Wright J, Callaghan J, Rush M, et al. Biosimilar infliximab in inflammatory bowel disease: outcomes of a managed switching programme. *J Crohns Colitis.* (2017) 11:690–6. doi: 10.1093/ecco-jcc/jjw216
148. Bergqvist V, Kadivar M, Molin D, Angelison L, Hammarlund P, Olin M, et al. Switching from originator infliximab to the biosimilar Ct-P13 in 313 patients with inflammatory bowel disease. *Therap Adv Gastroenterol.* (2018) 11:1756284818801244. doi: 10.1177/1756284818801244
149. Jorgensen KK, Olsen IC, Goll GL, Lorentzen M, Bolstad N, Haavardsholm EA, et al. Switching from originator infliximab to biosimilar Ct-P13 compared with maintained treatment with originator infliximab (nor-switch): a 52-week, randomised, double-blind, non-inferiority trial. *Lancet.* (2017) 389:2304–16. doi: 10.1016/S0140-6736(17)30068-5
150. Armuzzi A, Fiorino G, Variola A, Manetti N, Fries W, Orlando A, et al. The prosit cohort of infliximab biosimilar in ibd: a prolonged follow-up on the effectiveness and safety across Italy. *Inflamm Bowel Dis.* (2019) 25:568–79. doi: 10.1093/ibd/izy264
151. Ben-Horin S, Leszczyszyn J, Dudkowiak R, Lahat A, Gawdis-Wojnarska B, Pukitis A, et al. Op24 a novel subcutaneous infliximab (Ct-P13): 1-year results including switching results from intravenous infliximab (Ct-P13) in patients with active crohn's disease and ulcerative colitis. *J Crohns Colitis.* (2020) 14:S21–2.
152. Sandborn WJ, Baert F, Danese S, Krznaric Z, Kobayashi T, Yao X, et al. Efficacy and safety of vedolizumab subcutaneous formulation in a randomized trial of patients with ulcerative colitis. *Gastroenterology.* (2020) 158:562–72e12. doi: 10.1053/j.gastro.2019.08.027
153. Grinman AB, de Souza M, Bouskela E, Carvalho ATP, de Souza HSP. Clinical and laboratory markers associated with anti-tnf-alpha trough levels and anti-drug antibodies in patients with inflammatory bowel diseases. *Medicine.* (2020) 99:e19359. doi: 10.1097/MD.0000000000019359
154. Cappello M, Morreale GC. The role of laboratory tests in crohn's disease. *Clin Med Insights Gastroenterol.* (2016) 9:51–62. doi: 10.4137/CGast.S38203
155. Bressler B, Panaccione R, Fedorak RN, Seidman EG. Clinicians' guide to the use of fecal calprotectin to identify and monitor disease activity in inflammatory bowel disease. *Can J Gastroenterol Hepatol.* (2015) 29:369–72. doi: 10.1155/2015/852723
156. Moran CP, Neary B, Doherty GA. Endoscopic evaluation in diagnosis and management of inflammatory bowel disease. *World J Gastrointest Endosc.* (2016) 8:723–32. doi: 10.4253/wjge.v8.i20.723
157. Magro F, Langner C, Driessen A, Ensari A, Geboes K, Mantzaris GJ, et al. European consensus on the histopathology of inflammatory bowel disease. *J Crohns Colitis.* (2013) 7:827–51. doi: 10.1016/j.crohns.2013.06.001
158. Bruining DH, Zimmermann EM, Loftus EV Jr., Sandborn WJ, Sauer CG, Strong SA, et al. consensus recommendations for evaluation, interpretation, and utilization of computed tomography and magnetic resonance enterography in patients with small bowel crohn's disease. *Gastroenterology.* (2018) 154:1172–94. doi: 10.1053/j.gastro.2017.11.274
159. Calabrese E, Rispo A, Zorzi F, De Cristofaro E, Testa A, Costantino G, et al. Ultrasonography tight control and monitoring in crohn's disease during different biological therapies: a multicenter study. *Clin Gastroenterol Hepatol.* (2021) 20:e711–22. doi: 10.1016/j.cgh.2021.03.030
160. Castiglione F, Testa A, Rea M, De Palma GD, Diaferia M, Musto D, et al. Transmural healing evaluated by bowel sonography in patients with crohn's disease on maintenance treatment with biologics. *Inflamm Bowel Dis.* (2013) 19:1928–34. doi: 10.1097/MIB.0b013e31829053ce
161. Goodsall TM, Jairath V, Feagan BG, Parker CE, Nguyen TM, Guizzetti L, et al. Standardisation of intestinal ultrasound scoring in clinical trials for luminal crohn's disease. *Aliment Pharmacol Ther.* (2021) 53:873–86. doi: 10.1111/apt.16288
162. Maaser C, Petersen F, Helwig U, Fischer I, Roessler A, Rath S, et al. Intestinal ultrasound for monitoring therapeutic response in patients with ulcerative colitis: results from the trust&Uc study. *Gut.* (2020) 69:1629–36. doi: 10.1136/gutjnl-2019-319451
163. Cheifetz AS, Abreu MT, Afif W, Cross RK, Dubinsky MC, Loftus EV Jr., et al. A comprehensive literature review and expert consensus statement on therapeutic drug monitoring of biologics in inflammatory bowel disease. *Am J Gastroenterol.* (2021) 116:2014–25. doi: 10.14309/ajg.0000000000001396
164. Drobne D, Kurent T, Golob S, Svegl P, Rajar P, Terzic S, et al. Success and safety of high infliximab trough levels in inflammatory bowel disease. *Scand J Gastroenterol.* (2018) 53:940–6. doi: 10.1080/00365521.2018.1486882
165. Feuerstein JD, Nguyen GC, Kupfer SS, Falck-Ytter Y, Singh S. American gastroenterological association institute clinical guidelines C. American gastroenterological association institute guideline on therapeutic drug monitoring in inflammatory bowel disease. *Gastroenterology.* (2017) 153:827–34. doi: 10.1053/j.gastro.2017.07.032
166. Khan A, Berahmana AB, Day AS, Barclay ML, Schultz M. New Zealand society of gastroenterology guidelines on therapeutic drug monitoring in inflammatory bowel disease. *NZ Med J.* (2019) 132:46–62.
167. Ungar B, Levy I, Yavne Y, Yavzori M, Picard O, Fudim E, et al. Optimizing anti-tnf-alpha therapy: serum levels of infliximab and adalimumab are associated with mucosal healing in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol.* (2016) 14:550–7e2. doi: 10.1016/j.cgh.2015.10.025
168. Adedokun OJ, Xu Z, Marano CW, Strauss R, Zhang H, Johanns J, et al. Pharmacokinetics and exposure-response relationship of golimumab in patients with moderately-to-severely active ulcerative colitis: results from phase 2/3 pursuit induction and maintenance studies. *J Crohns Colitis.* (2017) 11:35–46. doi: 10.1093/ecco-jcc/jjw133
169. Berends SE, Strik AS, Jansen JM, de Boer NK, van Egmond PS, Brandse JF, et al. Pharmacokinetics of golimumab in moderate to severe ulcerative colitis: the go-kinetic study. *Scand J Gastroenterol.* (2019) 54:700–6. doi: 10.1080/00365521.2019.1619828
170. Magro F, Lopes S, Silva M, Coelho R, Portela F, Branquinho D, et al. Low golimumab trough levels at week 6 are associated with poor clinical, endoscopic and histological outcomes in ulcerative colitis patients: pharmacokinetic and pharmacodynamic sub-analysis of the evolution study. *J Crohns Colitis.* (2019) 13:1387–93. doi: 10.1093/ecco-jcc/jjz071
171. Vande Casteele N, Herfarth H, Katz J, Falck-Ytter Y, Singh S. American gastroenterological association institute technical review on the role of therapeutic drug monitoring in the management of inflammatory bowel diseases. *Gastroenterology.* (2017) 153:835–57e6. doi: 10.1053/j.gastro.2017.07.031
172. Miranda EF, Nones RB, Kotze PG. Correlation of serum levels of anti-tumor necrosis factor agents with perianal fistula healing in crohn's disease: a narrative review. *Intest Res.* (2021) 19:255–64. doi: 10.5217/ir.2020.00029
173. Berends SE, Strik AS, Lowenberg M, D'Haens GR, Mathot RAA. Clinical pharmacokinetic and pharmacodynamic considerations in the treatment of ulcerative colitis. *Clin Pharmacokinet.* (2019) 58:15–37. doi: 10.1007/s40262-018-0676-z
174. Ordas I, Mould DR, Feagan BG, Sandborn WJ. Anti-Tnf monoclonal antibodies in inflammatory bowel disease: pharmacokinetics-based dosing paradigms. *Clin Pharmacol Ther.* (2012) 91:635–46. doi: 10.1038/clpt.2011.328

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BRIEF RESEARCH REPORT
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Memory T Cell Subpopulations as Early Predictors of Remission to Vedolizumab in Ulcerative Colitis

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Background: Vedolizumab is a humanized monoclonal antibody targeting the $\alpha_4\beta_7$ integrin used for the treatment of ulcerative colitis. Few biomarkers related to vedolizumab response have been identified. The aim of this work was to assess whether baseline circulating $CD4^+$ and $CD8^+$ memory T-lymphocyte subpopulations could help to identify patients with response to vedolizumab treatment in ulcerative colitis.

Methods: Prospective pilot study in 15 patients with active ulcerative colitis and previous failure to anti-TNFα starting vedolizumab treatment. Peripheral blood samples were obtained before the first dose of vedolizumab and at week 6 and 14 of treatment. Clinical remission was defined as a Mayo Clinic partial score of $\leq$2 points without any concomitant dose of steroids. Biochemical remission or endoscopic improvement was defined as fecal calprotectin <250 mcg/g or Mayo endoscopic subscore $\leq$1.

Results: At week 14, nine patients achieved clinical remission and eight patients achieved biochemical remission or endoscopic improvement. Patients in clinical remission presented higher baseline CD8 $\alpha_4\beta_7^+$ memory T cells concentration when compared with patients with no remission. In addition, patients with biochemical remission or endoscopic improvement at week 14 presented higher baseline concentration of CD8 $\alpha_4\beta_7^+$ memory T cells. No differences were identified according to flare severity, extent of disease or type of anti-TNFα failure. There were no significant differences regarding changes in T cell subsets during vedolizumab induction.

Conclusion: $CD8^+$ $\alpha_4\beta_7^+$ memory T cells before starting vedolizumab therapy could be an early predictor of remission in ulcerative colitis patients and therefore help to select a subset of responders.

Keywords: inflammatory bowel disease, ulcerative colitis, biological therapy, integrins, T lymphocytes

INTRODUCTION

Ulcerative colitis (UC) is a chronic, relapsing, inflammatory disorder of the gastrointestinal tract affecting an increasing number of individuals in industrialized countries (1, 2). It is a subtype of inflammatory bowel disease (IBD), which also includes Crohn's disease (CD).

Treatment of UC includes salicylates, systemic corticosteroids, immunomodulators, and monoclonal antibodies (3). Treatment should be tailored to disease activity (mild, moderate, severe), extent and phenotype (4–6).

Vedolizumab (VDZ) is a humanized monoclonal antibody directed against gut-homing integrin $\alpha_4\beta_7$. It prevents T lymphocyte adhesion to the vascular endothelium [*mucosal addressing cell adhesion molecule 1* (MAdCAM-1) and fibronectin], expressed in the intestinal tract (7). VDZ has demonstrated a therapeutic effect in UC and CD (8, 9).

The administration of VDZ is followed by a significant expansion of $\alpha_4\beta_7^+$ memory helper T lymphocytes in peripheral blood while their frequency in gastrointestinal tissues decreases in primates (10). In humans, VDZ induces qualitative and quantitative changes in a subset of memory T cells (11) as well as several effects on innate immunity (changes in macrophage populations, pronounced alterations in the expression of molecules involved in microbial sensing, chemoattraction and regulation of the innate effector response) (12). Hence, studying the changes in circulating memory T cells in UC patients treated with VDZ could lead to identify molecular predictors of response to this treatment. Although recently some clinical and biochemical predictive factors of VDZ response in IBD have been described (13–16), data on molecular markers are still scarce in UC (17, 18). In this scenario, identifying biomarkers of response to VDZ in UC would be a useful tool to select a subset of patients who would be likely to respond to VDZ, rather than follow the current sequential treatment failure approach.

Therefore, the aim of this study was to assess whether baseline circulating $CD4^+$ and $CD8^+$ $\alpha4\beta7^+$ memory T cell subpopulations, several lymphocytic markers previously involved in the physiopathology of IBD (19, 20), and their changes during treatment could be predictors of response to VDZ in patients with UC.

METHODS

Study Population

We conducted a prospective observational study including UC patients recruited consecutively at the Hospital del Mar IBD Unit from January 2017 to June 2018. All patients were diagnosed of UC following ECCO criteria (21) and they received VDZ treatment in a standard induction plan (300 mg i.v. 0–2–6 weeks). Patients who were in clinical response at week 14 received VDZ 300 mg i.v. every 8 weeks as maintenance therapy. The washout period for previous anti-TNFα treatment was established per protocol as 4 weeks for infliximab i.v. and 2 weeks for adalimumab s.c. During the induction period, oral systemic corticosteroids and oral prolonged steroids (beclomethasone dipropionate) were allowed meanwhile any other immunosuppressant therapy were forbidden.

Before starting VDZ, disease activity was evaluated using the Mayo clinical score, including endoscopic activity confirmed by colonoscopy (Mayo endoscopic subscore 2 or 3). Bacterial and parasitic infections were ruled out by stool culture and cytomegalovirus was excluded in colonic biopsies by immunohistochemistry.

Data Collection

Peripheral blood samples were collected from patients prior to starting VDZ treatment and at week 6 and 14, immediately before VDZ administration. Stool sample collection was performed 1–3 days before starting VDZ treatment and before week 14. All stool samples were analyzed to measure fecal calprotectin (FC) by an automated immunoassay (Phadia EliA^{TM} Calprotectin; normal range from 0 to 50 mcg/g). In addition, the partial Mayo score was prospectively calculated at weeks 6 and 14. Demographic and clinical data including age, gender, disease duration, disease extent, concomitant medications, endoscopic activity, histology, albumin, and serological inflammatory marker levels were collected from medical records.

The primary endpoint was to evaluate whether baseline circulating $CD4^+/CD8^+$ $\alpha_4\beta_7^{\pm}$ memory T cells as well as several surface markers (HLA-DR, CCR9), Th17 phenotype marker IL23R and intracellular IL17A and IL9, predict clinical remission to VDZ at week 14.

The secondary end-points were:

- To assess whether the subsets of memory T cells ($\alpha_4\beta_7$, HLA-DR, IL23R, CCR9, IL17A, IL9, β_7, and β_7-CCR9) at baseline predict endoscopic and biochemical remission at week 14, and sustained clinical remission at week 52.
- To assess whether changes in the same memory T cell subsets during VDZ treatment are related to clinical and biochemical remission or endoscopic improvement.

Definitions of Response

Clinical response was defined as a decrease in the partial Mayo Clinic score of at least three points at week 14. Clinical remission was defined as a Mayo Clinic partial score of $\leq$2 points without any concomitant dose of steroids at week 14. Sustained clinical remission was defined as a Mayo Clinical partial score of $\leq$2 points without concomitant corticosteroid therapy at week 52. Biochemical remission was defined as FC $<$ 250 mcg/g, as considered in GETECCU Spanish guidelines (22, 23). Endoscopic improvement was defined as a Mayo endoscopic subscore $\leq$1 (24, 25).

Circulating Memory T Cell Isolation

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll gradient (GE Healthcare, Princeton, NJ, United States) and SepMate PBMC isolation tubes (STEMCELL Technologies, Grenoble, France). Then, memory T cells were purified after two sequential immunomagnetic separations consisting of the

$CD14^+$/$CD19^+$ and $CD45RA^+$/$CD16^+$ cell depletions (Miltenyi Biotec, Bergisch Gladbach, Germany). Purified circulating memory T cells were cryopreserved in aliquots in liquid nitrogen using established techniques.

Circulating Memory T Cell Populations Staining and Flow Cytometry Analysis

One day prior staining, circulating memory T cells were thawed and plated at 1M cells/ml in RPMI (Sigma-Aldrich, St. Louis, MO. United States) with 10% fetal bovine serum (Gibco, Grand Island, NY, United States) and 1% penicillin-streptomycin (Sigma-Aldrich), after cell viability assessment. Next day, cells were plated at 2M cells/ml in the presence of Brefeldin A solution (1 μl Brefeldin per 500 μl of final volume) (BioLegend, San Diego, CA, United States) and incubated at 37°C for 4 h.

The following antibodies were used for the multicolor flow cytometry staining: CD4-PE Texas Red (Life Technologies, Carlsbad, CA, United States), CD8-AF700 (BioLegend) α_4 integrin (CD49d)-BV510 (BioLegend), β_7 integrin-FITC (Affymetrix, eBioscience Inc., Santa Clara, CA, United States), HLA-DR-ACP/Cy7 (BioLegend), IL23R-PE (R&D Systems, United States), CCR9-PerCP-Cy5.5 (BioLegend), and intracellular IL17A-BV421 (BioLegend) and IL9-APC (Miltenyi Biotec). FOXP3 Fix/Perm Buffer Set (BioLegend) for the intracellular staining was used. Samples were resuspended in 400 μl sheath cytometer buffer and 100 μl AccuCheck Counting Beads (Invitrogen, Carlsbad, CA, United States) were added for the absolute cell subset counts.

Circulating memory T cell subsets were acquired through the Beckman Coulter Gallios Flow Cytometer at the core facility of the Parc Científic de Barcelona and FlowJo software was used for analysis gating using respective isotype control antibodies and adequate flow cytometer compensations.

Finally, we identified in peripheral blood the following memory T cell subpopulations: $\alpha_4\beta_7$, HLA-DR, IL23R, CCR9, IL17A, IL9, β_7, and β_7-CCR9.

Statistical Analysis

The study was designed as a proof of concept and standard sample size could not be calculated due to the absence of published previous data.

Dichotomous variables were presented as percentages, and *p*-value associations were determined with χ^2 or Fisher exact tests. For continuous variables, data were presented as median and interquartile range (IQR). Normally distributed data were analyzed by unpaired sample *t*-test. Abnormally distributed data were compared by non-parametrical tests (Mann–Whitney *U* test). Wilcoxon signed-rank test was used to compare immune subsets before and after VDZ therapy.

All *t*-tests were two-sided and *p*-values < 0.05 were considered statistically significant. No adjustments for multiple comparisons were performed, as this was a hypothesis-generating study and many of the outcomes measured were biologically related. Statistical analyses were performed using SPSS 25.0 software (Statistical Package for the Social Sciences Inc., Chicago, IL, United States).

Ethical Considerations

All study subjects provided written informed consent before enrollment. Research procedures were approved by the Hospital del Mar Clinical Research and Ethics Committee in 2016. This study were conducted according to the principles expressed in the 1975 Declaration of Helsinki (6th revision, 2008) in the Council of Europe Convention on Human Rights and Biomedicine.

RESULTS

Patients Characteristics

A total of fifteen UC patients starting VDZ treatment were included prospectively, seven with severe disease (partial Mayo score between 7 and 9 points).

All patients had received anti-TNFα previously: nine patients were primary non-responders and six patients presented loss of response.

Steroids were started at a standard dose simultaneously with VDZ: prednisone 1 mg/kg/day in 12 patients and beclomethasone dipropionate 5 mg/day in 1 patient. Two patients did not take any concomitant treatment. Steroids were tapered and completely discontinued between week 6 and 10.

After induction, 11 patients provided stool samples to measure to measure FC and 12 patients underwent and 12 patients underwent a colonoscopy.

At week 14, nine patients achieved clinical remission, five patients were in biochemical remission, six patients presented endoscopic improvement and eight patients achieved biochemical remission or endoscopic improvement.

At week 52, ten patients were in sustained clinical remission: nine received VDZ every 8 weeks and one patient received VDZ every 8 weeks, oral mesalazine and tacrolimus.

Clinical and demographic characteristics of patients depend on clinical remission are shown in **Table 1**.

Memory T Cell Subpopulations Before Treatment (at Baseline)

The concentration of the different $CD4^+$ and $CD8^+$ memory T cell subpopulations were studied in our cohort of UC patients before treatment. Results of one patient at baseline were excluded from the final statistic analysis due to technical problems with blood samples that led to massive cell death.

Patients who achieved clinical remission at week 14, presented significantly higher $CD4^+$ memory T cells and $CD8^+$ $\alpha_4\beta_7^+$ memory T cells concentration compared with those who were not in clinical remission [median: 394.47 cells/ml versus 304.73 cells/ml, $p = 0.02$ (**Figure 1A**); 19.27 cells/ml versus 11.63 cells/ml, $p = 0.02$ (**Figure 2A**), respectively]. No significant differences were found in $CD4^+$ memory T cells subsets between both groups. A representative flow cytometry plot is shown in **Figure 3**.

Patients who were in sustained clinical remission at week 52 presented higher $CD4^+$ memory T cells and CD8 $\alpha_4\beta_7^+$ memory T cells concentration compared with non-remitters (median:

TABLE 1 | Comparison of demographic and clinical characteristics between patients achieving or not clinical remission at week 14.

	Remitters (*n* = 9)	Non-remitters (*n* = 6)	*p*-value
Female sex, No. (%)	5 (55.6)	2 (33.3)	0.302
Age (years)- median (IQR)	45 (31.5–66)	41.5 (33.5–52.8)	0.750
Disease extent, No. (%)			0.411
E1 – Proctitis	1 (11.1)	0	
E2 – Left sided colitis	4 (44.4)	4 (66.7)	
E3 – Pancolitis	4 (44.4)	2 (33.3)	
Severity, No. (%)			
Severe (Mayo score 7–9)	4 (44.4)	3 (50)	0.696
Disease duration, No. (%)			0.441
<10 years	7 (77.8)	1 (16.7)	
Between 10 and 20 years	1 (11.1)	5 (83.3)	
>20 years	1 (11.1)	0	
TNF antagonist failure, No. (%)			0.418
Primary non-responders	7 (77.8)	2 (33.3)	
Loss of response	2 (22.2)	4 (66.7)	
Co-treatment at baseline, No. (%)			0.036[a]
Oral prednisone	9 (100)	3 (50)	
Oral beclomethasone	0	1 (16.7)	
None	0	2 (33.3)	
Albumin (g/dl), median (IQR)	4.2 (3.9–4.6)	4 (3.5–4.6)	0.331
C Reactive Protein (mg/dl), median (IQR)	0.51 (0.1–1.54)	0.56 (0.13–2.02)	0.801
Fecal Calprotectin (mcg/g), median (IQR)	619 (134–1767)	849 (311–3878)	0.308

[a]*p-value < 0.05.*

394.47 cells/ml versus 327.66 cells/ml, p = 0.02; 14.43 cells/ml versus 11.85 cells/ml, p = 0.02, respectively).

The $CD8^+$ $\alpha_4\beta_7{}^+$ memory T cells concentration in patients with biochemical remission was significantly higher (median: 24.75 cells/ml versus 11.87 cells/ml, p = 0.019) than in patients who did not achieved biochemical remission (**Figure 2B**). The $CD8^+$ $CCR9^+$ memory T cells concentration was significantly lower in biochemical remitters than in non-remitters (median: 0.29 cells/ml versus 1.12 cells/ml, p = 0.019). There were no statistically significant differences in $CD4^+$ memory T cell subsets between both groups (**Figure 1B**).

