# PERSONALIZING TREATMENT IN IBD: HYPE OR REALITY IN 2020?

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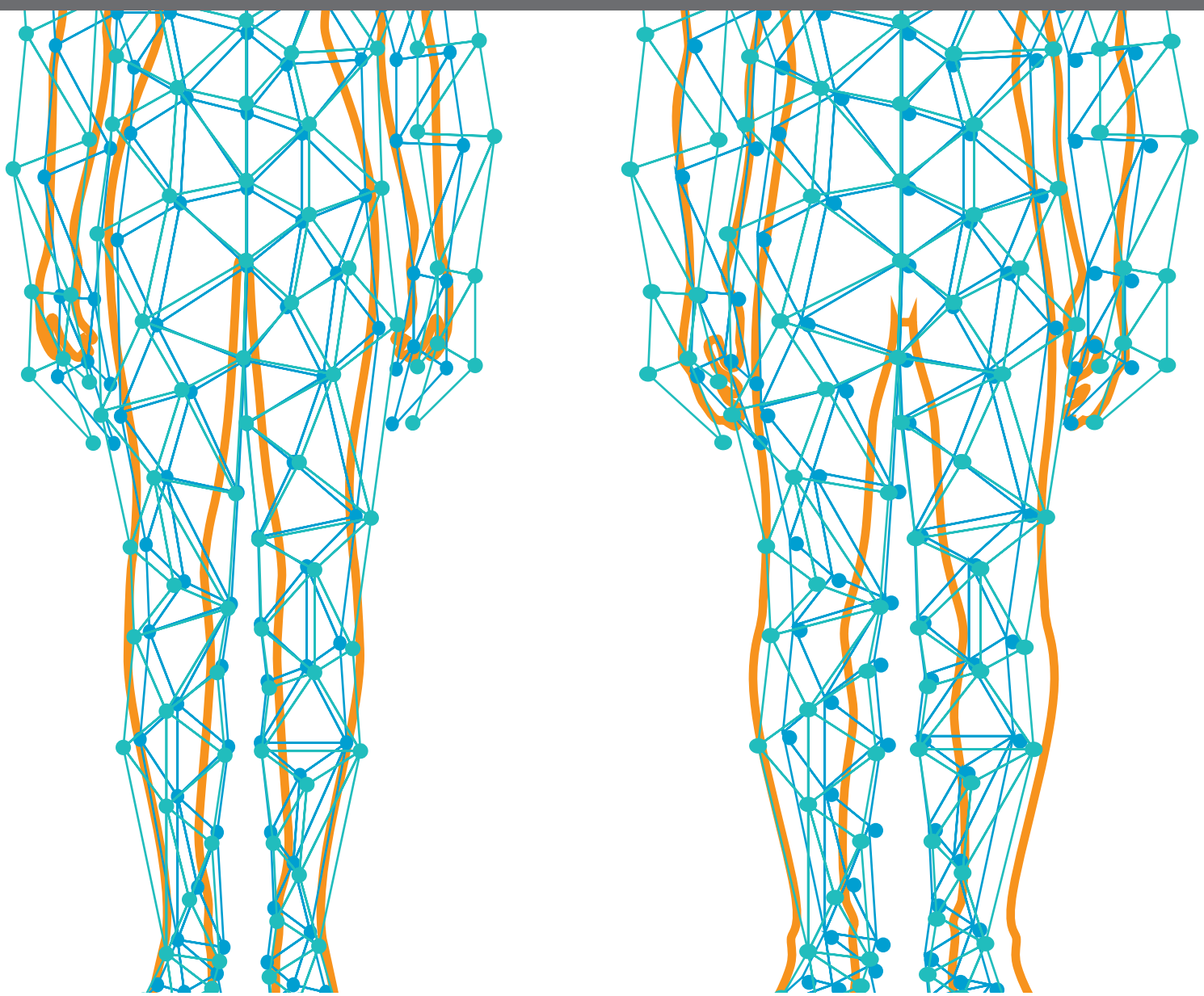

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# PERSONALIZING TREATMENT IN IBD: HYPE OR REALITY IN 2020?