Regarding endoscopic improvement, the $CD8^+$ $\alpha_4\beta_7{}^+$ memory T cells concentration was higher in patients with endoscopic improvement than in patients who did not show endoscopic improvement, but these differences did not reach statistical significance (median: 14.43 cells/ml versus 11.63 cells/ml, p = 0.43). $CD4^+$ memory T cells concentration was significantly higher in patients with endoscopic improvement compared with those without endoscopic improvement (median: 394.47 cells/ml versus 316.38 cells/ml, p = 0.004) (**Figure 1C**). Again, no statistically differences in $CD4^+$ memory T cell subsets were identified between both groups.

Finally, patients who were in biochemical remission or presented endoscopic improvement had significantly higher $CD4^+$ and $CD8^+$ $\alpha_4\beta_7{}^+$ memory T cells concentration compared with those without biochemical remission or endoscopic improvement [median: 394.47 cells/ml versus 304.73 cells/ml, p = 0.02 (**Figure 1D**); 14.43 cells/ml versus 11.63 cells/ml, p = 0.02 (**Figure 2C**), respectively].

In all $CD4^+$ and $CD8^+$ T cell subsets analyzed, no significant differences were identified according to flare severity, extent of disease or type of previous anti-TNFα failure.

Comparison between median of $CD4^+$ and $CD8^+$ T cell subsets depend on each type of remission is shown by bar graph in **Supplementary Figure 1**.

Memory T Cell Subpopulations at Weeks 6 and 14

Regarding clinical and biochemical remission, there were no statistically significant differences in all $CD4^+$ and $CD8^+$ T cell subsets at week 6. Likewise, no significant differences were identified in the same T cell subpopulations at week 6 between patients who presented endoscopic improvement and those without endoscopic improvement (**Supplementary Figure 2**).

In relation to T cell subpopulations at week 14, results of two patients were excluded from the final statistic analysis due to technical problems with blood samples that led to massive cell death. CD8 $\beta_7{}^+$ memory T cell concentration was significantly higher in the group of patients that achieved clinical remission and biochemical remission or endoscopic improvement, compared with those who did not present any type of remission (median: 21.10 cells/ml versus 7.07 cells/ml, p = 0.03) (**Supplementary Figure 3**).

Memory T Cell Subpopulations During Vedolizumab Treatment Induction Phase

During VDZ induction, since baseline until weeks 6 and 14, no statistically significant changes were observed in $CD4^+$ and $CD8^+$ memory T cell subsets concentration between patients presenting clinical remission and endoscopic improvement and patients who did not achieved remission.

DISCUSSION

In this prospective study, we investigated if several memory T cell subpopulations in peripheral blood could predict VDZ response in UC. A higher concentration of baseline $CD8^+$ $\alpha_4\beta_7{}^+$ memory T cells was positively associated with clinical remission, biochemical remission or endoscopic improvement in UC patients after VDZ induction. This association was not related to flare severity, extent of the disease or type of anti-TNFα failure. In addition, a higher total $CD4^+$ T cells concentration was also associated with clinical remission, biochemical remission or endoscopic improvement, although no statistically significant differences in $CD4^+$ T cell subsets were identified between remitters and non-remitters.

Different studies have explored the role of lymphocyte subpopulations in the response to VDZ. According to our results, Boden et al. demonstrated -in 26 IBD patients- an increased

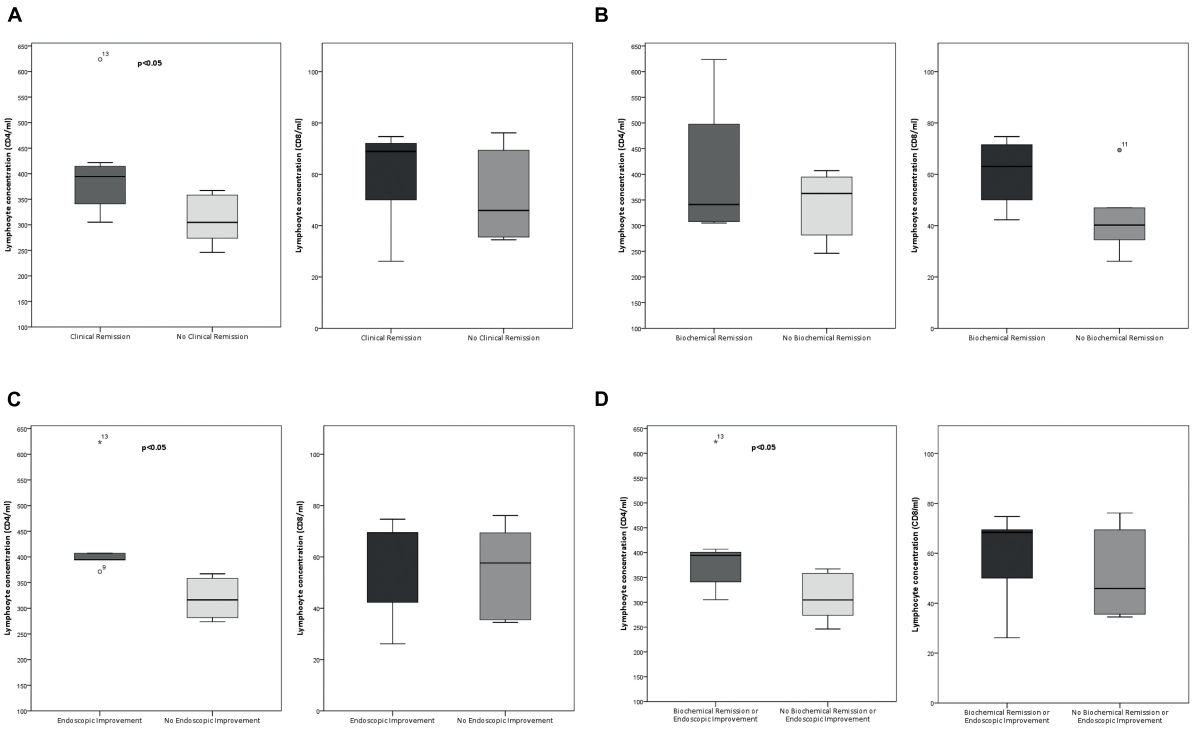

FIGURE 1 | Boxplots of total $CD4^+$ (left) and $CD8^+$ (right) lymphocytes concentration at baseline depend on each type of remission (values are shown in cells per milliliter). **(A)** Clinical remission at week 14. **(B)** Biochemical remission. **(C)** Endoscopic improvement. **(D)** Biochemical remission or endoscopic improvement. Outliers are shown as circles and extreme outliers, as *.

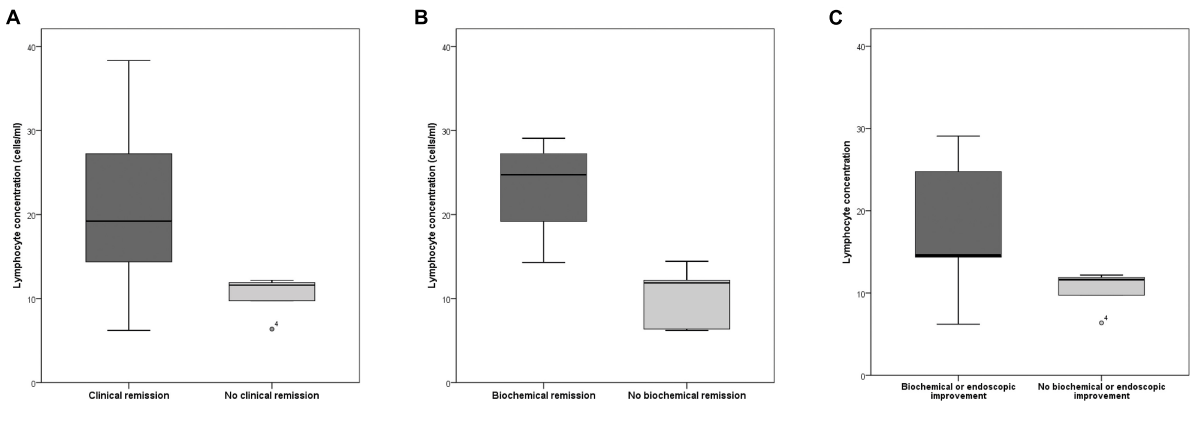

FIGURE 2 | Boxplots of CD8 $\alpha_4\beta_7^+$ memory T cells concentration at baseline depend on each type of remission (values are shown in cells per milliliter). All differences are statistically significant. **(A)** Clinical remission at week 14. **(B)** Biochemical remission. **(C)** Biochemical or endoscopic improvement. Outliers are shown as circles.

$\alpha_4\beta_7^+$ expression in IBD responders to VDZ in multiple subsets of T, B, and NK cells, with terminal effector memory T cells (CD4 and CD8) and NK cells best discriminating between responders and non-responders (17). Apart of pretreatment $\alpha_4\beta_7^+$ expression, they found that α4β7 receptor saturation during maintenance therapy could be a candidate biomarker for vedolizumab response.

Otherwise, Fuchs et al. (26) analyzed retrospectively integrins and chemokine receptors on T cells before and during VDZ treatment in 17 UC and 19 CD patients. They found that increasing $\alpha_4\beta_7$ levels in $CD4^+$ T cells during induction period in UC were associated with favorable clinical response. Although patients with clinical response at week 16 had lower pretreatment frequencies of $\alpha_4\beta_7$-expressing $CD4^+$ T cells, these results included CD and UC patients, and, as no specific alterations of α4β7 integrin expression were founded in CD in this study, UC and CD patients should be analyzed separately.

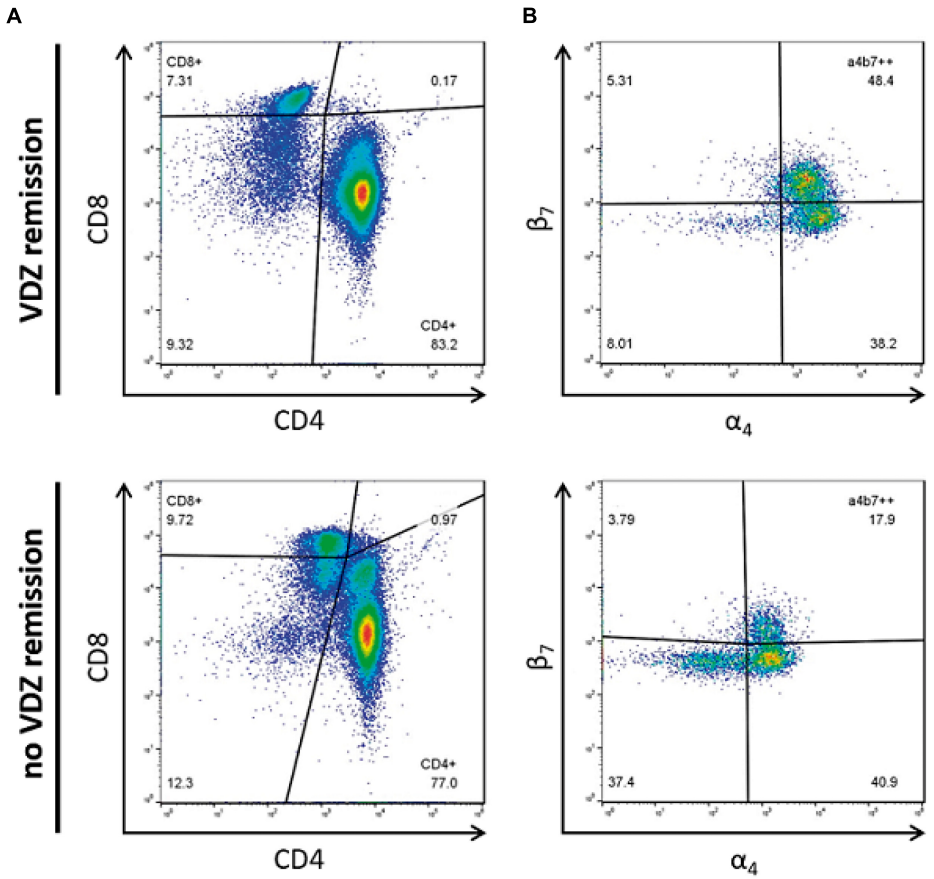

FIGURE 3 | Representative flow cytometric analysis of baseline memory T cell subsets. Differential expression of **(A)** total CD4 versus CD8 memory T cells and **(B)** CD8 $\alpha_4\beta_7^+$ memory T cell subset. Values are shown as percentages.

Furthermore, a Belgian group published recently results from a prospective study in 71 IBD patients focused on baseline T cell subsets (27). Unlike our results, they observed in the UC cohort differences in the baseline proportion of $CD4^+$ $\alpha_4\beta_7^+$ T cells between responders and non-responders, but not in the baseline proportion of $CD8^+$ $\alpha_4\beta_7^+$ T cells. Despite the differences between T cells subsets, results could not be compared directly given both studies had different endpoints -clinical remission in our study and clinical response in the Belgian group-.

Besides, some studies focused on B cells or soluble proteins also supported the role of $\alpha_4\beta_7$ as a predictor of response to VDZ. Uzzan et al. presented at the AGA Congress in 2018 a prospective study in 38 IBD patients (31 with UC) where a higher expression of pre-VDZ treatment $\alpha_4\beta_7^+$ on B cells predicted clinical remission at week 14 (28). Furthermore, a prospective study in 32 UC patients showed that patients who achieved clinical remission, soluble $\alpha_4\beta_7^+$ increased, whereas soluble MAdCAM-1, VCAM-1, ICAM-1, and TNF levels decreased rapidly (29).

Even though several groups have explored blood biomarkers, mucosal biomarkers had been broadly explored as predictors of response to VDZ treatment. Veny et al. analyzed the effect of VDZ treatment in the proportion of lymphocyte subsets and integrin expression both in colon biopsies and in blood samples (30). They included patients starting VDZ (n = 33), anti-TNFα (n = 45) and controls (n = 22). VDZ therapy specifically decreased $\alpha_4\beta_7^+$ $CD4^+$ and $CD8^+$ T cells in the colon, while preserving the proportion of $\alpha_4\beta_7^+$ plasma cells. However, this study was designed to understand the mechanism of action of VDZ and was not addressed to establish the association between baseline lymphocyte subpopulations and response to treatment.

Although mucosal biomarkers seemed very promising, we decided to investigate T cell subsets in peripheral blood as obtaining blood samples is minimally invasive for the patients and it can be easily applied in clinical routine. In addition, circulating $CD8^+$ memory T cells are starting to attract attention in UC since they are activated in periphery (31) and present a clonal expansion in colon mucosa (32–34), which supports the relevance of our results for colon homing $CD8^+$ T cells.

Some study limitations should be taken into account when interpreting our results: small sample size, as it was designed as an exploratory study, single-center cohort and differences in steroids treatment between groups. Therefore, additional studies will be needed to further validate our results in an independent and larger cohort and in order to elucidate if these results are associated exclusively with VDZ therapy.

Although it has also some strengths. It is a prospective study including a homogeneous and well-characterized cohort of UC patients with previous failure to anti-TNFα. The main goal, clinical remission at week 14, combined with an objective measurement of response (endoscopic improvement or calprotectin levels), was selected as a "real-life" endpoint. Likewise, T cell subpopulations were evaluated in peripheral blood as blood samples are routinely obtained in daily practice, which makes it easily reproducible.

In conclusion, in UC patients treated with VDZ, we have shown an association between high baseline $CD8^+$ $\alpha_4\beta_7^+$, $CD4^+$ T cells and clinical remission at week 14. Moreover, both are related to biochemical remission or endoscopic improvement. As a more specific subpopulation, assessing $CD8^+$ $\alpha_4\beta_7^+$ T cell subset in peripheral blood might be a predictor of response that would help to support therapeutic decisions in routine clinical practice.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Hospital del Mar Clinical Research and Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MA, LS-B, and LM-M contributed to the design of the study. MA, AF-C, AL-G, FM, LA, and LM-M included patients. MKLT, CJ-G, ER-R, and LS-dS analyzed the T cell subpopulations. MG-V, AF-C, MKLT, CJ-G, ER-R, and LS-dS collected the data. MG-V and LM-M analyzed the data. MG-V, LS-dS, and LM-M drafted the manuscript. CJ-G and LS-B critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.837294/full#supplementary-material

Supplementary Figure 1 | Median of $CD4^+$ (left) and $CD8^+$ (right) lymphocyte subpopulations concentration at baseline (values are shown in cells per milliliter). **(A)** Clinical remission. **(B)** Biochemical remission. **(C)** Endoscopic improvement. **(D)** Biochemical remission or endoscopic improvement.

Supplementary Figure 2 | Median of $CD4^+$ (left) and $CD8^+$ (right) lymphocyte subpopulations concentration at week 6 (values are shown in cells per milliliter) depend on clinical remission. Similar results were found between patients with biochemical or endoscopic improvement and patients without improvement.

Supplementary Figure 3 | Median of $CD4^+$ (left) and $CD8^+$ (right) lymphocyte subpopulations concentration at week 14 (values are shown in cells per milliliter) depend on clinical remission. Similar results were found between patients with biochemical or endoscopic improvement and patients without improvement.

REFERENCES

1. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel J-F. Seminar ulcerative colitis. *Lancet.* (2017) 389:1756–70. doi: 10.1016/S0140-6736(12)60150-0
2. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet.* (2017) 390:2769–78. doi: 10.1016/S0140-6736(17)32448-0
3. Harbord M, Eliakim R, Bettenworth D, Karmiris K, Katsanos K, Kopylov U, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 2: current management. *J Crohns Colitis.* (2017) 11:769–84.
4. Silverberg MS, Satsangi J, Ahmad T, Arnott IDR, Bernstein CN, Brant SR, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a working party of the 2005 Montreal world congress of gastroenterology. *Can J Gastroenterol.* (2005) 19:5A–36A. doi: 10.1155/2005/269076
5. Peyrin-Biroulet L, Sandborn W, Sands BE, Reinisch W, Bemelman W, Bryant RV, et al. Selecting therapeutic targets in inflammatory bowel disease (STRIDE): determining therapeutic goals for treat-to-target. *Am J Gastroenterol.* (2015) 110:1324–38.
6. Colombel JF, Narula N, Peyrin-Biroulet L. Management strategies to improve outcomes of patients with inflammatory bowel diseases. *Gastroenterology.* (2017) 152:351–61.e5. doi: 10.1053/j.gastro.2016.09.046
7. Wyant T, Fedyk E, Abhyankar B. An overview of the mechanism of action of the monoclonal antibody vedolizumab. *J Crohns Colitis.* (2016) 10:1437–44. doi: 10.1093/ecco-jcc/jjw092
8. Feagan BG, Rutgeerts P, Sands BE, Hanauer S, Colombel J-F, Sandborn WJ, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* (2013) 369:699–710.
9. Sandborn WJ, Feagan BG, Rutgeerts P, Hanauer S, Colombel JF, Sands BE, et al. Vedolizumab as induction and maintenance therapy for Crohn's disease. *N Engl J Med.* (2013) 369:711–21.
10. Fedyk ER, Wyant T, Yang L-L, Csizmadia V, Burke K, Yang H, et al. Exclusive antagonism of the α4 β7 integrin by vedolizumab confirms the gut-selectivity of this pathway in primates. *Inflamm Bowel Dis.* (2012) 18:2107–19. doi: 10.1002/ibd.22940
11. Lord JD, Long SA, Shows DM, Thorpe J, Schwedhelm K, Chen J, et al. Circulating integrin alpha4/beta7+ lymphocytes targeted by vedolizumab have

a pro-inflammatory phenotype. *Clin Immunol.* (2018) 193:24–32. doi: 10.1016/j.clim.2018.05.006
12. Zeissig S, Rosati E, Dowds CM, Aden K, Bethge J, Schulte B, et al. Vedolizumab is associated with changes in innate rather than adaptive immunity in patients with inflammatory bowel disease. *Gut.* (2019) 68:25–39. doi: 10.1136/gutjnl-2018-316023
13. Shmidt E, Kochhar G, Hartke J, Chilukuri P, Meserve J, Chaudrey K, et al. Predictors and management of loss of response to vedolizumab in inflammatory bowel disease. *Inflamm Bowel Dis.* (2018) 24:2461–7.
14. Dulai PS, Boland BS, Singh S, Chaudrey K, Koliani-Pace JL, Kochhar G, et al. Development and validation of a scoring system to predict outcomes of vedolizumab treatment in patients with Crohn's disease. *Gastroenterology.* (2018) 155:687–95.e10. doi: 10.1053/j.gastro.2018.05.039
15. Bertani L, Blandizzi C, Mumolo MG, Ceccarelli L, Albano E, Tapete G, et al. Fecal calprotectin predicts mucosal healing in patients with ulcerative colitis treated with biological therapies: a prospective study. *Clin Transl Gastroenterol.* (2020) 11:e00174. doi: 10.14309/ctg.0000000000000174
16. Dulai PS, Singh S, Vande Casteele N, Meserve J, Winters A, Chablaney S, et al. Development and validation of clinical scoring tool to predict outcomes of treatment with vedolizumab in patients with ulcerative colitis. *Clin Gastroenterol Hepatol.* (2020) 18:2952–61.e8. doi: 10.1016/j.cgh.2020.02.010
17. Boden EK, Shows DM, Chiorean MV, Lord JD. Identification of candidate biomarkers associated with response to vedolizumab in inflammatory bowel disease. *Dig Dis Sci.* (2018) 63:2419–29. doi: 10.1007/s10620-018-4924-8
18. Bertani L, Caviglia GP, Antonioli L, Pellicano R, Fagoonee S, Astegiano M, et al. Serum interleukin-6 and −8 as predictors of response to vedolizumab in inflammatory bowel diseases. *J Clin Med.* (2020) 9:1323. doi: 10.3390/jcm9051323
19. Danese S. Role of the vascular and lymphatic endothelium in the pathogenesis of inflammatory bowel disease: "brothers in arms". *Gut.* (2011) 60:998–1008. doi: 10.1136/gut.2010.207480
20. Park JH, Peyrin-Biroulet L, Eisenhut M, Shin JI. IBD immunopathogenesis: a comprehensive review of inflammatory molecules. *Autoimmun Rev.* (2017) 16:416–26. doi: 10.1016/j.autrev.2017.02.013
21. Magro F, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. *J Crohns Colitis.* (2017) 11:649–70. doi: 10.1093/ecco-jcc/jjx008
22. Lobatón T, Rodríguez-Moranta F, Lopez A, Sánchez E, Rodríguez-Alonso L, Guardiola J. A new rapid quantitative test for fecal calprotectin predicts endoscopic activity in ulcerative colitis. *Inflamm Bowel Dis.* (2013) 19:1034–42. doi: 10.1097/MIB.0b013e3182802b6e
23. D'Haens G, Ferrante M, Vermeire S, Baert F, Noman M, Moortgat L, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. *Inflamm Bowel Dis.* (2012) 18:2218–24. doi: 10.1002/ibd.22917
24. Mazzuoli S, Guglielmi FW, Antonelli E, Salemme M, Bassotti G, Villanacci V. Definition and evaluation of mucosal healing in clinical practice. *Dig Liver Dis.* (2013) 45:969–77. doi: 10.1016/j.dld.2013.06.010
25. D'Haens G, Sandborn WJ, Feagan BG, Geboes K, Hanauer SB, Irvine EJ, et al. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology.* (2007) 132:763–86. doi: 10.1053/j.gastro.2006.12.038
26. Fuchs F, Schillinger D, Atreya R, Hirschmann S, Fischer S, Neufert C, et al. Clinical response to vedolizumab in ulcerative colitis patients is associated with changes in integrin expression profiles. *Front Immunol.* (2017) 8:764. doi: 10.3389/fimmu.2017.00764
27. De Galan C, Gonzales GB, Van Welden S, Tavernier SJ, Lobaton T, Van Moerkercke W, et al. Role of integrin expression in the prediction of response to vedolizumab: a prospective real-life multicentre cohort study. *Clin Transl Med.* (2022) 12:e769. doi: 10.1002/ctm2.769
28. Uzzan M, Rosenstein A, Tokuyama M, Magri G, Martin J, Seki A, et al. Mo1832 - baseline level of integrin A4B7 on circulating plasmablasts predicts response to vedolizumab in patients with inflammatory bowel disease. *Gastroenterology.* (2018) 154:S–818.
29. Battat R, Dulai PS, Vande Casteele N, Evans E, Hester KD, Webster E, et al. Biomarkers are associated with clinical and endoscopic outcomes with vedolizumab treatment in ulcerative colitis. *Inflamm Bowel Dis.* (2019) 25:410–20. doi: 10.1093/ibd/izy307
30. Veny M, Garrido-Trigo A, Corraliza AM, Masamunt MC, Bassolas-Molina H, Esteller M, et al. Dissecting common and unique effects of anti-α4β7 and anti-tumor necrosis factor treatment in ulcerative colitis. *J Crohns Colitis.* (2021) 15:441–52. doi: 10.1093/ecco-jcc/jjaa178
31. Rabe H, Malmquist M, Barkman C, Östman S, Gjertsson I, Saalman R, et al. Distinct patterns of naive, activated and memory T and B cells in blood of patients with ulcerative colitis or Crohn's disease. *Clin Exp Immunol.* (2019) 197:111–29. doi: 10.1111/cei.13294
32. Smillie CS, Biton M, Ordovas-Montanes J, Sullivan KM, Burgin G, Graham DB, et al. Intra- and inter-cellular rewiring of the human colon during ulcerative colitis. *Cell.* (2019) 178:714–30.e22. doi: 10.1016/j.cell.2019.06.029
33. Boland BS, He Z, Tsai MS, Olvera JG, Omilusik KD, Duong HG, et al. Heterogeneity and clonal relationships of adaptive immune cells in ulcerative colitis revealed by single-cell analyses. *Sci Immunol.* (2020) 5:eabb4432. doi: 10.1126/sciimmunol.abb4432
34. Corridoni D, Antanaviciute A, Gupta T, Fawkner-Corbett D, Aulicino A, Jagielowicz M, et al. Single-cell atlas of colonic CD8+ T cells in ulcerative colitis. *Nat Med.* (2020) 26:1480–90. doi: 10.1038/s41591-020-1003-4

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Multi-utility of therapeutic drug monitoring in inflammatory bowel diseases

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Inflammatory bowel disease (IBD) treatment targets have progressed over time from clinical response to clinical and endoscopic remission. Several data have shown a positive correlation between serum biologic drug concentrations and favorable therapeutic outcomes. Therapeutic drug monitoring (TDM) has evolved as an important approach for optimizing the use of immunobiologics, especially antitumor necrosis factor therapy, in patients with IBD. The use of TDM is supported by medical societies and IBD experts in different contexts; however, challenges remain due to knowledge gaps that limit the widespread use of it. The aim of this review is to assess the role of TDM in IBD, focusing on the implementation of this strategy in different scenarios and demonstrating the multi-utility aspects of this approach in clinical practice.

KEYWORDS

Crohn's disease, ulcerative colitis, biologics, drug concentrations, therapeutic drug monitoring

Introduction

Treatment goals for patients with inflammatory bowel disease (IBD) have evolved over time from clinical response to deep remission (clinical and endoscopic remission), aiming for a change in the disease course (1). Therapeutic drug monitoring (TDM), which involves measuring serum drug concentrations and anti-drug antibody (ADA) concentrations, has been recognized as a useful tool for biological therapy optimization along with early and scheduled disease assessment to ensure maintenance of remission in IBD (2).

Several studies have demonstrated an association between serum biologic drug concentrations and favorable therapeutic outcomes, while subtherapeutic drug concentrations and immunogenicity can explain a substantial proportion of treatment failure (2). A recent large prospective observational multicenter study from the United Kingdom, PANTS, which enrolled 1,610 biologic-naïve patients with Crohn's disease (CD) treated with infliximab or adalimumab, demonstrated that treatment failure to infliximab and adalimumab is common and is predicted by low drug concentrations, mediated in part by immunogenicity (3). In multivariate analysis, drug concentration at week 14 was the major independent risk factor associated with time to

immunogenicity for both drugs. In addition, clinical covariates, such as inflammatory burden, albumin levels, and patient-related factors, have been recognized as factors that can influence pharmacokinetic variability for all biologics (4). Even though these circumstances may reasonably justify the adoption of TDM routinely in clinical practice, there are still many barriers to the widespread use of TDM (5).

The use of TDM is supported by medical societies and IBD experts in different situations (1, 2, 6–13). In 2017, the American Gastroenterology Association (AGA) recommended the use of reactive TDM to help treatment decisions in patients with IBD with active disease who are being treated with anti-tumor necrosis factor (anti-TNF). They make no suggestions about the use of routine proactive TDM (14). The American College of Gastroenterology (ACG) published a recent literature review and expert consensus that has advised the use of TDM in a reactive context for all biologics and proactive TDM for anti-TNF as well as following a drug holiday or previously to treatment de-escalation (12). **Table 1** summarizes recommendations regarding TDM of both guidelines. There are still many knowledge gaps in the literature, such as the most appropriate measurement timepoints, proper interpretation of the results, and the identification of the optimal thresholds to target.

In this review, we aim to explore the role of TDM in IBD, focusing on the applicability of this strategy in different scenarios, and illustrating the multi-utility aspects of this approach in clinical practice (**Figure 1**).

Proactive therapeutic drug monitoring

Proactive TDM is defined as the measurement of drug trough concentrations (measuring drug level just before the subsequent infusion) and ADA levels to optimize drug concentration at specific time points (i.e., induction, at the end of induction, or maintenance) (7, 8). It is performed to optimize therapy in order to improve response rates and likely prevent future flares and loss of response (LOR) (6). Moreover, some recent data suggest that proactive TDM could also improve the safety and cost-effectiveness of biologic therapy, by preventing undetectable or low drug levels (9, 10, 15–18).

Several exposure-outcome relationship data from prospective studies and *post hoc* analyses of randomized controlled trials (RCTs) have demonstrated that higher induction, postinduction, and maintenance anti-TNF drug levels are associated with more favorable outcomes, indicating that anti-TNF therapy may benefit from proactive TDM to guide dose optimization (9, 13, 19). Here, we explore the clinical scenarios where proactive TDM might be useful.

Induction

The induction phase has emerged as an important period to proactively adjust the biological serum concentrations. This phase is characterized by a high inflammatory burden, increased drug clearance, and consequently a greater risk of inadequate drug exposure. Thus, early optimization of biological therapy could potentially prevent primary non-response (PNR) and immunogenicity, providing clinical and pharmacoeconomic benefits (19).

Exposure–outcome relationship during induction

The relationship between inadequate serum drug levels and PNR has been explored in numerous studies. In a cohort of 25 patients with IBD initiating treatment with infliximab, Bar-Yoseph et al. identified that lower infliximab trough levels and higher antibody to infliximab titers were predictive of PNR (20). Verstockt et al. also demonstrated that adalimumab trough concentrations <8.3 μg/ml at week 4 were associated with a higher risk of detection of ADA at week 12 (21).

Moreover, proactive TDM at induction has been associated with better therapeutic outcomes at the end of the induction and during the maintenance period compared with empiric dose optimization, both in CD and ulcerative colitis (UC) (22–27). Papamichael et al. retrospectively evaluated 101 patients with UC and found that infliximab trough levels ≥15 μg/ml at week 6 and ≥2.1 μg/ml at week 14 were independent factors associated with short-term mucosal healing (22). Similarly, a *post hoc* analysis of 484 patients with UC from the active ulcerative colitis trials (ACT 1/2) demonstrated that infliximab trough levels ≥18.6 μg/ml at week 2 and ≥10.6 μg/ml at week 6 were associated with endoscopic remission at week 8 (23).

A *post hoc* analysis from the CLASSIC I/II trials also identified a positive relationship between adalimumab trough concentrations and clinical remission at week 4 in patients with moderate to severely active CD (24). Additionally, Davidov et al. identified that the infliximab trough level of >9.2 μg/ml at week 2 was associated with a fistula response at week 14 (25). Conversely, a recent RCT, NOR-DRUM study, evaluating 411 patients with chronic immune-mediated inflammatory diseases initiating infliximab therapy failed to demonstrate improvement in clinical remission rates at week 30 in the group undergoing TDM during induction compared with those on clinically based dosing. The trial did not have statistical power to test hypotheses within the IBD subgroup (28).

Furthermore, pharmacokinetics data have demonstrated that there is great interindividual variability in drug concentration vs. time profiles in biological fluids, and drug concentrations at induction can fluctuate more than during maintenance treatment (26, 29). Different studies have also demonstrated that the main covariates influencing infliximab trough level are the presence of ADA, evidence

TABLE 1 Summary of AGA and ACG guidelines.

AGA guideline (14)	**Suggested trough level (μ g/mL)**
Reactive TDM for anti-TNF treatment in active IBD	Infliximab > 5 Adalimumab ≥ 7.5 Certolizumab ≥ 20 Golimumab unknown
No recommendation about proactive TDM for anti-TNF treatment in quiescent IBD	
ACG guideline (12)	**Suggested trough level (μ g/mL)**
Reactive TDM for all biologics (primary non response and secondary loss of response)	Infliximab: At week 2: > 20–25 Week 6: > 15–20 Week 14: 7–10 Maintenance: 5–10 Adalimumab: Week 4: 8–12 Maintenance: 8–12
Proactive TDM for anti-TNF therapy (after induction, at least once in maintenance, treatment de-escalation, drug holiday, anti-TNF monotherapy)	

AGA, american gastroenterology association; ACG, american college of gastroenterology.

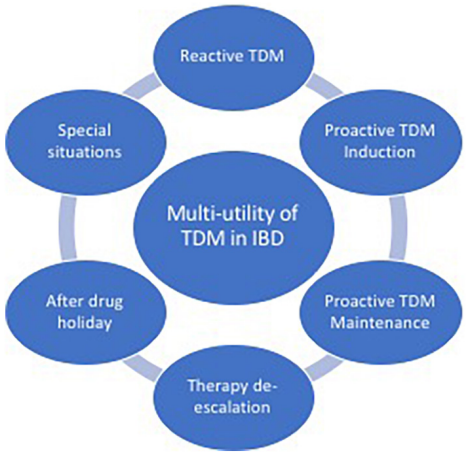

FIGURE 1
Aspects of multi-utility of therapeutic drug monitoring (TDM) in inflammatory bowel disease (IBD).

of a high inflammatory burden [elevated C-reactive protein (CRP), low albumin, and great extension of the disease], concomitant corticosteroid use, and infliximab monotherapy (26, 29).

It is important to point out that most of the data supporting the strategy of TDM during induction come from anti-TNF agents. Preliminary data related to other biologic drugs (vedolizumab and ustekinumab) has been emerging; however, it is still not possible to make recommendations regarding TDM with these specific agents (19).

Thresholds to target during induction

Although many observational studies reinforce the benefits of proactive TDM during induction, the threshold drug trough levels, as well as the best moment to measure it, have not yet been established. The target drug trough levels may vary according to the disease phenotype and desired therapeutic outcomes. A recent expert consensus statement on TDM of biologics in IBD by Cheifetz et al. supports the clinical utility of TDM during the induction phase for patients treated with anti-TNF agents, aiming at infliximab trough levels of 20–25 μg/ml at week 2 and 15–20 μg/ml at week 6, and adalimumab trough levels of 8–12 μg/ml at week 4 (12). Papamichael et al. proposed a simplified algorithm for TDM during infliximab induction therapy in IBD. They proposed that in the presence of an adequate infliximab trough level at week 2 or 6, patients in clinical response should continue on infliximab standard dose during the maintenance phase, but patients that show no response should switch the drug. In the group with therapeutic infliximab trough level, there is no recommendation for measuring antibody to infliximab since ADA is more clinically relevant when there is no detectable drug level. In contrast, individuals with undetectable or subtherapeutic infliximab trough levels should be assessed according to ADA levels. In this group, in the absence of antibody to infliximab or the presence of low titers of it, therapy optimization should be considered (either escalating the dose, decreasing the interval between the infusions, or adding immunosuppressants), while, in the presence of high-titer antibody to infliximab, switching therapy should be considered (30).

Table 2 summarizes the most relevant studies regarding TDM in the induction phase.

TABLE 2 Summary of main TDM studies in induction phase.

Observational studies	IBD type; *N*	Drug	Drug level target (μ g/mL)	Time point	Therapeutic outcome
Prospective					
Ungar et al. (POETIC)	CD; *N* = 91	Adalimumab	>6.7	Week 2	Clinical remission by week 14
Verstockt et al. (21)	CD; *N* = 116	Adalimumab	<8.3	Week 4	Presence of antibodies to adalimumab by week 12
Clarkston et al.	CD; *N* = 72	Infliximab	≥26.7	Week 2	Clinical response at week 14
			≥15.9	Week 6	
Buhl et al.	CD and UC; *N* = 166	Infliximab	>22.9	Week 2	Clinical response at week 14
			>11.8	Week 6	
Retrospective					
Dreesen et al.	CD; *N* = 122	Infliximab	>23.1	Week 2	Endoscopic remission at week 12
			>10	Week 6	
Vande Casteele et al. (23)	UC; *N* = 484	Infliximab	≥18.6	Week 2	Endoscopic remission at week 8
			≥10.6	Week 6	
Adedokun et al.	UC; *N* = 728	Infliximab	>22	Week 6	Clinical response at week 8

Maintenance

Many TDM studies are related to the maintenance phase of immunobiological therapy. A retrospective study by Perinbasekar et al. evaluating 127 patients with IBD treated with infliximab or adalimumab observed that clinical response rates at 60 days and 1 year were higher in the proactive group in comparison to the control group. The proactive group had higher rates of endoscopic response (31). Bernardo et al. retrospectively included 117 patients with IBD and found that the period to relapse was significantly longer in the drug monitoring group and there was a trend toward higher therapeutic failure in the clinical-based adjustment group (32).

A multicenter and retrospective cohort study evaluated 264 patients with IBD on infliximab maintenance therapy and found that the proactive group was associated with better clinical outcomes, such as greater drug durability, less need for IBD-related surgery or hospitalization, and a lower risk of antibodies to infliximab or serious infusion reactions. In this study, an infliximab level of 3.55 and 4.65 μg/ml were identified as the optimal cut-off values for treatment failure and IBD-related hospitalization, respectively (16). Moreover, Papamichael et al. evaluated 102 patients with IBD on infliximab maintenance therapy and compared long-term outcomes between patients who did proactive monitoring after reactive TDM with reactive testing only. This study demonstrated that the proactive group, in which more than 90% of patients had an infliximab trough concentration of >5 μg/ml, had a greater rate of treatment persistence and fewer IBD-related hospitalizations than the reactive testing group alone (10).

Another multicenter and retrospective study of 382 patients with IBD has shown that proactive TDM of adalimumab on maintenance therapy might be associated with a lower risk of treatment failure in comparison to the standard of care in patients with IBD. They found that an adalimumab serum level threshold of 11.7 μg/ml differentiates between patients with or without treatment failure (33). Also, Morita et al. have demonstrated that the cut-off value of the trough level for predicting mucosal healing was 2.7 μg/ml for infliximab and 10.3 μg/ml for adalimumab in patients with UC (34).

Recently, the aforementioned PANTS study reported that week 14 drug trough levels of 7 mg/L for infliximab and 12 mg/L for adalimumab were associated with clinical remission at both weeks 14 and 54 (3).

Therefore, both retrospective and prospective observational studies encourage the use of proactive TDM. Concerning RCT, two studies have been inconclusive, while three more recent ones indicate that proactive TDM could be associated with favorable outcomes.

The landmark TAXIT trial (the Trough Level Adapted Infliximab Treatment) did not achieve its primary endpoint, given that 69 vs. 66% of patients in the concentration vs. clinically based dosing groups achieved combined clinical and biochemical remission 1 year after optimization, respectively (p = 0.686). Even so, important secondary outcomes were observed in the proactive TDM group, such as lower frequency of undetectable drug levels, less antibody formation, and a lower chance of flares (17). Moreover, it was demonstrated that dose de-escalation did not affect disease activity and reduced drug costs by 28%.

A retrospective study from Pouillon et al. on the long-term outcomes of all 226 patients who completed the TAXIT maintenance phase reported that infliximab discontinuation happened earlier in patients treated in the clinically based dosing group than in patients treated in the proactive TDM group

during a follow-up of 41 months. In addition, concentration-based dosing was associated with longer treatment responses, low surgical rates, and corticosteroid use (35).

Another prospective, double-blind, and randomized study evaluating 122 patients with CD, the TAILORIX trial, showed that there was no difference in corticosteroid-free clinical remission between an increasing dose of infliximab based on a combination of symptoms, biomarkers, and serum drug levels and an increasing dose based on symptoms alone, starting at week 14. There were important limitations concerning the study design that could explain the unexpected results. For instance, in the control group, 60% of dose escalations based on symptoms had normal biomarkers, whereas 53% of possible dose escalations based on symptoms in the interventional arm were avoided as biomarkers were not elevated. Moreover, a minority of patients were dose escalated based on trough concentration (36).

The PAILOT trial was a prospective and randomized controlled study conducted with 78 biologic-naive children with CD who were randomly assigned into proactive vs. reactive TDM groups following response to adalimumab induction. The authors found that the proactive dose adjustment of adalimumab was associated with a higher rate of corticosteroid-free clinical remission at all visits from weeks 8 to 72 when compared with the reactive group (37).

Strik et al. conducted the PRECISION trial, enrolling 80 patients with IBD in clinical remission treated with infliximab in the maintenance phase. They were randomized into two groups; one received infliximab dosing guided by a Bayesian pharmacokinetic model, targeting the infliximab trough level of >3 μg/ml, and the other received conventional treatment. After 1 year, the study demonstrated that a higher proportion of patients from the infliximab dosing model group were in sustained clinical remission compared to the control group. In addition, the TDM group had lower median FCP levels (38).

Recently, a Norwegian multicenter trial conducted with 458 patients with immune-mediated inflammatory diseases undergoing maintenance therapy with infliximab has demonstrated that the proportion of patients with sustained disease control over 52 weeks of follow-up was significantly higher in the proactive TDM group compared with the standard therapy group. The cost-effectiveness, as well as the superiority of this strategy as compared with the reactive approach, remains to be demonstrated (39).

Concerning TDM in biologics other than the anti-TNF mechanism, there are only a few studies evaluating the exposure-response relationship, reinforcing that higher vedolizumab and ustekinumab concentrations are associated with favorable outcomes (40–43). There is an expert agreement that more data are needed to support the use of proactive TDM for biologics other than anti-TNF therapies (12). **Tables 3**, **4** summarize RCTs and observational studies regarding TDM in the maintenance phase, respectively.

Guiding treatment de-escalation

Another important role for proactive TDM is to guide treatment de-escalation of biological therapy. A prospective study by Amiot et al. reported that in patients with IBD in clinical remission, TDM-based adjustment is predictive of LOR following infliximab dose reduction. The authors concluded that therapy de-escalation of infliximab in patients in clinical remission should be guided by TDM rather than according to symptoms and CRP (44). Recently, a retrospective observational single-center study of 96 patients with IBD in remission showed that TDM-based adjustment (with infliximab trough levels of more than 7 mg/L) was associated with a decreased risk of relapse when compared to clinically based de-escalation (45).

A real-world cohort from Petitcollin et al. with 91 patients with IBD in remission showed that TDM could be beneficial for follow-up of patients after infliximab de-escalation (46). Furthermore, a prospective observational study of 87 patients with IBD suggested that a cut-off adalimumab level of 12.2 mg/ml could be appropriate in guiding dose reduction (47). The recent expert consensus statement on TDM recommended that dose de-escalation should be considered for infliximab or adalimumab trough concentrations that are consistently higher than 10–15 mg/ml (12).

Correspondingly, proactive TDM should be considered after withdrawal of immunosuppressive therapy (48, 49). A study by Drobne et al. that evaluated patients with CD using infliximab in combination with immunosuppressants observed that detectable infliximab trough level at the time of immunomodulator removal is associated with long-term response (49).

Reactive therapeutic drug monitoring

Reactive TDM should be performed in the context of active disease to elucidate the mechanism of primary or secondary loss-of-response (SLR) to immunobiological therapy. Thus, this approach helps to guide treatment decisions, such as dose optimization, combination therapy with an immunomodulator, or switch in or out of class (14, 50).

Whether reactive TDM compared to empiric care is associated with better outcomes remains controversial. However, there are intuitive benefits to using TDM to elucidate the mechanism underlying anti-TNF LOR, such as the avoidance of futile, and potentially hazardous, dose intensification in patients with high titer antidrug antibodies (50).

A retrospective observational cohort study by Kelly et al. showed that the reactive TDM approach is associated with higher post-adjustment clinical response and endoscopic remission compared to clinical decision-making alone

TABLE 3 Summary of RCTs assessing the role of TDM in IBD.

RCT	IBD type; *N*	Groups	Drug	Drug level target (μ g/mL)	Primary endpoint
Steenholdt et al. (57)	CD *N* = 69	Reactive TDM vs. standard care	Infliximab	≥0.5	Cost-effectiveness and Crohn's disease activity index response after 12 weeks
Vande Casteele et al. (17) (TAXIT)	CD and UC *N* = 263	Proactive TDM vs. clinically based	Infliximab	>3	Clinical and biochemical remission at 1 year after the optimization phase
D'Haens et al. (36) (TAILORIX)	CD *N* = 122	Dose optimization based on clinical symptoms and biomarkers and/or proactive TDM vs. clinical symptoms alone	Infliximab	>3	Sustained corticosteroid-free clinical remission from weeks 22 to 54 with mucosal healing at week 54
Assa et al. (37) (PAILOT)	Pediatric CD *N* = 78	Proactive vs. reactive TDM	Adalimumab	≥5	Sustained corticosteroid-free clinical remission from weeks 8 to 72
Strik et al. (38) (PRECISION)	CD and UC *N* = 80	Proactive TDM based on pharmacokinetic dashboard vs. standard dosing	Infliximab	>3	Sustained clinical remission after 1 year
Syversen et al. (39) (NOR-DRUM)	Rheumatoid arthritis, spondyloarthritis, psoriatic arthritis, UC, CD, and psoriasis *N* = 458	Part A – proactive TDM in induction phase vs. standard therapy Part B – proactive TDM in maintenance phase vs. standard therapy	Infliximab	>20 at the second infusion >15 at the third infusion Maintenance IFX 3–8	Part A – clinical remission at week 30 Part B – sustained disease control without disease worsening during 52 weeks

(51). Yanai et al. demonstrated that at the time of SLR, infliximab and adalimumab trough concentrations of more than 3.8 and 4.5 mg/ml, respectively, identified patients who benefited more from a switch to another mechanism than to dose escalation or switching to another antitumor necrosis factor (52).

Similarly, an interesting prospective study by Roblin et al. showed that, in patients with IBD presenting secondary LOR to adalimumab, low drug trough levels without antibodies are strongly predictive of clinical response in 67% of cases after adalimumab optimization. In addition, adalimumab trough concentrations of >4.9 μg/ml were associated with the failure of two anti-TNF agents (adalimumab and infliximab) in 90% of cases, and switching to another drug class should be cogitated (53).

Given that there are still limited treatment options for IBD, especially for certain phenotypes such as perianal fistulizing CD, the optimization of the first biologic is usually recommended as it typically results in a higher rate of efficacy when compared to subsequent biologic therapies (54, 55). Thus, the most recent expert consensus on TDM suggests that treatment discontinuation should not be considered until a trough level of at least 10–15 μg/ml is achieved for both infliximab and adalimumab therapies (12).

A recognizable unmet need when performing reactive TDM is the proper interpretation of ADA, as titers are often expressed in arbitrary units and cannot be directly compared between different assays (2). As such, to avoid the inappropriate withdrawal of a biologic due to hypothetical high-titer ADA, it is crucial to differentiate levels that can be overcome by treatment optimization (dose escalation, dose interval shortening, and/or addition of an immunomodulator) from high-titer ADA that can lead to undetectable or low drug concentrations, infusion reactions, and treatment failure (12). Although the specific cut-off identifying high-titer ADA remains uncertain for each assay, experts agree that low-titer antibodies to infliximab can be defined as 10 U/ml for the homogeneous mobility shift assay (12).

Besides guiding better therapeutic management, some studies have suggested that TDM-based dosing is less costly and more effective than empiric dose escalation in the setting of secondary LOR (56). Moreover, an RCT by Steenholdt et al. reported that reactive TDM was associated with

TABLE 4 Summary of most relevant observational proactive TDM studies in maintenance phase.

Observational studies	IBD type; *N*	Drug	Drug level target (μ g/mL)	Time point	Therapeutic outcome
Prospective					
Kennedy et al. (3) (PANTS)	CD; *N* = 1610	Infliximab	≥7.0	Week 14	Clinical remission at week 14 and 54
		Adalimumab	≥12		
Retrospective					
Perinbasekar et al. (31)	CD and UC; *N* = 127	Infliximab	≥3	At least once in maintenance	Clinical response at 60 days, clinical response at 1 year, endoscopic response and persistence with anti-TNF at 1 year
		Adalimumab	≥5		
Bernardo et al. (32)	CD and UC; *N* = 117	Infliximab	3–7 in CD; 5–10 in UC	Every 6 months	Clinical remission at week 48
		Adalimumab	5–7 in CD; 7–9 in UC		
Papamichael et al. (30)	CD and UC; *N* = 264	Infliximab	5–10	Any frequency during maintenance phase	Treatment failure (IFX discontinuation due to LOR or serious adverse event or surgery)
Papamichael et al. (18)	CD and UC; *N* = 102	Infliximab	5–10	Median of 3 (range 1–7) proactive infliximab monitoring evaluations	Treatment failure and IBD-related surgery and hospitalization
Papamichael et al. (33)	CD and UC; *N* = 382	Adalimumab	>10	At least once	Treatment failure from the start of adalimumab until the end of follow-up (3 years)

important cost savings at 12 and 20 weeks and 1 year (57, 58). Therefore, most gastroenterology societies and expert groups recommend the use of reactive TDM for both PNR and secondary LOR (6, 12, 14). **Figure 2** summarizes the approach to secondary LOR when TDM is available. **Table 5** summarizes the most relevant observational studies regarding reactive TDM.

Therapeutic drug monitoring in special situations

Following a drug holiday

In patients who have already experienced the LOR to a biologic agent, reexposure to the same drug is associated with a high risk of failure to treatment. In this specific scenario, TDM has been recognized as a promising strategy to optimize drug levels and avoid pharmacokinetic failure due to inadequate drug exposure (12, 59).

Assuming drug holiday as a delay (intentional or not) of at least 3 doses of a biological agent, an expert panel study published by Melmed et al. considers appropriate checking drug and ADA after the first reinduction dose (59). The ACG consensus also endorses proactive TDM after a long drug holiday as an approach to efficiently guide treatment decisions, and it recommends that TDM should be performed in patients restarting treatment with infliximab before the second dose. As there is no sufficient evidence, the authors made no statement regarding drug holidays with other biologic agents (12).

In a retrospective study by Baert et al. that evaluated 128 patients with IBD who restarted infliximab after a median 15-month discontinuation, the absence of antibody to infliximab before the second infusion and reinitiation therapy with concomitant immunomodulator were associated with the clinical response at weeks 10–14. This study also showed that the early detection of antibodies to infliximab (before second or third doses) after reexposure to infliximab was associated with higher rates of infusion reactions. For preventing severe infusion reactions, the authors suggest concomitant immunomodulator therapy (azathioprine/6-mercaptopurine or methotrexate) when reinitiating infliximab after a drug holiday, and it may also be reasonable not to administer subsequent doses if there is evidence of circulating ADA after the first reinduction dose (60).

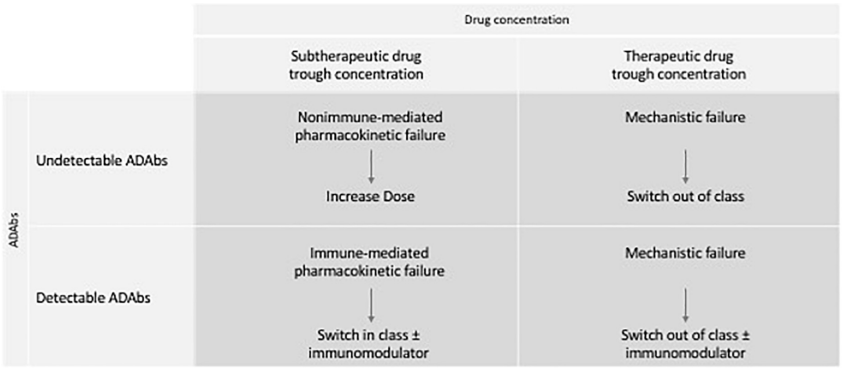

FIGURE 2
Approach of secondary loss of response of reactive TDM.

TABLE 5 Summary of most relevant reactive TDM studies.

Observational studies	IBD type; *N*	Drug	Drug level target (μ g/mL)	Time point	Therapeutic outcome
Prospective					
Guidi et al.	CD and UC; *N* = 148	Infliximab	>3	Loss of response including active endoscopic disease	Clinical outcomes 12 weeks after the therapeutic intervention
Retrospective					
Yanai et al. (52)	CD and UC	Infliximab	>3.8	Loss of response	Clinical efficacy of each intervention strategy instituted for loss of response
		Adalimumab	>4.5		
Kelly et al. (51)	CD and UC; *N* = 271	Infliximab	>4.5	Loss of response	Endoscopic remission 6 months after readjustment

Perioperative care

Despite significant improvements in the medical management of IBD, surgery is still needed in a significant subset of patients during the course of the disease (61–63). Given that most patients who undergo surgery have been previously treated with biologics (64), the proper understanding of the impact of serum drug concentrations on perioperative outcomes is paramount. However, data regarding serum concentrations of biologics in the perioperative period are still conflicting.

A retrospective Canadian study by Waterman et al. analyzed the results of 473 CD-related surgical procedures (195 in patients under previous anti-TNFs and 278 in matched controls) (65). No significant differences were observed in the length of stay, rates of urinary tract infection, pneumonia, bacteremia, readmission, reoperation, or mortality between groups. The authors also showed that detectable infliximab levels did not increase the rates of postoperative wound infection ($p = 0.21$).

A prospective study by Lau et al. evaluating 123 patients with CD undergoing abdominal surgery demonstrated that infliximab concentration above 3 μg/ml was associated with an increased rate of overall complications (OR 2.5; $p = 0.03$) and infectious complications (OR 3.0; $p = 0.03$) (66). The increase in overall complications and readmission rates was more significant in patients with drug concentrations above 8 μg/ml. Conversely, no difference was observed in postoperative morbidity in patients with UC with undetectable concentrations [31/77 (40%)] and patients with detectable infliximab concentrations [8/17 (41%)], $p = 0.61$.

The largest prospective multicenter trial assessing the risk of surgery and biologics (*The Postoperative Infection in Inflammatory Bowel Disease*—PUCCINI) was presented at Digestive Disease Week (DDW) 2019 (67). Among a total of 955 procedures (382 with the use of anti-TNFs up to 12 weeks before surgery), the rates of overall infectious complications did not differ between patients with previous exposure to anti-TNFs and controls (20 vs. 19.4%, $p = 0.801$) or detectable serum anti-TNF concentrations (19.7 vs. 19.6%, $p = 0.985$). Accordingly, no differences in the rates of surgical site infections were found in patients with exposure to anti-TNFs (12.4 vs. 11.5%, $p = 0.692$) or detectable drug concentrations (10.3 vs. 12.1%, $p = 0.513$).

There is only one study assessing the effect of preoperative vedolizumab drug concentrations on postoperative outcomes in patients with IBD undergoing major abdominal surgery (68). Among 72 patients with IBD (42 UC and 27 CD), no differences in postoperative morbidity were observed between patients with detectable (>1.6 mcg/ml) and undetectable vedolizumab

concentrations. Likewise, there is just a single report assessing the impact of preoperative ustekinumab concentrations on postoperative surgical outcomes in 36 patients with IBD (31 CD, 4 UC, and 1 IBD-unclassified). Ustekinumab concentrations were detectable (≥0.9 μg/ml) in 25 (69%) and undetectable in 11 (31%) patients (69). There were no significant differences between groups regarding overall postoperative morbidity (27 vs. 28%, $p = 0.72$), 30-day readmission rate (18 vs. 8%, $p = 0.57$), postoperative ileus (18 vs. 8%, $p = 0.57$), or wound infection (9 vs. 4%, $p = 0.52$).

Perianal fistulizing Crohn's disease

The perianal fistulizing CD comprises a disabling phenotype of IBD whose clinical course may tremendously affect patients' quality of life. Studies have demonstrated that higher serum concentrations of anti-TNF agents are associated with higher rates of fistula closure. A *post hoc* analysis of ACCENT-II showed that infliximab trough concentrations at week 14 were associated with fistula response at weeks 14 and 54 (70). Higher concentrations of infliximab at week 14 were independently associated with both fistula response and normalization of CRP at week 14 (OR: 2.32; 95% CI: 1.55–3.49; $p < 0.001$). Infliximab trough levels predictive of fistula response and CRP normalization at week 14 were ≥20.2 μg/ml at week 2, ≥15 μg/ml at week 6, and ≥7.2 μg/ml at week 14.

Early induction infliximab levels were also associated with perianal fistula response. A retrospective observational study evaluating 36 patients with perianal fistulas demonstrated that infliximab drug levels of 9.25 μg/ml at week 2 and 7.25 μg/ml at week 6 were the best predictors of cessation or significant improvement of fistula drainage (25). Moreover, a cross-sectional study that included 117 patients with CD with perianal fistula found that levels of infliximab ≥10 μg/ml were also associated with higher fistula healing rates (71).

Acute severe ulcerative colitis

Despite the introduction of salvage therapies such as cyclosporine and infliximab, management of acute severe UC remains challenging and colectomy is still required in a subset of refractory patients (72, 73). Failure to infliximab treatment has been associated with low drug exposure as a consequence of increased inflammatory burden, high drug clearance, and fecal loss (74–77).

Emerging data support that the achievement of higher drug levels during induction correlates with endoscopic remission for UC. In a *post hoc* analysis from the ACT 1 and 2 trials including 484 patients with UC, infliximab levels of ≥18.6 μg/ml at week 2 and ≥10.6 μg/ml at week 6 were associated with endoscopic remission at week 8 (23).

A recent retrospective study by Battat et al. showed that higher clearance of infliximab and, consequently, lower serum concentrations are associated with a greater chance of colectomy in 39 patients with acute severe UC. The median baseline calculated clearance of infliximab was higher in patients with colectomy at 6 months than in patients without (0.733 vs. 0.569 L/day; $p = 0.005$) (76). A clearance threshold of infliximab of 0.627 L/day identified patients who required colectomy with 80.0% sensitivity and 82.8% specificity (AUC, 0.80). In addition, the multivariable analysis identified that the baseline infliximab clearance value was the only factor associated with colectomy.

Based on the current data, emphasis should be given to studying the role of TDM in acute severe UC and choosing the optimal infliximab dosing aiming for improvements in clinical outcomes.

Conclusion

Therapeutic drug monitoring is supported by both retrospective and prospective studies, and this approach has progressively evolved as the standard of care for patients with IBD on any biologics. Although there is some conflicting data, proactive TDM is beneficial for improving outcomes for patients with IBD on anti-TNFs. Patients with a higher risk of increased clearance and immunogenicity are more likely to benefit from proactive drug monitoring. Future prospective studies assessing the role of TDM in special situations are eagerly awaited.

Author contributions

CM, KG, and NQ wrote the manuscript. All authors critically reviewed the content of the manuscript and approved the submission of the manuscript.

Conflict of interest

NQ had served as a speaker and advisory board member for Janssen, Takeda, and Abbvie.

The remaining 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.

Publisher's note

References

1. Turner D, Ricciuto A, Lewis A, D'Amico F, Dhaliwal J, Griffiths AM, et al. STRIDE-II: an Update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) Initiative of the International Organization for the Study of IBD (IOIBD): determining therapeutic goals for treat-to-target strategies in IBD. *Gastroenterology.* (2021) 160:1570–83. doi: 10.1053/j.gastro.2020.12.031

2. Papamichael K, Afif W, Drobne D, Dubinsky MC, Ferrante M, Irving PM, et al. Therapeutic drug monitoring of biologics in inflammatory bowel disease: unmet needs and future perspectives. *Lancet Gastroenterol Hepatol.* (2022) 7:171–85. doi: 10.1016/S2468-1253(21)00223-5

3. Kennedy NA, Heap GA, Green HD, Hamilton B, Bewshea C, Walker GJ, et al. Predictors of anti-TNF treatment failure in anti-TNF-naive patients with active luminal Crohn's disease: a prospective, multicentre, cohort study. *Lancet Gastroenterol Hepatol.* (2019) 1253:1–13. doi: 10.1016/S2468-1253(19)30012-3

4. Lefevre PLC, Shackelton LM, Vande Casteele N. Factors influencing drug disposition of monoclonal antibodies in inflammatory bowel disease: implications for personalized medicine. *BioDrugs.* (2019) 33:453–68. doi: 10.1007/s40259-019-00366-1

5. Nigam GB, Nayeemuddin S, Kontopantelis E, Hayee B, Limdi JK. UK National Survey of Gastroenterologists' attitudes and barriers toward therapeutic drug monitoring of anti-TNF therapy in inflammatory bowel disease. *Frontline Gastroenterol.* (2021) 12:22–9. doi: 10.1136/flgastro-2019-101372

6. Mitrev N, Vande Casteele N, Seow CH, Andrews JM, Connor SJ, Moore GT, et al. Review article: consensus statements on therapeutic drug monitoring of anti-tumour necrosis factor therapy in inflammatory bowel diseases. *Aliment Pharmacol Therapeut.* (2017) 46:1037–53.

7. Papamichael K, Cheifetz AS. Therapeutic drug monitoring in inflammatory bowel disease. *Curr Opin Gastroenterol.* (2019) 35:302–10.

8. de Almeida Martins C, Moss AC, Sobrado CW, Queiroz NSF. Practical aspects of proactive TDM for Anti-TNF agents in IBD: defining time points and thresholds to target. *Crohn's Colitis 360.* (2019) 1:1–7.

9. Papamichael K, Cheifetz AS, Melmed GY, Irving PM, Van de Casteele N, Kozuch PL, et al. Appropriate therapeutic drug monitoring of biologic agents for patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol.* (2019) 17:1655–68.e3. doi: 10.1016/j.cgh.2019.03.037.

10. Papamichael K, Vajravelu RK, Vaughn BP, Osterman MT, Cheifetz AS. Proactive infliximab monitoring following reactive testing is associated with better clinical outcomes than reactive testing alone in patients with inflammatory bowel disease. *J Crohn's Colitis.* (2018) 12:804–10. doi: 10.1093/ecco-jcc/jjy039

11. Vande Casteele N, Herfarth H, Katz J, Falck-Ytter Y, Singh S. American gastroenterological association institute technical review on the role of therapeutic drug monitoring in the management of inflammatory bowel diseases. *Gastroenterology.* (2017) 153:835–57.e6. doi: 10.1053/j.gastro.2017.07.031

12. Cheifetz AS, Abreu MT, Afif W, Cross RK, Dubinsky MC, Loftus EV, et al. A comprehensive literature review and expert consensus statement on therapeutic drug monitoring of biologics in inflammatory bowel disease. *Am J Gastroenterol.* (2021) 116:2014–25.

13. Vermeire S, Dreesen E, Papamichael K, Dubinsky MC. How, when, and for whom should we perform therapeutic drug monitoring? *Clin Gastroenterol Hepatol.* (2020) 18:1291–9. doi: 10.1016/j.cgh.2019.09.041

14. Feuerstein JD, Nguyen GC, Kupfer SS, Falck-Ytter Y, Singh S, Gerson L, et al. American gastroenterological association institute guideline on therapeutic drug monitoring in inflammatory bowel disease. *Gastroenterology.* (2017) 153:827–34. doi: 10.1053/j.gastro.2017.07.032

15. Vaughn BP, Martinez-Vazquez M, Patwardhan VR, Moss AC, Sandborn WJ, Cheifetz AS. Proactive therapeutic concentration monitoring of infliximab may improve outcomes for patients with inflammatory bowel disease: results from a pilot observational study. *Inflamm Bowel Dis.* (2014) 20:1996–2003. doi: 10.1097/MIB.0000000000000156

16. Papamichael K, Chachu KA, Vajravelu RK, Vaughn BP, Ni J, Osterman MT, et al. Improved long-term outcomes of patients with inflammatory bowel disease receiving proactive compared with reactive monitoring of serum concentrations of infliximab. *Clin Gastroenterol Hepatol.* (2017) 15:1580–8.e3. doi: 10.1016/j.cgh.2017.03.031

17. Vande Casteele N, Ferrante M, Van Assche G, Ballet V, Compernolle G, Van Steen K, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. *Gastroenterology.* (2015) 148:1320–9.e3.

18. Papamichael K, Osterman MT, Siegel CA, Melmed GY, Dubinsky MC, Colombel J-F, et al. Using proactive therapeutic drug monitoring of anti-tumor necrosis factor therapy in inflammatory bowel disease: from an old concept to a future standard of care? *Gastroenterology.* (2018) 154:1201–2. doi: 10.1053/j.gastro.2018.01.001

19. Sparrow MP, Papamichael K, Ward MG, Riviere P, Laharie D, Paul S, et al. Therapeutic drug monitoring of biologics during induction to prevent primary non-response. *J Crohn's Colitis.* (2020) 14:543–56. doi: 10.1093/ecco-jcc/jjz162

20. Bar-Yoseph H, Levhar N, Selinger L, Manor U, Yavzori M, Picard O, et al. Early drug and anti-infliximab antibody levels for prediction of primary nonresponse to infliximab therapy. *Aliment Pharmacol Ther.* (2018) 47:212–8. doi: 10.1111/apt.14410

21. Verstockt B, Moors G, Bian S, Van Stappen T, Van Assche G, Vermeire S, et al. Influence of early adalimumab serum levels on immunogenicity and long-term outcome of anti-TNF naive Crohn's disease patients: the usefulness of rapid testing. *Aliment Pharmacol Ther.* (2018) 48:731–9. doi: 10.1111/apt.14943

22. Papamichael K, Van Stappen T, Vande Casteele N, Gils A, Billiet T, Tops S, et al. Infliximab concentration thresholds during induction therapy are associated with short-term mucosal healing in patients with ulcerative colitis. *Clin Gastroenterol Hepatol.* (2016) 14:543–9. doi: 10.1016/j.cgh.2015.11.014

23. Vande Casteele N, Jeyarajah J, Jairath V, Feagan BG, Sandborn WJ. Infliximab exposure-response relationship and thresholds associated with endoscopic healing in patients with ulcerative colitis. *Clin Gastroenterol Hepatol.* (2019) 17:1814–21.e1. doi: 10.1016/j.cgh.2018.10.036

24. Chiu YL, Rubin DT, Vermeire S, Louis E, Robinson AM, Lomax KG, et al. Serum adalimumab concentration and clinical remission in patients with Crohn's disease. *Inflamm Bowel Dis.* (2013) 19:1112–22.

25. Davidov Y, Ungar B, Yoseph HB, Carter D, Haj-Natour O, Yavzori M, et al. Association of induction infliximab levels with clinical response in perianal Crohn's disease. *J Crohn's Colitis.* (2015) 11:549–55.

26. Dreesen E, Faelens R, Van Assche G, Ferrante M, Vermeire S, Gils A, et al. Optimising infliximab induction dosing for patients with ulcerative colitis. *Br J Clin Pharmacol.* (2019) 85:782–95.

27. Kobayashi T, Suzuki Y, Motoya S, Hirai F, Ogata H, Ito H, et al. First trough level of infliximab at week 2 predicts future outcomes of induction therapy in ulcerative colitis—results from a multicenter prospective randomized controlled trial and its post hoc analysis. *J Gastroenterol.* (2016) 51:241–51. doi: 10.1007/s00535-015-1102-z

28. Syversen SW, Goll GL, Jørgensen KK, Sandanger Ø, Sexton J, Olsen IC, et al. Effect of therapeutic drug monitoring vs standard therapy during infliximab induction on disease remission in patients with chronic immune-mediated inflammatory diseases: a randomized clinical trial. *JAMA.* (2021) 325:1744–54. doi: 10.1001/jama.2021.4172

29. Fasanmade AA, Adedokun OJ, Blank M, Zhou H, Davis HM. Pharmacokinetic properties of infliximab in children and adults with Crohn's Disease: a retrospective analysis of data from 2 Phase III clinical trials. *Clin Ther.* (2011) 33:946–64. doi: 10.1016/j.clinthera.2011.06.002

30. Papamichael K, Vande Casteele N, Ferrante M, Gils A, Cheifetz AS. Therapeutic drug monitoring during induction of anti-tumor necrosis factor therapy in inflammatory bowel disease: defining a therapeutic drug window. *Inflamm Bowel Dis.* (2017) 23:1510–5.

31. Perinbasekar RA, Brown SA, Syed N, Lonsako S, Cross RK. Proactive monitoring of infliximab (IFX) and Adalimumab (ADA) drug and anti-drug antibody concentration utilizing the labcorp assay in inflammatory bowel disease (IBD) patients. *Gastroenterology.* (2017) 152:S392. doi: 10.1016/S0016-5085(17)31549-4

32. Bernardo S, Fernandes S, Gonçalves AR, Baldaia C, Valente A, Moura Santos P, et al. Efficacy of therapeutic drug monitoring of anti-TNF therapy in the control of patients with inflammatory bowel disease. *J Crohn's Colitis.* (2017) 11(Suppl_1):S308–9.

33. Papamichael K, Juncadella A, Wong D, Rakowsky S, Sattler LA, Campbell JP, et al. Proactive therapeutic drug monitoring of adalimumab is associated with better long-term outcomes compared with standard of care in patients with inflammatory bowel disease. *J Crohn's Colitis.* (2019) 13:976–81. doi: 10.1093/ecco-jcc/jjz018

34. Morita Y, Bamba S, Takahashi K, Imaeda H, Nishida A, Inatomi O, et al. Prediction of clinical and endoscopic responses to anti-tumor necrosis factor-α antibodies in ulcerative colitis. *Scand J Gastroenterol.* (2016) 51:934–41. doi: 10.3109/00365521.2016.1144781

35. Pouillon L, Ferrante M, Van Assche G, Rutgeerts P, Noman M, Vande Casteele N, et al. OP006 correlation of durability of response, serum trough concentrations and outcome parameters: long-term follow-up of the trough concentration adapted

infliximab treatment (TAXIT) trial. *J Crohn's Colitis.* (2017) 11(Suppl_1):S4–4. doi: 10.1016/S0016-5085(17)30494-8

36. D'Haens G, Vermeire S, Lambrecht G, Baert F, Bossuyt P, Pariente B, et al. Increasing infliximab dose based on symptoms, biomarkers, and serum drug concentrations does not increase clinical, endoscopic, and corticosteroid-free remission in patients with active luminal Crohn's disease. *Gastroenterology.* (2018) 154:1343–51.e1.

37. Assa A, Matar M, Turner D, Broide E, Weiss B, Ledder O, et al. Proactive monitoring of adalimumab trough concentration associated with increased clinical remission in children with crohn's disease compared with reactive monitoring. *Gastroenterology.* (2019) 157:985–96.e2. doi: 10.1053/j.gastro.2019.06.003

38. Strik AS, Löwenberg M, Mould DR, Berends SE, Ponsioen CI, van den Brande JMH, et al. Efficacy of dashboard driven dosing of infliximab in inflammatory bowel disease patients; a randomized controlled trial. *Scand J Gastroenterol.* (2021) 56:145–54. doi: 10.1080/00365521.2020.1856405

39. Syversen SW, Jørgensen KK, Goll GL, Brun MK, Sandanger Ø, Bjørlykke KH, et al. Effect of therapeutic drug monitoring vs standard therapy during maintenance infliximab therapy on disease control in patients with immune-mediated inflammatory diseases: a randomized clinical trial. *JAMA.* (2021) 326:2375–84. doi: 10.1001/jama.2021.21316

40. Adedokun OJ, Xu Z, Gasink C, Jacobstein D, Szapary P, Johanns J, et al. Pharmacokinetics and exposure response relationships of ustekinumab in patients with Crohn's Disease. *Gastroenterology.* (2018) 154:1660–71. doi: 10.1053/j.gastro.2018.01.043

41. Dreesen E, Verstockt B, Bian S, de Bruyn M, Compernolle G, Tops S, et al. Evidence to support monitoring of vedolizumab trough concentrations in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol.* (2018) 16:1937–46.e8. doi: 10.1016/j.cgh.2018.04.040

42. Verstockt B, Mertens E, Dreesen E, Outtier A, Noman M, Tops S, et al. Influence of drug exposure on vedolizumab-induced endoscopic remission in anti-tumour necrosis factor [TNF] naïve and anti-TNF exposed IBD patients. *J Crohn's Colitis.* (2020) 14:332–41.

43. Battat R, Kopylov U, Bessissow T, Bitton A, Cohen A, Jain A, et al. Association between ustekinumab trough concentrations and clinical, biomarker, and endoscopic outcomes in patients with Crohn's Disease. *Clin Gastroenterol Hepatol.* (2017) 15:1427–34.e2.

44. Amiot A, Hulin A, Belhassan M, Andre C, Gagniere C, Le baleur Y, et al. Therapeutic drug monitoring is predictive of loss of response after de-escalation of infliximab therapy in patients with inflammatory bowel disease in clinical remission. *Clin Res Hepatol Gastroenterol.* (2016) 40:90–8. doi: 10.1016/j.clinre.2015.05.019

45. Lucidarme C, Petitcollin A, Brochard C, Siproudhis L, Dewitte M, Landemaine A, et al. Predictors of relapse following infliximab de-escalation in patients with inflammatory bowel disease: the value of a strategy based on therapeutic drug monitoring. *Aliment Pharmacol Ther.* (2019) 49:147–54. doi: 10.1111/apt.15046

46. Petitcollin A, Brochard C, Siproudhis L, Tron C, Verdier MC, Lemaitre F, et al. Pharmacokinetic parameters of infliximab influence the rate of relapse after de-escalation in adults with inflammatory bowel diseases. *Clin Pharmacol Ther.* (2019) 106:605–15. doi: 10.1002/cpt.1429

47. Aguas Peris M, Bosó V, Navarro B, Marqués-Miñana MR, Bastida G, Beltrán B, et al. Serum adalimumab levels predict successful remission and safe deintensification in inflammatory bowel disease patients in clinical practice. *Inflamm Bowel Dis.* (2017) 23:1454–60. doi: 10.1097/MIB.0000000000001182

48. Colombel JF, Adedokun OJ, Gasink C, Gao LL, Cornillie FJ, D'Haens GR, et al. Combination therapy with infliximab and azathioprine improves infliximab pharmacokinetic features and efficacy: a post hoc analysis. *Clin Gastroenterol Hepatol.* (2019) 17:1525–32.e1. doi: 10.1016/j.cgh.2018.09.033

49. Drobne D, Bossuyt P, Breynaert C, Cattaert T, Vande Casteele N, Compernolle G, et al. Withdrawal of immunomodulators after co-treatment does not reduce trough level of infliximab in patients with crohn's disease. *Clin Gastroenterol Hepatol.* (2015) 13:514–21.e4. doi: 10.1016/j.cgh.2014.07.027

50. Ricciuto A, Dhaliwal J, Walters TD, Griffiths AM, Church PC. Clinical outcomes with therapeutic drug monitoring in inflammatory bowel disease: a systematic review with meta-analysis. *J Crohn's Colitis.* (2018) 12:1302–15.

51. Kelly OB, O'Donnell S, Stempak JM, Steinhart AH, Silverberg MS. Therapeutic drug monitoring to guide infliximab dose adjustment is associated with better endoscopic outcomes than clinical decision making alone in active inflammatory bowel disease. *Inflamm Bowel Dis.* (2017) 23:1202–9. doi: 10.1097/MIB.0000000000001126

52. Yanai H, Lichtenstein L, Assa A, Mazor Y, Weiss B, Levine A, et al. Levels of drug and antidrug antibodies are associated with outcome of interventions after loss of response to infliximab or adalimumab. *Clin Gastroenterol Hepatol.* (2015) 13:522–30.e2. doi: 10.1016/j.cgh.2014.07.029

53. Roblin X, Rinaudo M, Del Tedesco E, Phelip JM, Genin C, Peyrin-Biroulet L, et al. Development of an algorithm incorporating pharmacokinetics of adalimumab in inflammatory bowel diseases. *Am J Gastroenterol.* (2014) 109:1250–6. doi: 10.1038/ajg.2014.146

54. Singh S, George J, Boland BS, Vande Casteele N, Sandborn WJ. Primary non-response to tumor necrosis factor antagonists is associated with inferior response to second-line biologics in patients with Inflammatory bowel diseases: a systematic review and meta-analysis. *J Crohn's Colitis.* (2018) 12:635–43. doi: 10.1093/ecco-jcc/jjy004

55. Sands BE, Feagan BG, Rutgeerts P, Colombel JF, Sandborn WJ, Sy R, et al. Effects of vedolizumab induction therapy for patients with Crohn's disease in whom tumor necrosis factor antagonist treatment failed. *Gastroenterology* (2014) 147:618–27.e3. doi: 10.1053/j.gastro.2014.05.008

56. Velayos FS, Kahn JG, Sandborn WJ, Feagan BGA. Test-based strategy is more cost effective than empiric dose escalation for patients with crohn's disease who lose responsiveness to infliximab. *Clin Gastroenterol Hepatol.* (2013) 11:654–66. doi: 10.1016/j.cgh.2012.12.035

57. Steenholdt C, Brynskov J, Thomsen OØ, Munck LK, Fallingborg J, Christensen LA, et al. Individualised therapy is more cost-effective than dose intensification in patients with Crohn's disease who lose response to anti-TNF treatment: a randomised, controlled trial. *Gut.* (2014) 63:919–27. doi: 10.1136/gutjnl-2013-305279

58. Steenholdt C, Brynskov J, Thomsen O, Munck LK, Fallingborg J, Christensen LA, et al. Individualized therapy is a long-term cost-effective method compared to dose intensification in crohn's disease patients failing infliximab. *Dig Dis Sci.* (2015) 60:2762–70. doi: 10.1007/s10620-015-3581-4

59. Melmed GY, Irving PM, Jones J, Kaplan GG, Kozuch PL, Velayos FS, et al. Appropriateness of testing for anti–tumor necrosis factor agent and antibody concentrations, and interpretation of results. *Clin Gastroenterol Hepatol.* (2016) 14:1302–9. doi: 10.1016/j.cgh.2016.05.010

60. Baert F, Drobne D, Gils A, Vande Casteele N, Hauenstein S, Singh S, et al. Early trough levels and antibodies to infliximab predict safety and success of reinitiation of infliximab therapy. *Clin Gastroenterol Hepatol.* (2014) 12:1474–81.e2. doi: 10.1016/j.cgh.2014.01.033

61. Cosnes J, Gowerrousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology.* (2011) 140:1785–94.e4. doi: 10.1053/j.gastro.2011.01.055

62. Bernell O, Lapidus A, Hellers G. Risk factors for surgery and postoperative recurrence in Crohn's disease. *Ann Surg.* (2000) 231:38–45.

63. El Ouali S, Click B, Holubar SD, Rieder F. Natural history, diagnosis and treatment approach to fibrostenosing Crohn's disease. *United Eur Gastroenterol J.* (2020) 8:263–70. doi: 10.1177/2050640620901960

64. Roda G, Chien Ng S, Kotze PG, Argollo M, Panaccione R, Spinelli A, et al. Crohn's disease. *Nat Rev Dis Prim.* (2020) 6:22. doi: 10.1038/s41572-020-0156-2

65. Waterman M, Xu W, Dinani A, Steinhart AH, Croitoru K, Nguyen GC, et al. Preoperative biological therapy and short-term outcomes of abdominal surgery in patients with inflammatory bowel disease. *Gut.* (2013) 62:387–94.

66. Lau C, Dubinsky M, Melmed G, Vasiliauskas E, Berel D, McGovern D, et al. The impact of preoperative serum anti-TNFα therapy levels on early postoperative outcomes in inflammatory bowel disease surgery. *Ann Surg.* (2015) 261:487–96.

67. Cohen BL, Fleshner P, Kane SV, Herfarth HH, Palekar N, Farraye FA, et al. 415a – anti-tumor necrosis factor therapy is not associated with post-operative infection: results from prospective cohort of ulcerative colitis and crohn's disease patients undergoing surgery to identify risk factors for postoperative infection I (Puccin). *Gastroenterology.* (2019) 156:S–80. doi: 10.1016/S0016-5085(19)36987-2

68. Parrish AB, Lopez NE, Truong A, Zaghiyan K, Melmed GY, McGovern DPB, et al. Preoperative serum vedolizumab levels do not impact postoperative outcomes in inflammatory bowel disease. *Dis Colon Rectum.* (2021) 10:1259–66. doi: 10.1097/DCR.0000000000001920

69. Kumar R, Syal G, Ha C, Melmed G. Su457 Does preoperative serum ustekinumab concentration predict thirty-day postoperative outcomes in patients with inflammatory bowel disease undergoing abdominal surgery? *Gastroenterology.* (2021) 160:S699–700.

70. Papamichael K, Vande Casteele N, Jeyarajah J, Jairath V, Osterman MT, Cheifetz AS. Higher postinduction infliximab concentrations are associated with improved clinical outcomes in fistulizing Crohn's Disease: an ACCENT-II post hoc analysis. *Am J Gastroenterol.* (2021) 116:1007–14. doi: 10.14309/ajg.0000000000001111

71. Yarur AJ, Kanagala V, Stein DJ, Czul F, Quintero MA, Agrawal D, et al. Higher infliximab trough levels are associated with perianal fistula healing in patients with Crohn's disease. *Aliment Pharmacol Ther.* (2017) 45:933–40.

72. Mak WY, Zhao M, Ng SC, Burisch J. The epidemiology of inflammatory bowel disease: east meets west. *J Gastroenterol Hepatol.* (2020) 35:380–9.

73. Holvoet T, Lobaton T, Hindryckx P. Optimal management of acute severe ulcerative colitis (Asuc): challenges and solutions. *Clin Exp Gastroenterol.* (2021) 14:71–81. doi: 10.2147/CEG.S197719

74. Ungar B, Mazor Y, Weisshof R, Yanai H, Ron Y, Goren I, et al. Induction infliximab levels among patients with acute severe ulcerative colitis compared with patients with moderately severe ulcerative colitis. *Aliment Pharmacol Ther.* (2016) 43:1293–9. doi: 10.1111/apt.13631

75. Brandse JF, Van Den Brink GR, Wildenberg ME, Van Der Kleij D, Rispens T, Jansen JM, et al. Loss of infliximab into feces is associated with lack of response to therapy in patients with severe ulcerative colitis. *Gastroenterology.* (2015) 149:350–5.e2. doi: 10.1053/j.gastro.2015.04.016

76. Battat R, Hemperly A, Truong S, Whitmire N, Boland BS, Dulai PS, et al. Baseline clearance of infliximab is associated with requirement for colectomy in patients with acute severe ulcerative colitis. *Clin Gastroenterol Hepatol.* (2021) 19:511–8.e6. doi: 10.1016/j.cgh.2020.03.072

77. Kevans D, Murthy S, Mould DR, Silverberg MS. Accelerated clearance of infliximab is associated with treatment failure in patients with corticosteroid-refractory acute ulcerative colitis. *J Crohn's Colitis.* (2018) 12:662–9. doi: 10.1093/ecco-jcc/jjy028

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The impact of biologic therapies on extra-intestinal manifestations in inflammatory bowel disease: A multicenter study

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Introduction: Patients with inflammatory bowel disease (IBD) have a high risk of developing extra-intestinal manifestations (EIMs). We aimed to assess the cumulative incidence and clinical course of EIMs in patients treated with Vedolizumab (VDZ) and non-gut selective biologic drugs.

Materials and methods: In this multicenter observational study, we enrolled 1,182 patients with IBD under biologic treatment in tertiary care centers, collecting the rate of new-onset EIMs and the clinical course of new and pre-existing EIMs since the introduction of the ongoing biologic drug (259 VDZ vs. 923 non-gut selective agents, median time 3 vs. 4 years).

Results: Among 1,182 patients with IBD (median age of 46 years; 55% men) on biologics, the overall cumulative incidence of new onset EIMs was 4.1% (49/1,182), in particular 6.6% (17/259) on VDZ vs. 3.5% (32/923) on non-gut selective biologics (p = 0.02). Among 224 patients reporting new or pre-existing EIMs, those on VDZ showed a higher rate of clinical worsening compared with non-gut selective therapies (15.5 vs. 7.3%, p = 0.08). However, both showed a similar rate of modification of the therapeutic regimen. Female gender [hazard ratio (*HR*) 2.18], a longer course of ongoing biologic therapy (*HR* 1.18), ulcerative colitis (UC) (*HR* 1.83), and VDZ therapy (*HR* 1.85) were significant risk factors for developing new EIMs.

Discussion: Our study suggests that the type of biologic treatment might affect the risk of developing EIMs, with a slightly higher risk in patients on gut-selective therapies. However, a similar clinical course is observed in the two groups.

KEYWORDS

inflammatory bowel disease, extra-intestinal manifestations (EIMs), biologic therapy, Vedolizumab, TNF inhibitors, Ustekinumab

Introduction

Both ulcerative colitis (UC) and Crohn's disease (CD) have a high risk of developing extra-intestinal manifestations (EIMs) since almost 40% of patients will develop an EIM during the course of gastrointestinal disease (1–3). EIMs may affect different organs of the musculoskeletal, skin, ocular, and hepatobiliary system, accounting for a relevant clinical problem, (4) that may occur both during clinical activity and remission phases of an intestinal disease (4, 5).

Since the high prevalence of EIMs in inflammatory bowel diseases (IBD) and their negative influence on patients' quality of life and the healthcare system, assessment of EIMs should be undertaken on a regular basis during the follow-up of these patients to ensure adequate treatment.

The main goals of IBD medical treatment are the induction and maintenance of clinical and endoscopic remission to treat symptoms, guarantee an improved quality of life, and prevent complications leading to hospital admission and surgery. Currently, medications used to treat IBD include various agents which are tailored based on treatment indication, disease extent, and severity. Since the introduction of the first biologic agents, the therapeutic scenario has deeply evolved. Biologics were initially restricted to multi-failure patients who had already experienced mesalamine, corticosteroids, or immunosuppressants, such as azathioprine, in a so-called "step-up" therapy. Actually, an early approach with biologic agents with a "top-down" therapeutic strategy has shown substantial benefit in the management of selected patients with moderate-to-severe IBD, both in terms of clinical and endoscopic outcomes (6).

Biologic drugs currently available are monoclonal antibodies that target different inflammatory pathways, such as antibodies against tumor necrosis factor-alpha (anti-TNF-α), interleukin 12/23, anti-integrins, and small molecules Janus kinase (JAK) inhibitors, such as tofacitinib. Choosing which biologic is the most appropriate for each individual patient may sometimes be challenging, especially in presence of concomitant EIMs.

Vedolizumab (VDZ), a monoclonal antibody against $\alpha_4\beta_7$ integrin, acts by preventing leucocyte migration and homing toward the gut mucosa and has proven its efficacy as induction and maintenance therapy in both UC and CD (7, 8). The specific gut-selective effect of VDZ makes its safety profile extremely favorable (9). Conversely, its action may not influence the course of EIMs (8, 10, 11). Indeed, data on the efficacy of VDZ on EIMs are scarce and often discordant (12). A recent study by Dubinsky et al. suggests that treatment with VDZ may actually increase the likelihood of developing *de novo* EIMs (13).

Therefore, we aimed to analyze the incidence of new onset EIMs and the clinical course of new and pre-existing EIMs comparing patients treated with VDZ with those on non-gut selective biologic drugs in a large cohort of patients with IBD under biologic therapy.

Materials and methods

We retrospectively collected data about all the adult patients with IBD on biologic therapy in their regular clinical follow-up at 6 tertiary referral centers in Lombardy.

Eligible patients were adults (>18 years old) with a confirmed diagnosis of IBD (CD, UC, and undetermined IBD) under treatment with any of the currently available biologic therapies for at least 2 months. All patients had a periodic and updated follow-up visit in the previous months.

Demographical and clinical data (age, gender, IBD type, ongoing and previous biologic therapies, smoking status, and the presence of EIMs before the start of the ongoing treatment) were retrieved from medical records.

According to the European Crohn's and Colitis Organization (ECCO) guidelines, (14) the main EIMs included were: rheumatologic (peripheral and axial arthropathies), mucocutaneous (stomatitis, pyoderma gangrenosum, erythema nodosum, and psoriasis), ophthalmologic (episcleritis and uveitis), hepatobiliary [primary sclerosing cholangitis (PSC)], and others (such as pancreatitis and central nervous system manifestations).

The diagnosis of EIMs was confirmed by other specialists' medical reports (rheumatologists, ophthalmologists, dermatologists, and hepatologists) and/or objective data from imaging, histology, and laboratory tests.

Data about the onset and the clinical course of EIMs were retrospectively collected and retrieved from medical records.

We included the "new onset" EIMs (intended as any EIMs occurred after the introduction of the ongoing biologic therapy) and "pre-existing" EIMs (intended as any EIMs already mentioned before the introduction of the ongoing biologic therapy).

The course was defined as improvement or worsening of EIM-related symptoms during the follow-up. In the case of clinical worsening, we assessed the need to modify the therapeutic regimen by introducing an adjunctive therapy (corticosteroids/anti-inflammatory drugs/immunomodulators) or by switching/optimizing the ongoing biologic treatment.

The primary endpoints of this study were to assess the cumulative incidence of new onset EIMs in two cohorts of patients with IBD on biologic treatment (gut selective vs. non-gut selective) in clinical follow-up since the start of each treatment and to identify any potential risk factor for developing new EIMs.

The secondary endpoint of this study was to assess the clinical course of new onset and pre-existing EIMs in these two cohorts of patients. In particular, we aimed to analyze whether VDZ was associated with a higher incidence of *de novo* EIMs or with the clinical worsening of pre-existing EIMs, needing adjunctive and/or switching therapy.

Data were inserted into a database accessible to all participating centers. This study was an observational, retrospective study, using de-identified data from medical records and the research was conducted according to the principles of the Declaration of Helsinki; therefore, it was exempted from the Institutional Review Board approval. The data underlying this article will be shared upon reasonable request to the corresponding author.

Statistical analysis

Demographic and clinical data were expressed as numbers or percentages for discrete variables and as mean and standard deviation (SD) or median and interquartile range (IQR) for continuous variables, according to their distribution.

The prevalence of EIMs was defined as the "number of persons with EIMs/overall population" at the time of the data collection.

The cumulative incidence of EIMs was defined as the "number of new onset cases/overall population" since the introduction of the ongoing biologic drug.

The gut-selective (VDZ) and non-gut selective groups were compared with the chi-square or Fisher's exact test for categorical variables and the Mann–Whitney analysis for continuous variables. A p-value of < 0.05 value was considered statistically significant.

When variables were not available for some patients, these were excluded for percentage calculation. Univariate and multivariate analyses with logistic regression were performed, and hazard ratio (*HR*) was calculated.

Statistical analyses were done using IBM SPSS Statistics (release 23; IBM corporation, United States).

Results

We retrospectively collected data on 1,182 patients with IBD (797 CD and 385 UC) in clinical follow-up on treatment with biologics. Demographic and clinical data are shown in **Table 1**.

The overall prevalence of patients with at least one EIM in our IBD cohort was 19.8% (234/1,182) (**Figure 1**), including both the pre-existing EIMs and the ones which developed after starting the last biologic treatment. They were 44% men, 65% CD, and 35% UC, with a mean age of 51 ± 14 years. Of them, about one-third (79/234) reported multiple concomitant EIMs.

Patients were under treatment with different biologic agents: 307 on intravenous anti-TNF (Infliximab), 505 on subcutaneous anti-TNF (Adalimumab, Golimumab), 111 on Ustekinumab, and 259 on Vedolizumab. The median duration of the ongoing therapy was 4 years (with a range of 1–16 years). In this follow-up period, the incidence of new onset EIMs was 4.1% (49/1,182): 33 rheumatic (5 axial, 26 peripheral, and 2 both axial and peripheral arthropathies), 14 cutaneous (10 cases of psoriasis, 2 cases of aphthous stomatitis, one case of pyoderma gangrenosum, and one case of suppurative hidradenitis), one case of idiopathic pancreatitis, and one case of autoimmune hemolytic anemia.

The overall incidence of new EIMs in patients on treatment with VDZ was statistically higher compared with patients under non-gut selective therapies (6.6 vs. 3.5%, 17/259 vs. 32/923, $p = 0.02$). Interestingly, this difference mainly depends on the highest incidence of rheumatic diseases among patients in the gut selective group (4.6 vs. 2.4%, 12/259 vs. 22/923, $p = 0.05$), while the incidence of cutaneous diseases was comparable in the two cohorts (1.9 vs. 1.1%, 5/259 vs. 10/923, $p = 0.4$).

According to the univariate analysis, older and female patients, suffering from UC, with a longer course of biologic therapy, and under treatment with gut-selective agents showed a higher risk of developing a new EIM (**Table 2**). In the multivariate analysis, only the female gender and the duration of the ongoing biologic treatment maintained statistical significance (**Table 2**).

In the whole cohort of patients, 194 patients reported at least one EIM even before the start of the ongoing treatment. Indeed, about one-third of patients (66/194, 34%) were already on therapy with an ongoing adjunctive treatment, including 35 patients on steroids (topical or systemic), 11 on methotrexate (MTX), 9 on salazopyrin (SASP), 9 on analgesics, and 2 on other therapies (hydroxychloroquine and ciclosporin). Independent of biologic agents, of 20 patients with pre-existing EIMs already on disease-modifying antirheumatic drugs (DMARDs), one patient (5%) developed an EIM flare, compared with 6.9%

TABLE 1 Demographic and clinical parameters of included patients.

	Overall	Gut-selective therapies[1]	Non-gut selective therapies[2]	*P*
N	1182	259	923	–
Age, mean ± SD	46 ± 15	52 ± 17	45 ± 14	<0.01
Male pts, *n* (%)	653 (55)	141 (54)	512 (56)	0.77
IBD, *n* (%)				
CD	797 (67)	105 (41)	692 (75)	<0.01
UC	385 (33)	154 (59)	231 (25)	
Smokers, *n* (%)*	305 (26)	56 (25)	249 (32)	0.21
Disease duration, years (mean ± SD)	14 ± 9	14 ± 9	14 ± 9	0.98
Ongoing biologic treatment duration, years (mean ± SD)	4 ± 2	3 ± 1	4 ± 3	<0.01
Previous biologic therapy, n (%)	535 (45)	162 (63)	305 (33)	<0.01

*Data not available are excluded from the calculation.
[1] Vedolizumab,
[2] Infliximab, Adalimumab, Golimumab, Ustekinumab.

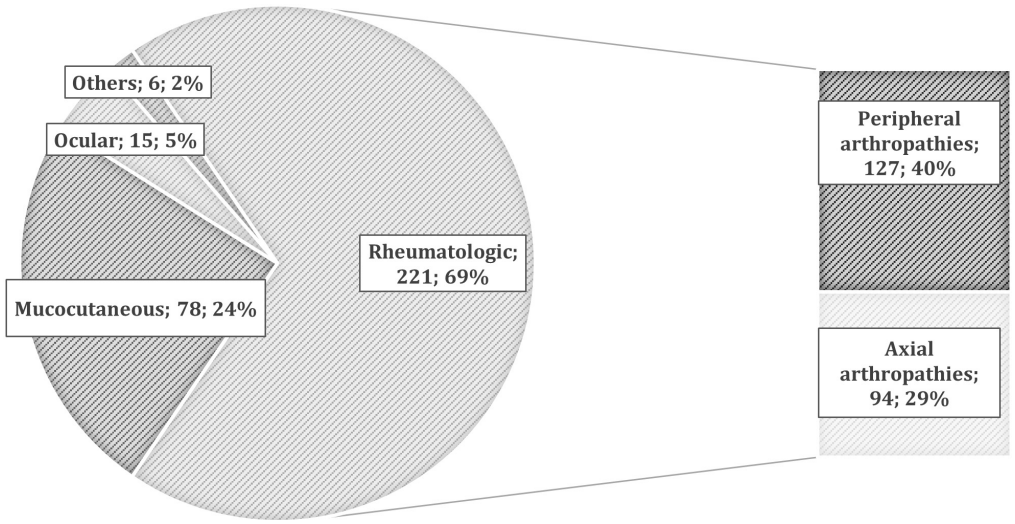

FIGURE 1
The prevalence of different extra-intestinal manifestations (EIMs) in patients with inflammatory bowel disease (IBD). According to the European Crohn's and Colitis Organization (ECCO) definitions (11), we collected 221 rheumatologic EIMs (127 peripheral and 94 axial arthropathies), 78 mucocutaneous (10 pyoderma gangrenosum, 36 erythema nodosum, 21 psoriasis, and 11 others), 15 ocular (6 episcleritis and 9 uveitis), and 6 others [such as primary sclerosing cholangitis (PSC), pancreatitis, and central nervous system manifestations].

(12/174) of patients who were not taking DMARDs (p = 0.7). Globally, we retrieved the clinical course of the 224 patients reporting a new or pre-existing EIM since the introduction of the ongoing biologic therapy. Of them, a clinical improvement was observed in about 90% of patients (204/224, partial in 52, and complete in 152), while a worsening of disease was reported in 20 patients.

Patients on VDZ showed a higher rate of clinical worsening since the introduction of the biologic agent compared with non-gut selective therapies, even if the results did not reach statistical significance (15.5 vs. 7.3%, 7/45 vs. 13/179, p = 0.08). This trend of worsening was observed mainly in the case of rheumatic EIMs (18.5 vs. 6.9%, 5/27 vs. 9/131, p = 0.05), while this association was not observed in cutaneous EIMs (7.7 vs. 6.2%, 1/13 vs. 3/48, p = 0.8). Thus, we analyzed the need for a modification of the ongoing therapeutic regimen to control the clinical worsening: over 80% of patients did not undergo any modification in both groups (87% of patients on VDZ vs. 81% on other treatments, 39/45 vs. 145/179, p = 0.1). Moreover, the rate of DMARDs addition or change/optimization of biologic therapy was comparable (4/45 vs. 25/179, p = 0.5, and 1/45 vs. 14/179, p = 0.2).

Discussion

In the current scenario of IBD treatments, multiple biologic options are available with high and comparable

TABLE 2 The univariate and multivariate analysis of clinical variables predicting the new onset of extra-intestinal manifestations (EIMs) in our cohort of patients with inflammatory bowel disease (IBD).

			Univariate analysis			Multivariate analysis		
	Cases of new EIMs	Controls	HR	95% CI	P	HR	95% CI	P
N	49	1133	–	–	–			
Age, mean ± SD	51 ± 14	46 ± 15	1.02	1.00–1.04	**0.02**	1.02	1.00–1.04	0.10
Female pts, *n* (%)	31 (63)	498 (44)	2.20	1.21–3.97	**0.009**	2.18	1.19–3.98	**0.01**
IBD type, *n* (%) UC	23 (47)	362 (32)	1.89	1.06–3.35	**0.03**	1.83	0.98–3.41	0.06
Smokers, *n* (%)*	15 (34)	290 (30)	1.2	0.64–2.29	0.60			
Disease duration, median (IQR)	15 ± 10	14 ± 9	1.01	0.98–1.05	0.58			
Type of biologic, *n* (%)VDZ	17 (35)	242 (21)	1.96	1.07–3.58	**0.03**	1.85	0.91–3.72	0.08
Duration of ongoing biologic, median (IQR)	5 ± 3	4 ± 2	1.15	1.04–1.27	**0.005**	1.18	1.07–1.31	**0.002**
Previous EIM, *n* (%)	9 (18)	185 (16)	1.15	0.55–2.41	0.71			
Previous biologic treatment, *n* (%)	19 (39)	449 (43)	0.95	0.53–1.71	0.87			

*Data not available are excluded from the calculation. Bolded values represent statistically significant <0.05 values.

levels of efficacy on IBD activity. Hence, choosing which biologic is the most appropriate for a specific patient can sometimes be a difficult task, especially in the presence of EIMs. In this regard, the available evidence on the efficacy of gut-selective therapies, such as VDZ on EIMs, is often conflicting and results only from case series, prospective or retrospective cohort studies, or the *post-hoc* analysis of randomized controlled trials (RCT) (13, 15, 16). To date, no comparative head-to-head RCTs between VDZ and non-gut selective biologics are available to define their efficacy in EIMs.

In a recent retrospective cohort study, Dubinsky et al. analyzed large databases of insurance claims and identified a 28% higher incidence of EIMs in patients with CD on VDZ compared with patients on anti-TNF agents. On the contrary, this effect was not statistically significant in patients with UC even if a higher incidence of aphthous stomatitis, pyoderma gangrenosum, and PSC was described. However, this study was limited by the use of ICD-9 and ICD-10 diagnosis codes and de-identified insurance claims data (13).

In our real-life multicenter study, based on a large cohort of patients with IBD on clinical follow-up in tertiary referral centers, we found a statistically higher incidence of new onset EIMs among patients on VDZ compared with patients on non-gut selective therapies, despite a shorter observation time. Including all available clinical variables, the univariate analysis showed a positive correlation between the risk of developing new EIMs and female sex, older age, IBD type, longer duration of current biologic treatment, and VDZ therapy. In multivariate analysis, female sex proved to be the strongest predictive factor for the onset of new EIMs (*HR* 2.18). Moreover, a slightly higher risk of developing new EIMs in patients with a longer course of the ongoing biologic therapy (*HR* 1.18), UC (*HR* 1.83), and VDZ therapy (*HR* 1.85) was observed. Instead, no correlation was observed between the risk of developing new EIMs and long duration of disease and smoking status. Moreover, neither a concomitant therapy with steroids and/or immunosuppressants nor a previous biologic treatment influences the risk of developing EIMs.

Musculoskeletal and cutaneous diseases are the most frequently observed EIMs. Regarding these types of EIMs, controversial data are available in the literature. A systematic review by Chateau et al. recently demonstrated that treatment with VDZ may have no effect on preexisting arthralgia and arthritis but it may play a role in reducing the incidence of new rheumatic manifestations compared with placebo (15). In our study, patients treated with VDZ showed a higher rate of worsening of pre-existing rheumatic EIMs, even though the trend did not reach statistical significance. Moreover, the need for adjunctive therapy (as DMARDs) or withdrawal/change of biologic agents was similar between the two treatment groups. Indeed, in most cases, the clinical worsening did not require a major modification of the maintenance therapy. Patients' symptoms were managed with on-demand analgesic or anti-inflammatory drugs in case of rheumatic manifestations or topical agents in the case of cutaneous manifestations.

These results are in line with Ramos et al., who reported that almost one-third of 201 patients under VDZ had a worsening of preexisting EIMs, and peripheral arthritis was the most affected (17). In addition, in the multicenter cohort study by the GETAID OBSERV-IBD, about 14% of patients developed arthralgia (16). On the contrary, in a *post-hoc* analysis of the GEMINI Trials, long-term treatment with VDZ was found to be associated with a reduced incidence of worsening/new arthralgia and arthritis (18). This effect could be explained, especially in the EIM linked to the activity of the disease, by the intestinal remission of the disease induced by VDZ.

Cutaneous manifestations seem to be less affected by the introduction of VDZ: in particular, in the OBSERV-IBD study, up to 75% of cutaneous EIMs were in remission after 54 weeks of VDZ (13). In addition, Ramos et al. reported stability of disease in 77% of cutaneous EIMs despite the introduction of VDZ as biologic therapy (17). Similarly, according to our study, the effect of VDZ on the incidence of new onset EIMs was observed only in rheumatic manifestations, since the incidence of cutaneous manifestations was not statistically different between the two treatment groups.

Furthermore, in the case of previous biologic treatment, the cumulative effect of multiple therapeutic lines on pre-existing EIMs was difficult to retrieve and analyze. However, in multivariate analysis, previous biologic treatment did not demonstrate an impact on the risk of developing EIMs.

To our knowledge, this is the first multicenter study that evaluated the cumulative incidence of new onset EIMs after VDZ initiation in a very large cohort of patients with IBD, all in clinical follow-up at tertiary referral IBD units. Moreover, we evaluated the clinical course of EIM in this very large cohort of patients with IBD under biological therapy.

Our study was limited by the retrospective design, making it difficult to collect data regarding the activity of intestinal inflammation at the time of EIM occurrence. Nonetheless, when analyzing the incidence of new rheumatic EIMs by the proportion of axial vs. peripheral arthropathy, the latter typically following intestinal disease activity, no difference was found between the two treatment groups. To clarify this point, future prospective and targeted studies should be performed.

Finally, the cohort included patients with IBD with different follow-ups. As expected, the time of exposure to anti-TNF was superior to VDZ as it is available for a longer time. However, it is noteworthy that, even after a shorter time of exposure, higher rates of EIM onset were observed in the gut-selective cohort compared with the non-gut-selective cohort.

In conclusion, our study suggests that the type of biologic treatment may have an impact on the risk of developing *de novo* EIMs, especially rheumatologic manifestations. Thus, in patients presenting concomitant risk factors for EIMs, if possible, therapeutic strategies other than VDZ should be taken into consideration as the first-line approach. Otherwise, in the case of VDZ treatment, it is advisable to closely monitor for the occurrence of rheumatic symptoms, which may prompt further workup and/or adjunctive therapies. Of course, the design of specific RCTs and prospective studies is advisable for offering more robust evidence in the future.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

SA, FF, MM, and GM: planning the study, drafting the article, data collection, and interpretation. AD, CB, FCa, FCo, LP, ML, MV, RC, SS, SC, and SM: data collections and critical revision of the manuscript for important intellectual content. All authors approved the final version of the manuscript including the authorship list.

Conflict of interest

CB received lecture fees from Takeda, AbbVie, and Janssen. SS received lecture fees and was an advisory board member for Takeda and Janssen. LP received Lecture fees from AbbVie, Takeda, Sandoz, and Janssen Pharmaceuticals, and served as a member of the Advisory Board of Fresenius-Kabi. FCa served as a consultant to: Mundipharma, Abbvie, MSD, Takeda, Janssen, Roche, and Celgene and received lecture fees from Abbvie, Ferring, Takeda, Allergy Therapeutics, and Janssen, and unrestricted research grants from Giuliani, Sofar, MSD, Takeda, and Abbvie. GM received lecture fees from Janssen-Cilag, Roche, Gilead, Abbvie, and Alfa-Sigma. SA served as a speaker, consultant, and/or advisory board member for the following organizations: AbbVie, MSD, Takeda, Janssen, Pfizer, Sandoz, and Enthera.

The remaining 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.

Publisher's note

References

1. Baumgart DC, Sandborn WJ. Crohn's disease. *Lancet.* (2012) 380:1590–605. doi: 10.1016/S0140-6736(12)60026-9

2. Ordás I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. *Lancet.* (2012) 380:1606–19. doi: 10.1016/S0140-6736(12)60150-0

3. Ardizzone S, Puttini PS, Cassinotti A, Porro GB. Extraintestinal manifestations of inflammatory bowel disease. *Dig Liver Dis.* (2008) 40(Suppl. 2):S253–9. doi: 10.1016/S1590-8658(08)60534-4

4. Vavricka SR, Schoepfer A, Scharl M, Lakatos PL, Navarini A, Rogler G. Extraintestinal manifestations of inflammatory bowel disease. *Inflamm Bowel Dis.* (2015) 21:1982–92. doi: 10.1097/MIB.0000000000000392

5. Ott C, Schölmerich J. Extraintestinal manifestations and complications in IBD. *Nat Rev Gastroenterol Hepatol.* (2013) 10:585–95. doi: 10.1038/nrgastro.2013.117

6. Berg DR, Colombel JF, Ungaro R. The role of early biologic therapy in inflammatory bowel disease. *Inflamm Bowel Dis.* (2019) 25:1896–905. doi: 10.1093/ibd/izz059

7. Feagan BG, Sandborn WJ, Gasink C, Jacobstein D, Lang Y, Friedman JR, et al. Ustekinumab as induction and maintenance therapy for Crohn's Disease. *N Engl J Med.* (2016) 375:1946–60. doi: 10.1056/NEJMoa1602773

8. Sandborn WJ, Feagan BG, Rutgeerts P, Hanauer S, Colombel JF, Sands BE, et al. Vedolizumab as induction and maintenance therapy for Crohn's disease. *N Engl J Med.* (2013) 369:711–21. doi: 10.1056/NEJMoa1215739

9. Loftus EV Jr., Feagan BG, Panaccione R, Colombel JF, Sandborn WJ, Sands BE, et al. Long-term safety of vedolizumab for inflammatory bowel disease. *Aliment Pharmacol Ther.* (2020) 52:1353–65. doi: 10.1111/apt.16060

10. Feagan BG, Rutgeerts P, Sands BE, Hanauer S, Colombel JF, Sandborn WJ, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* (2013) 369:699–710. doi: 10.1056/NEJMoa1215734

11. Wyant T, Leach T, Sankoh S, Wang Y, Paolino J, Pasetti MF, et al. Vedolizumab affects antibody responses to immunisation selectively in the gastrointestinal tract: randomised controlled trial results. *Gut.* (2015) 64:77–83. doi: 10.1136/gutjnl-2014-307127

12. Hanzel J, Ma C, Casteele NV, Khanna R, Jairath V, Feagan BG. Vedolizumab and extraintestinal manifestations in inflammatory bowel disease. *Drugs.* (2021) 81:333–47. doi: 10.1007/s40265-020-01460-3

13. Dubinsky MC, Cross RK, Sandborn WJ, Long M, Song X, Shi N, et al. Extraintestinal manifestations in vedolizumab and anti-TNF-treated patients with inflammatory bowel disease. *Inflamm Bowel Dis.* (2018) 24:1876–82. doi: 10.1093/ibd/izy065

14. Harbord M, Annese V, Vavricka SR, Allez M, Barreiro-de Acosta M, Boberg KM, et al. The first European evidence-based consensus on extra-intestinal manifestations in inflammatory bowel disease. *J Crohns Colitis.* (2016) 10:239–54. doi: 10.1093/ecco-jcc/jjv213

15. Chateau T, Bonovas S, Le Berre C, Mathieu N, Danese S, Peyrin-Biroulet L. Vedolizumab treatment in extra-intestinal manifestations in inflammatory bowel disease: a systematic review. *J Crohns Colitis.* (2019) 13:1569–77. doi: 10.1093/ecco-jcc/jjz095

16. Tadbiri S, Peyrin-Biroulet L, Serrero M, Filippi J, Pariente B, Roblin X, et al. Impact of vedolizumab therapy on extra-intestinal manifestations in patients with inflammatory bowel disease: a multicentre cohort study nested in the OBSERV-IBD cohort. *Aliment Pharmacol Ther.* (2018) 47:485–93. doi: 10.1111/apt.14419

17. Ramos GP, Dimopoulos C, McDonald NM, Janssens LP, Hung KW, Proctor D, et al. The impact of vedolizumab on pre-existing extraintestinal manifestations of inflammatory bowel disease: a multicenter study. *Inflamm Bowel Dis.* (2021) 27:1270–6. doi: 10.1093/ibd/izaa293

18. Feagan BG, Sandborn WJ, Colombel JF, Byrne SO, Khalid JM, Kempf C, et al. Incidence of arthritis/arthralgia in inflammatory bowel disease with long-term vedolizumab treatment: post hoc analyses of the GEMINI trials. *J Crohns Colitis.* (2019) 13:50–7. doi: 10.1093/ecco-jcc/jjy125

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Untargeted serum metabolomics reveals specific metabolite abnormalities in patients with Crohn's disease

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Crohn's disease (CD) is a subtype of inflammatory bowel disease (IBD) characterized by skip intestinal lesions that can occur in any part of the gastrointestinal tract. Currently, the diagnosis of CD is based on clinical history, physical examination and complementary diagnostic tests. It is challenging for physicians to make a definitive diagnosis. This study aimed to analyze the variation in metabolites in CD serum and identify potential predictive biomarkers of CD diagnosis. We collected serum samples from 316 subjects, including patients with CD and healthy controls (HCs). Serum metabolomics was conducted using liquid chromatography coupled to mass spectrometry. Potential biomarkers were screened and evaluated by univariate and multivariate analyses. A panel of two metabolites (deoxycholic acid and palmitic amide) was identified as a specific biomarker of CD. Receiver operating characteristic analysis (ROC) showed that the panel had a sensitivity of 80.25% with a specificity of 95.54% in discriminating CD patients from healthy controls. The biomarkers identified are increased in CD compared with healthy controls. Our approach successfully identified serum biomarkers associated with CD patients. The potential biomarkers indicated that CD metabolic disturbance might be associated with bile acid biosynthesis, fatty acids and energy metabolism.

KEYWORDS

serum metabolomics, Crohn's disease, biomarker, LC-MS, metabolite profile

Introduction

Crohn's disease (CD), a subtype of inflammatory bowel disease (IBD), is characterized by skip intestinal lesions that can occur in any part of the gastrointestinal tract (1). CD usually typically presents with chronic, relapsing, progressive and destructive transmural inflammation. Patients with CD usually present with chronic abdominal pain, diarrhea, obstruction and/or perianal lesions (1).

The precise etiology and pathogenesis of CD remain poorly understood. The current understanding mainly involves environmental factors in a genetically susceptible host, genetic factors, a defective host mucosal immune system, and gut microbial dysbiosis (2). Mucosal inflammation is a consequence of a multifaceted interaction. Currently, the diagnosis of CD is mainly based on clinical history, physical examination and complementary diagnostic tests, including assays for serological and fecal biomarkers, cross-sectional and endoscopic imaging, and histological evaluation of biopsy specimens (3, 4). In addition, quantitative magnetic resonance imaging (MRI) biomarkers combined with magnetic resonance enterography (MRE) qualitative assessment has also been applied to the diagnosis of CD (5). However, there are potential drawback in the addition of quantitative sequences to MRE examinations including increased scan time and the need for further validation before use in therapeutic drug trials and clinical trials. Thus, it is challenging for physicians to make a definitive diagnosis. Identification of biomarkers to discriminate CD patients from healthy individuals and other IBDs patients is highly desirable.

Metabolomics, which refers to an analytical study with high-throughput profiling of metabolites with the size of <1,500 Da including biofluids, cells and tissues, can revealed high-abundance molecules in various states such as disease and treatment states (6). Therefore, metabolomics has been widely used for the early screening of metabolic biomarkers in numerous diseases as well as providing new insights into the pathophysiology of diseases (7–9). Currently, there are several approaches that are applied to metabolomics, such as nuclear magnetic resonance (NMR) spectroscopy (10), quantitative NMR (11) gas chromatography-mass spectrometry (GC-MS) (12), liquid chromatography-mass spectrometry (LC-MS) (13), and capillary electrophoresis mass spectrometry (CE-MS) (14). In the last few years, the metabolomic approach has been used to identify metabolites in breath (15), fecal (16), serum (17), and urine (18) samples to discriminate inflammatory disease patients from healthy volunteers. However, there are few reports on CD serum metabolomics to distinguish patients with CD from healthy individuals. Therefore, there is a need to perform more studies on patients with CD.

The primary aim of this study was to identify serum metabolite profiles that could be used to differentiate CD patients from healthy controls (HCs) and to identify predictive potential biomarkers. We also aimed to investigate whether metabolomics could provide new insight into the complex pathophysiology of CD.

Materials and methods

Participants

This cross-sectional study examined adult CD patients and HCs. One hundred and eight CD patients were Asian inpatients from the Affiliated Hospital of Nanjing University of Traditional Chinese Medicine from September 2017 to April 2019. At the same time, 158 healthy volunteers came from the physical examination center of the Affiliated Hospital of Nanjing University of Chinese Medicine as a healthy control group. The diagnosis of CD was confirmed by previously established clinical, radiological and endoscopic criteria as well as histological findings (2). Disease activity was assessed using the simplified CD activity index (CDAI). Active disease was defined as a simplified CDAI of >4 for CD (19). The patients with other comorbidities that might affect metabolic characteristics, such as diabetes and cardiovascular diseases were excluded from the study. Patients who are taking oral hormones or other therapeutic drugs such as immunosuppressant's will also be excluded. A group of healthy adult volunteers ($n = 158$) matched for age, gender, and ethnicity served as controls. All participants gave informed consent, and the study was approved by the Institutional Review Board and the Ethics Committee of the First Affiliated Hospital of Nanjing University of Traditional Chinese Medicine (approval number, 2015NL-126-03) and complied with the principles of the Declaration of Helsinki.

Sample preparation

The serum samples were stored at −80°C until analysis. Each serum sample had a volume of 45 μL. After centrifugation for 30 s, 135 μL of acetonitrile was added to precipitate the protein. The samples were vortexed for several seconds and then rested for 3 h. Then, the samples were centrifuged for 10 min at 13,000 rcf at 4°C, and 153 μL of supernatant was dried in a Speed Vac sample concentrator at 45°C for 2 h and re-dissolved in 120 μL of 50% acetonitrile solution. Eighty microliters were placed in the injection vial after centrifugation. At the same time, 10 μL of each serum sample was combined to form a quality control (QC) sample and was processed with the same procedure used for the experimental samples. During analysis of the samples, one quality control (QC) sample was run after every 10 injections.

Metabolomic assays

The serum samples were assayed using an Agilent Technologies 1290 infinity liquid chromatograph coupled with an AB Sciex 4600 TripleTOF (AB Sciex, Framingham, MA, USA). For the detection of metabolites, 3 μL aliquots of sample solution maintained at 4°C in an autosampler were injected onto a reversed-phase ACQUITY UPLC HSS T3 C_{18} column (100 ×2.1 mm, 1.8 μm) maintained at 40°C. Mobile phase A was 1 ‰ formic acid in water, and mobile phase B was acetonitrile. The flow rate was 0.4 mL/min, and the gradient elution program was as follows: 0.5 min, 3% B; 0.5–1.5 min, 20% B; 1.5–6 min,

TABLE 1 Clinical characteristics of the subjects.

Characteristic	Discovery set ($n = 221$)		Validation set ($n = 95$)	
	CD	HC	CD	HC
Number	106	115	52	43
Male	75	81	36	30
Female	31	34	16	13
Age (year)	28.6 ± 9.6	35.6 ± 10.8	26.3 ± 8.4	35.8 ± 9.7
CRP (mg/L)	12.1 ± 20.8	—	8.1 ± 12.5	—
ESR (mm/h)	19.3 ± 19.3	—	16.6 ± 16.4	—
PLT (10^9/L)	253.4±79.8	—	233.8 ± 103.3	—
WBC (10^9/L)	6.1 ± 2.7	—	6.6 ± 3.6	—
HGB(g/L)	128.1 ± 21.7	—	126 ± 32	—
GWDB(μg/mL)	180.1 ± 347.3	—	249.9 ± 372.2	—
ALB(g/L)	36.1 ± 8.9	—	34 ± 12.8	—
UA(μmol/L)	295.1 ± 111.2	—	321.3 ± 139.8	—

Results are expressed as the means ± standard deviation.
CRP, C-reactive protein; ESR, erythrocyte sedimentation rate;PLT, platelet; WBC, white blood cell; HGB, hemoglobin; GWDB, Fecal Calprotectin; ALB, Albumin; UA, Uric acid.

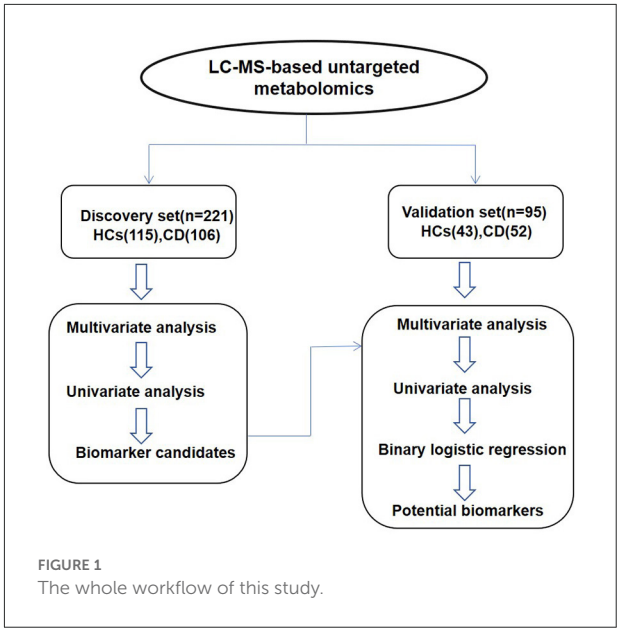

FIGURE 1
The whole workflow of this study.

60% B; 6–9 min, 95% B; 9–12 min, 95% B; 12–12.1 min, 3% B; and 12.1–16 min, 3% B.

All MS experiments were performed in positive and negative ion modes using a heated electrospray ionization (ESI) source. The mass spectrum parameters were as follows: mass range for time-of-flight mass spectrometry (TOF-MS), 60–1,000 m/z; source temperature, 550°C; atomization gas pressure, 50 psi; auxiliary gas pressure, 50 psi; curtain gas pressure, 35 psi; ion scanning voltage, 5,500 V (positive ion mode); and mass range for MS/MS, 40–1,000 m/z.

Data processing and statistical analysis

After obtaining the UPLC-MS chromatograms, the original data were derived by Analyst® TF 1.7 Software (AB Sciex, USA), the abnormal peaks were removed by PeakView (v.2.0, AB SCIEX), and the peaks were aligned by Markview (version 1.2.1AB Sciex). Finally, a three-dimensional data table including the mass-to-charge ratio, retention time and peak area was obtained, and all peaks were corrected by QC samples.

In this study, there were two types of variables, continuous and categorical variables, which are presented as the mean ± SD and number (%), respectively. For continuous variables, Shapiro-Wilk tests were used to test the normality of the distribution. Student's *t*-test and Mann-Whitney U-test were used for normally and non-normally distributed data, respectively. For categorical variables, chi-square tests were applied. The correlation between the levels of metabolites and the severity of CD was performed using Spearman's rank correlation (Rs). These analyses were performed using SPSS 20.0 software (IBM, Armonk, NY, USA), and $P < 0.05$ was considered statistically significant.

For metabolomic analysis, we reduced the resulting matrix by replacing all the missing values with a small value. The data were normalized using logarithmic transformation and Pareto scaling in MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/). Identified metabolites were subjected to further statistical analysis by univariate and multivariate statistical methods. For univariate statistical analysis, a non-parametric test was applied to measure the significance of each metabolite. The *P*-values for each metabolite in all comparisons were corrected by MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/), in which the threshold was set as 0.05. Correction for multiple comparisons was performed by testing the false discovery rate, and the Q value was reported. Multivariate statistical analysis involving principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) was performed using SIMCA version 14.0 (Umetrics, Umea, Sweden). In the OPLS-DA model, the goodness of fit and predictive capacity was evaluated by the values of R^2 and Q^2. The model is deemed stable and reliable if the values are close to 1. Moreover, a permutation test was performed to assess the goodness of fit of the OPLS-DA model. The model was considered valid if all Q^2 and R^2 values to the left were lower than the original points to the right. The variable importance in the projection (VIP) value, which was calculated in the OPLS-DA model, indicated the contribution

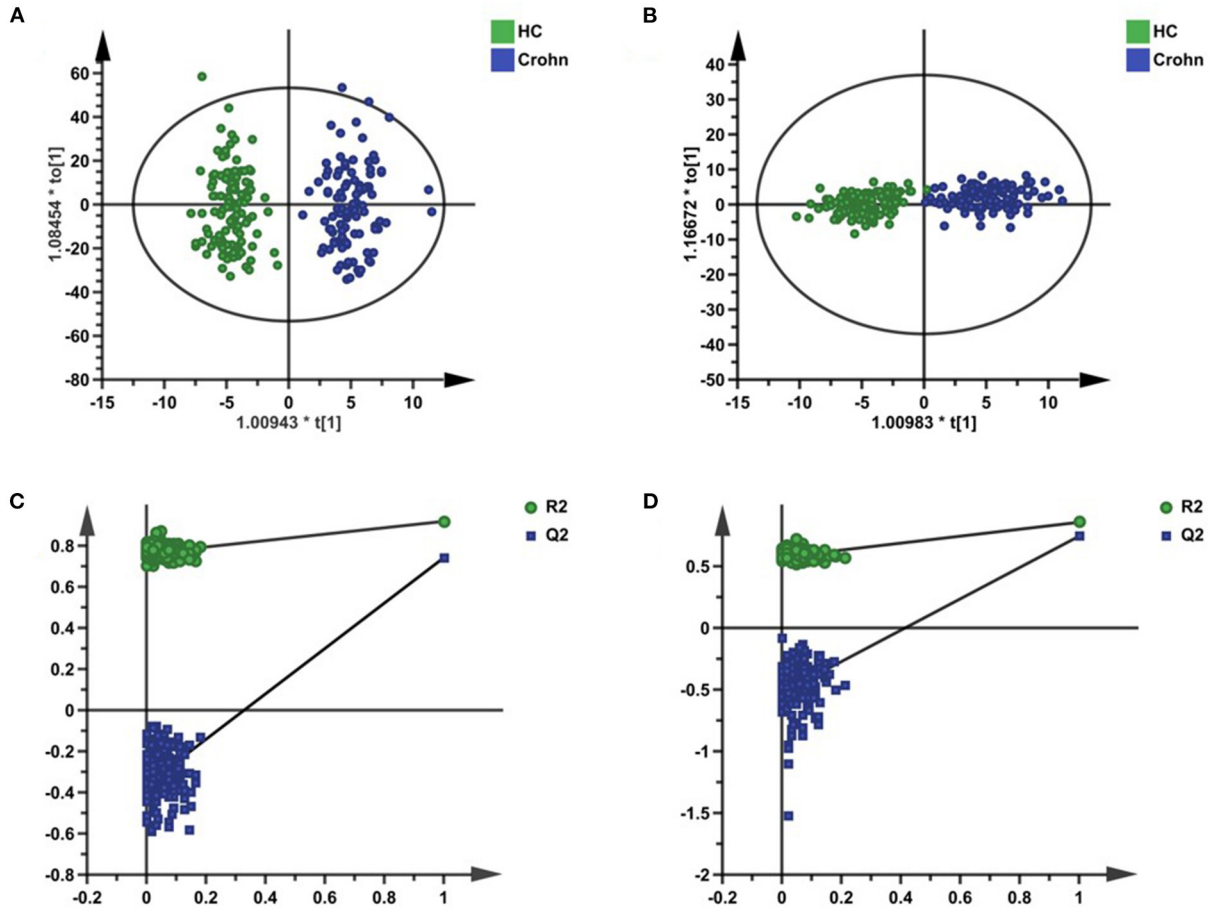

FIGURE 2
Multivariate statistical analysis of serum metabolites in the discovery set. **(A,B)** The OPLS-DA scatter plots were based on the serum metabolic profiles of CD patients and HCs in positive ion mode and negative ion mode. **(C,D)** The 200-time permutation plots of two OPLS-DA models.

of each feature to the regression model. The higher the VIP scores were, the greater the contribution was. Metabolites with VIP scores above 1 and false discovery rates (Q values) <0.05 were selected as metabolite candidates that were the major contributors to discrimination between the two groups of participants. Through multivariate and univariable analysis, the metabolites are searched in the database including the Human Metabolome Database (http://www.hmdb.cn), METLIN (http://metlin.scripps.edu) and KEGG (http://www.kegg.com).

To explore the best combination of significantly altered metabolites, a binary logistic regression (BLR) model was built on the basis of the binary outcome of patients with CD and HCs as dependent variables. The forward stepwise regression method and the Wald test were used to select altered metabolites and assess significance in the BLR prediction model, respectively. Moreover, the area under the receiver operating characteristic (ROC) curve (AUC) as well as sensitivity and specificity values were calculated to identify the performance of logistical regression models. This method was used to discover the most important metabolites until there were no more significant predictors from the data in SPSS 20.0 software (IBM, Armonk, NY, USA).

Results

Basic characteristics of the participants

In this study, 221 subjects (106 with CD and 115 HCs) were allocated to the discovery set to evaluate biomarkers, and 95 subjects (52 with CD and 43 HCs) were allocated to the validation set to test candidate biomarkers. The clinical characteristics of the subjects are listed in Table 1. We recorded age, gender information of all volunteers, and recorded multiple clinical test indicators of patients with CD, including: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), platelet (PLT), white blood cell (WBC), hemoglobin (HGB), fecal calprotectin, albumin (ALB) and uric acid (UA) It can be

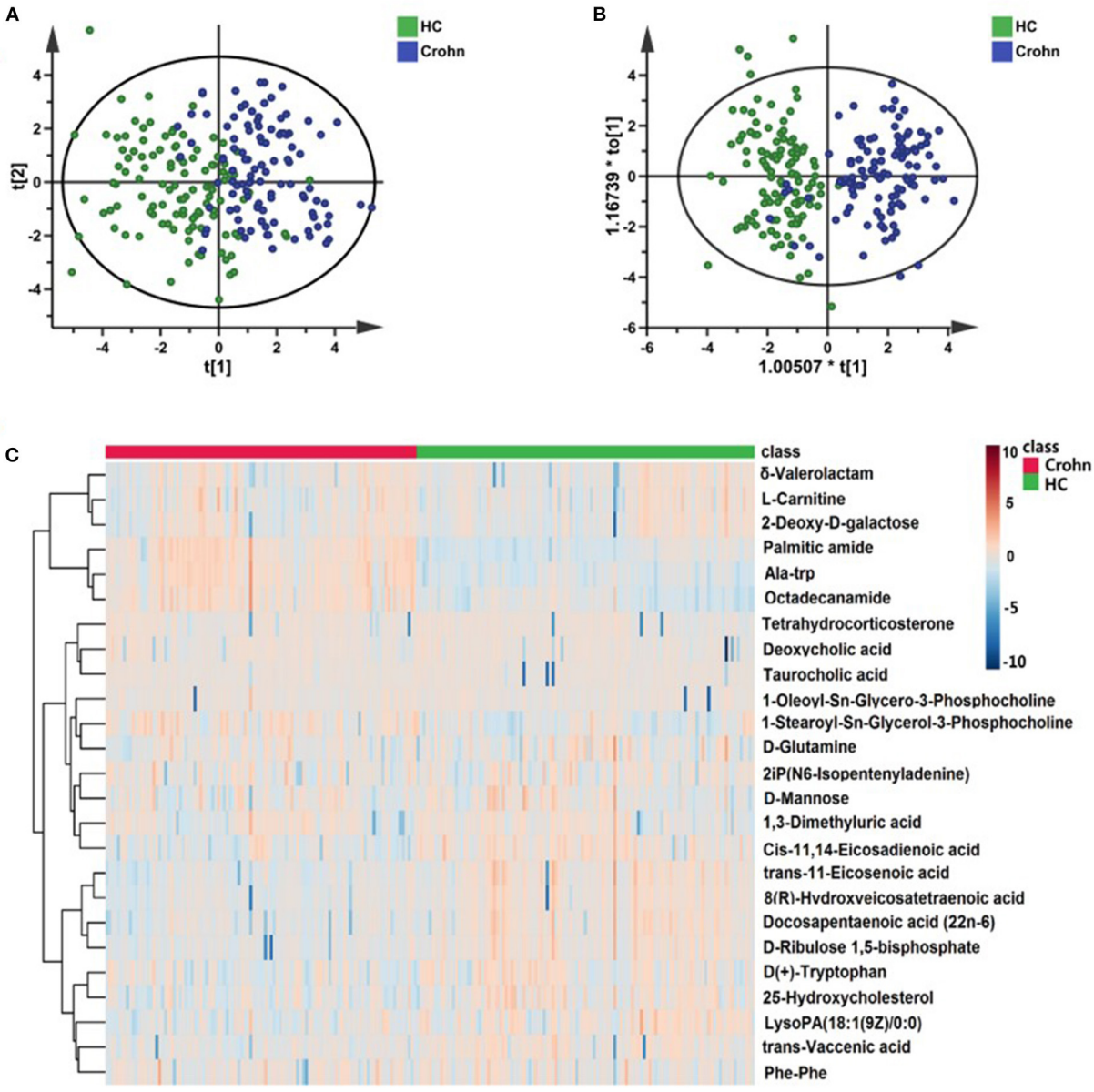

FIGURE 3
The performance of 25 differential metabolites for classification in the discovery set. **(A)** Heatmap of cluster analysis of each metabolite. **(B)** The scatter plot of PCA analysis was based on 25 metabolites. **(C)** The OPLS-DA scatter plot of 25 metabolites.

seen that these representative indicators of CD patients are abnormally elevated.

Identification of serum differential metabolites

The whole workflow of this study is shown in Figure 1. A total of 1,690 features in positive mode and 1,021 features in negative mode were detected using UPLC-TOF-MS. After data processing, we conducted multivariate statistical analysis using SIMCA. The PCA could outline the original distribution of metabolites, but the scatter plot failed to show a clear distribution in both modes. As show in Figures 2A,B, the OPLS-DA scatter plot could be divided into two clusters, which indicated the differentiation between the groups. The models presented satisfactory fit ($R^2 = 0.917$, $Q^2 = 0.741$ in positive ion mode; $R^2 = 0.856$, $Q^2 = 0.743$ in negative ion mode). Furthermore, the 200-permutation test indicated all Q^2 and R^2 values to the left were lower than the original

TABLE 2 Differentially altered metabolites identified between patients with CD and HCs.

Metabolite	Discovery set				Validation set			
	P-value	FDR	VIP	FC	*P*-value	FDR	VIP	FC
Deoxycholic acid	<0.001	<0.001	4.257	6.733	<0.001	<0.001	3.043	6.836
Docosapentaenoic acid (22n-6)	<0.001	<0.001	1.474	1.264	<0.001	<0.001	1.624	1.428
Octadecanamide	<0.001	<0.001	3.698	2.967	<0.001	<0.001	1.695	4.549
Palmitic amide	<0.001	<0.001	4.171	4.202	<0.001	<0.001	2.184	5.678
Tetrahydrocorticosterone	<0.001	<0.001	1.746	0.669	<0.001	<0.001	1.370	0.631

Five metabolites which were verified in the validation cohort. VIP was obtained from OPLS-DA model with a threshold of 1.0. P-values from one-way ANOVA. Value of FDR was obtained from the adjusted P-value calculated using MetaboAnalyst 5.0 software. FC was obtained by comparing those metabolites in patients with CD with the healthy controls; FC with a value >1 indicated a relatively higher intensity presenting in patients with CD, whereas a value <1 indicated a relatively lower intensity compared with the healthy controls.

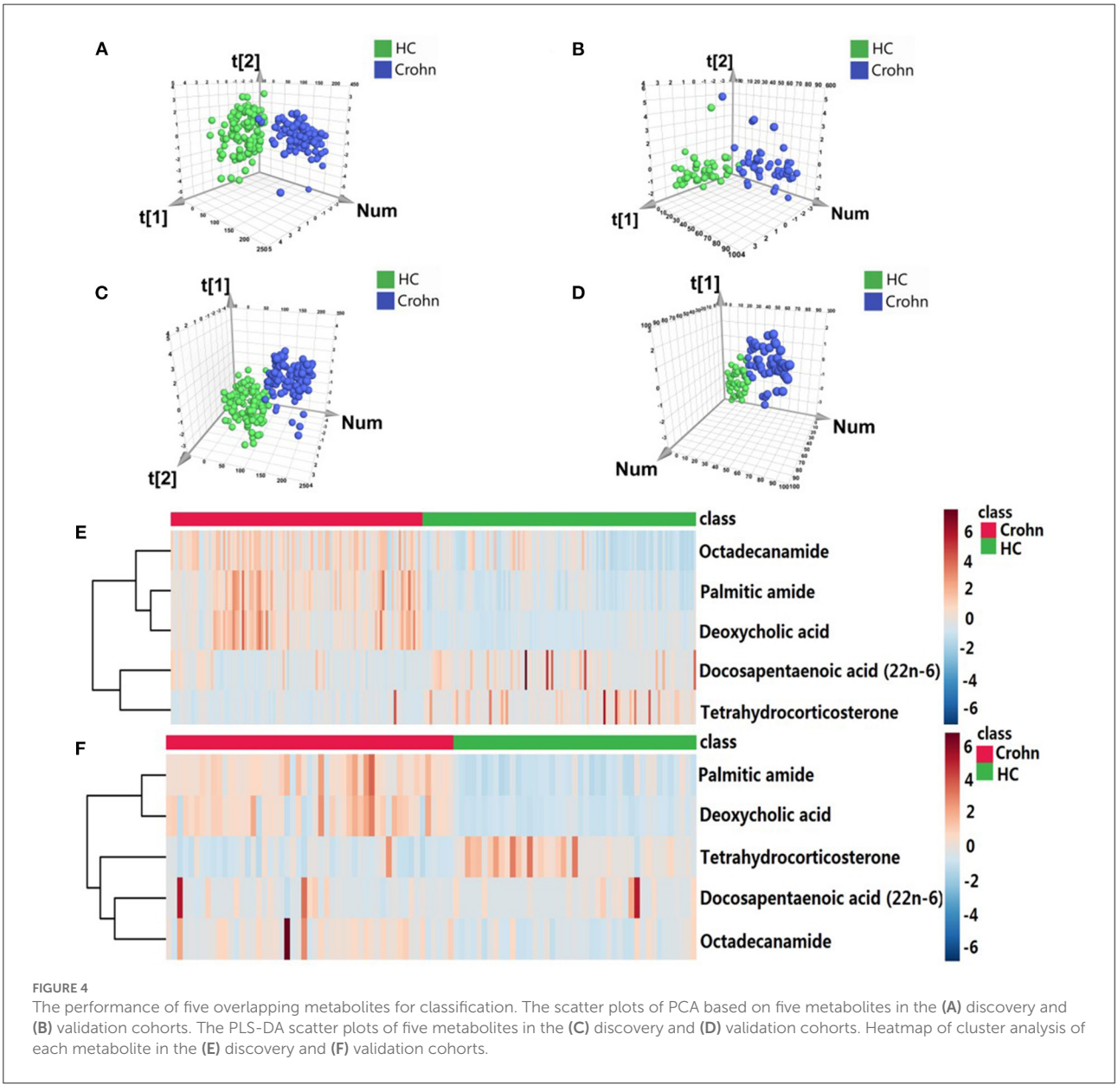

FIGURE 4
The performance of five overlapping metabolites for classification. The scatter plots of PCA based on five metabolites in the **(A)** discovery and **(B)** validation cohorts. The PLS-DA scatter plots of five metabolites in the **(C)** discovery and **(D)** validation cohorts. Heatmap of cluster analysis of each metabolite in the **(E)** discovery and **(F)** validation cohorts.

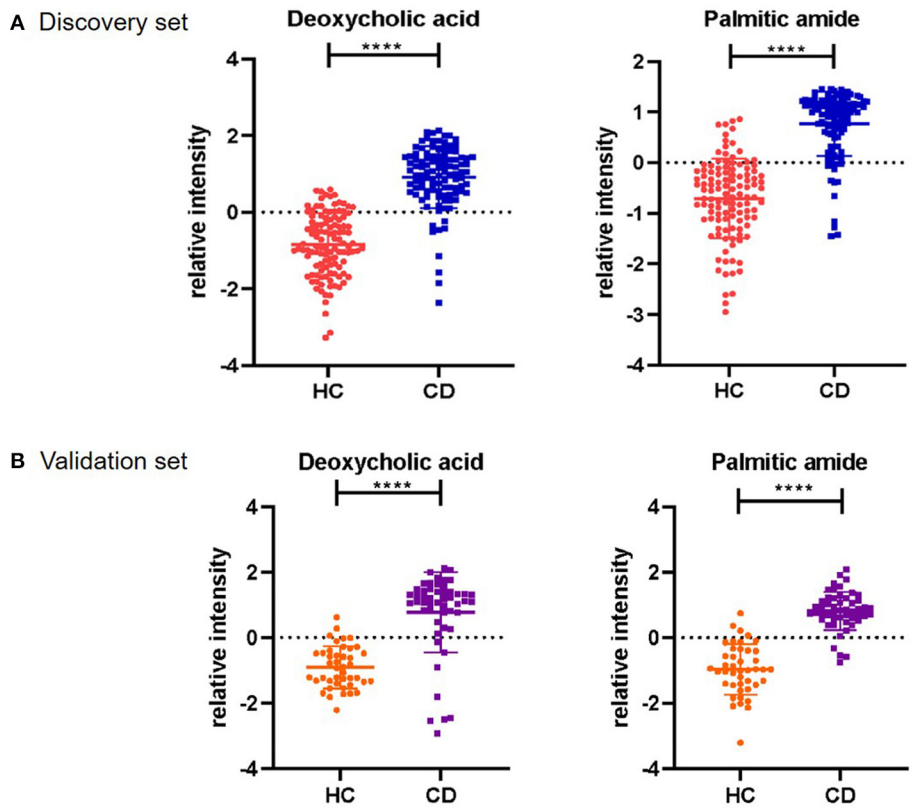

FIGURE 5
Serum relative intensity of the defined potential biomarkers. Discrimination of CD patients and healthy individuals with the combination of two potential biomarkers. Serum relative intensities of Deoxycholic acid and Palmitic amide in the discovery set **(A)** and validation set **(B)**. Statistical differences are marked by an asterisk, ****$P < 0.0001$.

points to the right which results validated the OPLS-DA models (Figures 2C,D). Combined with univariate Wilcoxon rank-sum test (adjusted $P < 0.05$), we screened 25 differential metabolites (Supplementary Table S1). In addition, the 25 differential metabolites were subjected to PCA, OPLS-DA and cluster analysis. As show in Figure 3, both in the PCA, OPLS-DA, the samples were clearly separated between the two groups. Heatmap shows that these 25 metabolites are significantly different. Therefore, the 25 differential metabolites could well-distinguish CD patients from HCs.

Validation of three potential biomarkers with a test set

An independent test cohort of 95 individuals (Figure 1) was used to evaluate the reliability of 25 biomarker candidates and confirm the application potential of the biomarkers. The OPLS-DA scatter plots (Supplementary Figures S1A,B) show the separation between the two groups, with good fitness ($R^2 = 0.921$, $Q^2 = 0.641$ in positive ion mode; $R^2 = 0.908$, $Q^2 = 0.490$ in negative ion mode), and both the models are validated (Supplementary Figures S1C,D). Five metabolites were verified in the validation cohort (Table 2). The table shows the *P*-value, FDR value, VIP value, and FC value of these five metabolites in the discovery set and validation set, respectively. These metabolites satisfied the following criteria: (1) VIP scores above 1 and false discovery rates (*Q*-values) below 0.05 and (2) maintaining the same change trend as the discovery set.

To construct the optimal diagnostic model, firstly, we detected the classification performance of the five metabolites. As shown in Figure 4, PCA, OPLS-DA and cluster analysis indicated that the five metabolites could separate CD patients from HCs. Additionally, we conducted BLR to optimize the model further. Through forward BLR analysis, two of the five metabolites remained in the logistics regression model: deoxycholic acid (DCA) and palmitic amide. Both the DCA and palmitic amide levels were significantly increased in patients with CD (Figure 5). The ROC values of the two metabolites and their combination are presented in Figure 6. For DCA, palmitic amide and their combination showed AUCs of 0.914, 0.921, and 0.948, sensitivities of 87.26, 84.08, and 80.25%, and specificities of 94.27, 89.17, and 95.54%, respectively.

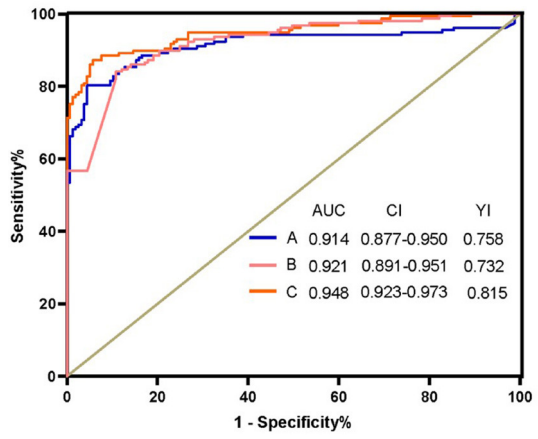

FIGURE 6
Receiver operating characteristic curve analysis (ROC) of Deoxycholic acid, Palmitic amide and their combination. (A) ROC of Deoxycholic acid (B) ROC of Palmitic amide. (C) ROC of the combination Deoxycholic acid, Palmitic amide. AUC, area under the curve; CI, confidence interval; YI, Youden index.

Next, we tried to diagnose the disease activity of CD using DCA and palmitic amide. According to the latest CD staging standard, scores of <4, 5–8 and >9 are considered mild (or remission), moderate and severe disease activity, respectively. The results showed that there were significant differences in the levels of the two metabolites in different disease status of CD. As shown in Figures 7A,B, the combination of DCA and palmitic amide distinguishes HCs from remission CD and active CD, with a coincidence rate of 89.49 and 94.88%, and cut-off value of 0.798 and 0.879, respectively. The results indicate that the two differentially expressed metabolites could separate CD patients from HCs with high sensitivity, specificity and diagnostic performance. Finally, we confirmed the combination of DCA and palmitic amide as the ideal biomarker panel to distinguish patients with CD from HCs.

Discussion

In this study, we demonstrated that there were significant differences in serum metabolic profiles between CD patients and HCs. We also confirmed the ideal biomarker panel to distinguish patients with CD from subjects without CD. The strengths of this paper are the relatively high number of cases and controls and the use of independent discovery and validation sets in building a discriminatory model. To the best of our knowledge, this is the first report on screening a specific biomarker panel to distinguish CD patients from HCs using untargeted serum metabolomics.

DCA is a secondary bile acid produced in the liver and is usually conjugated with glycine or taurine. This acid facilitates fat absorption and cholesterol excretion. DCA independently induces NLRP3 inflammasome activation and high proinflammatory cytokine-IL-1β production in macrophages (20). DCA triggers the activation of NLRP3 inflammasome by at least partially promoting the release of cathepsin B through sphingosine-1-phosphate receptor 2. In this study, compared with that in the healthy group, the DCA level in the CD group was significantly higher, by more than 6-fold. Thus, this result indicated that CD might be activate NLRP3 inflammasome induced by high levels of DCA, which could provide insights into the pathogenesis of CD in the future.

Palmitic amide is a primary fatty acid amide coming from palmitic acid (C16:0). Palmitic amide competes with other active substances such as cannabinoids or fatty acid amide hydrolase (degrading endocannabinoids), thereby increasing its concentration by preventing its degradation (21). William (22) et al. found that the activation of cannabinoid receptors both on immune cells and colonocytes is essential to prevent colitis and can be used as a preventive and treatment method for colitis. In our study, the concentration of palmitic amide in the CD group was significantly higher than that in the healthy group; thus, we proposed that the activation of cannabinoid receptors in CD patients was blocked.

In a recent publication, Lai et al. (23) reported that there was a unique metabolic pattern in patients with CD compared to that in HCs, and the identified differential compounds were structurally diverse, pointing to important pathway perturbations ranging from energy metabolism (e.g., β-oxidation of fatty acids) to signaling cascades of lipids (e.g., DHA) and amino acids (e.g., L-tryptophan). In our study, most of the metabolic pathway changes were also in lipid, amino acid and energy metabolism; in addition, disturbances in steroidogenesis were discovered. The metabolites of tetrahydrocorticosterone were significantly decreased in patients with CD. Tetrahydrocorticosterone is one of the major metabolites of corticosterone. Glucocorticoids are steroid hormones that decrease the severity of IBD by suppressing the immune response. In a previous study, Huang et al. (24) showed that during chronic intestinal inflammation, intestinal glucocorticoid synthesis was inhibited. In our study, CD patient steroid synthesis was blocked, and glucocorticoids were downregulated, which was consistent with previous studies.

Previous studies have demonstrated abnormal lipid and bile acid metabolism in patients with CD. In patients with CD, the terminal ileum is damaged, leading to malabsorption of bile acids and lipids, which in turn leads to diarrhea. Ineffective absorption of n-3 polyunsaturated fatty acids (PUFAs) reduces its anti-inflammatory effect (25). In our study, docosapentaenoic acid which is one of the n-3 PUFAs was significantly elevated in CD patients, indicating that its absorption was significantly reduced and thus the anti-inflammatory effect was reduced and the disease occurred. In addition, 7α-hydroxy-4-cholesten-3-one (C4) is a stable bile acid precursor. Robert et al. (26)

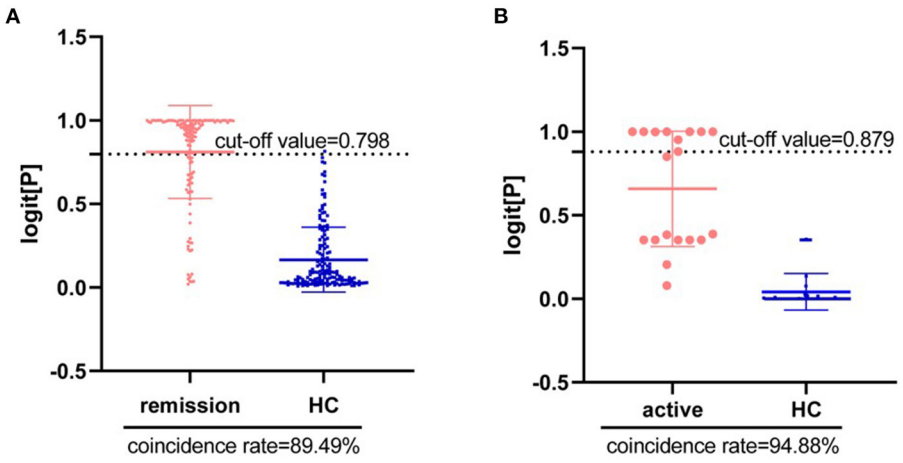

FIGURE 7
Diagnosis of disease activity in Crohn's Disease using Deoxycholic acid and Palmitic amide. **(A)** Diagnostic coincidence rate for the comparison between remission CD vs. HCs. **(B)** Diagnostic coincidence rate for the comparison between active CD vs. HCs. The vertical axis is the predicted probability. The horizontal axis represents the group.

observed significantly increased serum concentrations of C4 in patients with CD and indicated C4 may be a biomarker to identify patients with diarrhea attributable to bile acid malabsorption. Vitamin D regulates the immune system by reducing Th1/Th17 T cells, inflammatory cytokines, etc, and vitamin D absorption is significantly reduced in patients with CD. Therefore, abnormal metabolism of vitamin D also perturbs lipid metabolism in CD patients.

There are more research about biomarkers in the field of CD. In a value review, Mohsen et al. (27) address CD biomarkers including serologic biomarkers, genetic predisposing markers and Interleukin-24. However, their specificity and accuracy need to be further improved, for example, CRP and ESR, which are cheap and reliable, but hugely non-specific. At the end of the article the author mentioned the need for more novel biomarkers like metabolomics technology in the future, leading to highly accurate testing. In our study, an LC-MS/MS metabolomic method was applied to compare the serum metabolic characteristics between CD patients and HCs and a panel of two metabolites (DCA and palmitic amide) was identified as a specific biomarker of CD.

In recent years, research on fecal metabolites and IBD is rapidly increasing and improving with the development of technology. However, the characterization of the human fecal metabolome still lags behind these other metabolomes because of various reasons, such as standardized methods and freely available resources (28). In a past review study on colorectal cancer, we found that serum and tissue are the preferred biological samples, and the analysis of stool samples only accounts for a small part (29). Since the acquisition of human intestinal tissue samples is invasive, the acquisition of serum samples is very convenient, and serum metabolomics is a classic metabolomics study. Therefore, we choose serum for metabolomics analysis.

According to the lesion site, CD can be divided into L1, terminal ileum; L2, colon; L3, ileocolon and L4, upper gastrointestinal tract. In our study, there is L1, L2, L3, L1L4, L2L4, and L3L4. The result of association between regio specific CD sites and markers is showed in Supplementary Table S2. It can be seen from the results that there is not significant association between regio specific CD sites and markers. It may be caused by the distribution of different types of samples or other reasons. In future research, we should pay attention to the selection of sample types and other factors that may affect the results. We have also conducted correlation analysis between clinical characteristics and biomarker (Supplementary Table S3). Because the results of the correlation analysis are not good, we do not add in the text. It may be due to the limitation of sample size. In the future research, we will try to expand the number of samples to design experiments.

There were three limitations in this study. Firstly, there were all CD patients from one center in our study. In the future, a large cohort of multicenter participants will be required to verify the reliability of the results. In this study, we are limited to cross-sectional studies to screen biomarkers, and further studies provide more longitudinal data to prove the role of these biomarkers in early diagnosis, monitoring disease evolution or time to relapse of the patient. Secondly, all patients were from Asia. We should include CD patients of different races for research in future studies. Lastly, we know that IBD mainly includes two subtypes, UC and CD. In our study, only CD patient samples were included. Therefore, performing further studies focused on metabolic profiling using UC patient samples are required. So, we can try to include UC patients to identify the

metabolite profile that could be used to differentiate CD and UC patients, it will be a more challenging study.

Conclusion

In conclusion, an LC-MS/MS metabolomic method was applied to compare the serum metabolic characteristics between CD patients and HCs in this study. A panel of two metabolites (DCA and palmitic amide), which were both upregulated, was identified as a specific biomarker of CD. These serum metabolites are mainly related to bile acid biosynthesis, fatty acids and energy metabolism. Our findings will provide a new method for the diagnosis of CD and new insights into CD pathogenesis.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Nanjing University of Traditional Chinese Medicine. The patients/participants provided their written informed consent to participate in this study.

Author contributions

HL, MX, and SL were responsible for the conception and design of the study. QH performed the study retrieval. PW and MK collected samples. HL and MX contributed to the data collection and statistical analysis. HL drafted the manuscript. MX and SL were responsible for the revision of the manuscript. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.814839/full#supplementary-material

References

1. Torres J, Mehandru S, Colombel JF, Peyrin-Biroulet L. Crohn's disease. *Lancet*. (2017) 389:1741–55. doi: 10.1016/S0140-6736(16)31711-1.3

2. Roda G, Chien Ng S, Kotze PG, Argollo M, Panaccione R, Spinelli A, et al. Crohn's disease. *Nat Rev Dis Primers*. (2020) 6:22. doi: 10.1038/s41572-020-0156-2

3. Schmitt H, Neufert C, Neurath MF, Atreya R. Resolution of Crohn's disease. *Semin Immunopathol*. (2019) 41:737–46. doi: 10.1007/s00281-019-00756-1

4. Epidemiology and management. *Mayo Clin Proc*. (2017) 92:1088–103. doi: 10.1016/j.mayocp.2017.04.010

5. Bane O, Gee MS, Menys A, Dillman JR, Taouli B. Emerging Imaging Biomarkers in Crohn Disease. *Top Magn Reson Imaging*. (2021) 30:31–41. doi: 10.1097/RMR.0000000000000264

6. Muthubharathi BC, Gowripriya T, Balamurugan K. Metabolomics: small molecules that matter more *Mol Omics*. (2021) 17:210-−29. doi: 10.1039/d0mo00176g

7. Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomarkers and towards mechanisms. *Nat Rev Mol Cell Bio*. (2016) 17:451–9. doi: 10.1038/nrm.2016.25

8. Patti GJ, Yanes O, Siuzdak G. Innovation: Metabolomics: the apogee of the omics trilogy. *Nat Rev Mol Cell Biol*. (2012) 13:263–9. doi: 10.1038/nrm3314

9. De Preter V, Verbeke K. Metabolomics as a diagnostic tool in gastroenterology. *World J Gastrointest Pharmacol Ther*. (2013) 4:97–107. doi: 10.4292/wjgpt.v4.i4.97

10. Tiziani S, Lopes V, Gunther UL. Early stage diagnosis of oral cancer using ^{1}H NMR-based metabolomics. *Neoplasia*. (2009) 11:269–76. doi: 10.1593/neo.81396

11. Crook AA, Powers R. Quantitative NMR-based biomedical metabolomics: current status and applications. *Molecules*. (2020) 25:5128. doi: 10.3390/molecules25215128

12. Kind T, Tolstikov V, Fiehn O, Weiss RH. A comprehensive urinary metabolomic approach for identifying kidney cancer. *Anal Biochem*. (2007) 363:185–95. doi: 10.1016/j.ab.2007.01.028

13. Sieber M, Wagner S, Rached E, Amberg A, Mally A, Dekant W. Metabonomic study of ochratoxin a toxicity in rats after repeated administration: phenotypic anchoring enhances the ability for biomarker discovery. *Chem Res Toxicol*. (2009) 22:1221–31. doi: 10.1021/tx800459q

14. Sugimoto M, Wong DT, Hirayama A, Soga T, Tomita M. Capillary electrophoresis mass spectrometry-based saliva metabolomics identified oral, breast, and pancreatic cancer-specific profiles. *Metabolomics*. (2010) 6:78–95. doi: 10.1007/s11306-009-0178-y

15. Habeeb AA, El-Tarabany AA, Gad AE, Atta MA. Volatile organic compounds in breath as markers for irritable bowel syndrome: a metabolomic approach. *Aliment Pharmacol Ther*. (2016) 44:45–56. doi: 10.1111/apt.13654

16. Tana C, Umesaki Y, Imaoka A, Handa T, Kanazawa M, Fukudo S. Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. *Neurogastroenterol Motil*. (2010) 22:512–9, e114-515. doi: 10.1111/j.1365-2982.2009.01427.x

17. Kohashi M, Nishiumi S, Ooi M, Yoshie T, Matsubara A, Suzuki M, et al. A novel gas chromatography mass spectrometry-based serum diagnostic and assessment approach to ulcerative colitis. *J Crohns Colitis*. (2014) 8:1010–21.doi: 10.1016/j.crohns.2014.01.024

18. Alonso A, Julia A, Vinaixa M, Domenech E, Fernandez-Nebro A, Canete JD, et al. Urine metabolome profiling of immune-mediated inflammatory diseases. *BMC Med*. (2016) 14:133. doi: 10.1186/s12916-016-0681-8

19. Harvey RF, Bradshaw JM. A simple index of Crohn's-disease activity. *Lancet*. (1980) 1:514. doi: 10.1016/s0140-6736(80)92767-1.20

20. Zhao S, Gong Z, Zhou J, Tian C, Gao Y, Xu C, et al. Deoxycholic acid triggers NLRP3 inflammasome activation and aggravates DSS-induced colitis in mice. *Front Immunol*. (2016) 7:53. doi: 10.3389/fimmu.2016.00536

21. Cui Y, Liu X, Wang M, Liu L, Sun X, Ma L, et al. Lysophosphatidylcholine and amide as metabolites for detecting alzheimer disease using ultrahigh-performance liquid chromatography-quadrupole time-of-flight mass spectrometry-based metabonomics. *J Neuropathol Exp Neurol*. (2014) 73:954–63. doi: 10.1097/NEN.0000000000000116

22. Alrafas HR, Busbee PB. Walla MD, Wilson K, Miranda K, et al. Cannabinoid receptor activation on hematopoietic cells and enterocytes protects against colitis. *J Crohns Colitis*. (2020) 15:1032–48. doi: 10.1093/ecco-jcc/jjaa253

23. Lai Y, Xue J, Liu CW, Motley AK, Peyton SC, Ferguson SL, et al. Serum metabolomics identifies altered bioenergetics, signaling cascades in parallel with exposome markers in Crohn's disease. *Molecules*. (2019) 24:449. doi: 10.3390/molecules24030449

24. Huang SC, Lee CT, Chung BC. Tumor necrosis factor suppresses NR5A2 activity and intestinal glucocorticoid synthesis to sustain chronic colitis. *Sci Signal*. (2014) 7:ra20. doi: 10.1126/scisignal.2004786

25. Uchiyama K, Kishi H, Komatsu W, Nagao M, Ohhira S, Kobashi G. Lipid and bile acid dysmetabolism in Crohn's disease. *J Immunol Res*. (2018) 36:7270486. doi: 10.1155/2018/7270486

26. Battat R, Duijvestein M, Vande Casteele N, Singh S, Dulai PS, Valasek MA, et al. Serum Concentrations of 7α-hydroxy-4-cholesten-3-one are associated with bile acid diarrhea in patients with Crohn's disease. *Clin Gastroenterol Hepatol*. (2019) 17:2722–30. doi: 10.1016/j.cgh.2018.11.012

27. Norouzinia M, Chaleshi V, Alizadeh AHM, Zali MR. Biomarkers in inflammatory bowel diseases: insight into diagnosis, prognosis, and treatment. *Gastroenterol Hepatol Bed Bench*. (2017) 10:155–67.

28. Naama K, Lu D, Mordechai S, Guo AC, Sajed T, Huynh H, et al. A review on human fecal metabolomics: methods, applications, and the human fecal metabolome database. *Anal Chim Acta*. (2018) 1030:1–24. doi: 10.1016/j.aca.2018.05.031

29. Ni Y, Xie GX, Jia W. Metabonomics of human colorectal cancer: new approaches for early diagnosis and biomarker discovery. *J Proteome Res*. (2014) 13:3857–70. doi: 10.1021/pr500443c

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