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EDITORIAL
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# Editorial: Personalizing Treatment in IBD: Hype or Reality in 2020?

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**Keywords: personalized, IBD, Crohn's disease, ulcerative colitis, prediction**

**Editorial on the Research Topic**

**Personalizing Treatment in IBD: Hype or Reality in 2020?**

Let us go to the daily clinic. Ana Isabel, Raúl, and José Luis are the real names of three of IBD (inflammatory bowel disease) patients to be seen tomorrow at the office. We will share information on their symptoms, tests results, treatment plans and worries for the following months. We are confident, for instance, that they will ask about the convenience of the COVID vaccination. Some of their questions will be very easy to answer, but things will get complicated if they ask me about the future. For instance, Ana Isabel could ask: Can I stop my infliximab? And our response would, should, and will be: we do not know. An apparently simple question is not so simple. As we do love books, we will, first, quote some recent ones for establishing context.

First, communication between patients and physicians is not always easy (1). Making decisions is also complicated (2, 3). Besides, much medical advice does not resist the test of time (4). As humans, we have complex behaviors, sometimes "at our best" sometimes "at our worst" (5). We should be conscious of our limitations, and experts on Healthcare Systems have given us excellent guidelines to improve our systems (6, 7). In a world where artificial intelligence is taking the lead (8), "predicting and preempting disease" remains a very complex matter, as Eric Topol tells us in his provocative and inspiring books (9, 10). In the foreword of the last book, Abraham Verghese quotes this sentence from the Danish philosopher Soren Kierkegaard: "Life can only be understood backwards; but it must be lived forwards." We cannot imagine a better description of our daily clinical task.

Coming back to IBD, two excellent recent reviews summarize the concept and possibilities of personalized medicine in Crohn's disease (11) and in IBD (12). Our goal in the present Research Topic is to help practical clinicians by providing some clues for prediction in some typical scenarios of an everyday IBD clinic: using biomarkers, microbiota clues, and responses to antiTNF, vedolizumab, and ustekinumab are some examples. For the exact question of my patient, we would need to study Edouard's views in his review (Louis), revisiting the clinical record of Ana Isabel, and being ready to listen to her opinion. Our current available tools can give estimates of "a risk of relapse of 25% in 3 years." This data is of scientific interest, but is of a very relative value in a given person. Our ability to predict on an *individual* basis is poor, excepting very specific circumstances (11, 12). The fears, previous experiences, and very personal optics and circumstances of the patient will affect the conversation and the final decision (1). Of course, the current state of knowledge could change, and even be completely reverted (4). A patient's and physician's conversation will not be isolated from system and social circumstances (7). For instance, if a patient is under infliximab and azathioprine combination, a rather typical one in IBD, when considering withdrawal of one of them, efficacy and presumed toxicity should be the main issues, but insurers and payers will see

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price as a very important issue, and limit (sometimes decisively) the election of the cheapest one.

We think that the aspects we have discussed in this Research Topic are of maximal interest for IBD clinicians and patients. However, as should be the norm in good science, there remain more questions than answers. We would like to finish by asking for more investigator driven research, making randomized clinical trials with high ethical standards, the only way to make prediction easier and reversal rare (4). For Ana Isabel, the results of the SPARE trial are eagerly awaited.

## AUTHOR CONTRIBUTIONS

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

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ORIGINAL RESEARCH
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# Administration Timing Is the Best Clinical Outcome Predictor for Adalimumab Administration in Crohn's Disease

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Biological intervention for Crohn's Disease (CDs) patients, mainly using anti-TNF antibodies, is often an efficient therapeutic solution. Nonetheless, data defining the administration timing to maximize the chances of clinical remission are lacking. The objective of this "real-life" retrospective study was to evaluate if early Adalimumab (ADA) administration (<12 months) was an efficient strategy to improve patients' clinical outcome. This single center study included 157 CD patients, of which 80 received the first ADA administration within the first 12 months from the diagnosis. After 1 year of therapy, clinical remission was observed in 50.32% of patients, mucosal healing in 37.58%. Clinical remission was observed in 66.25% of the early ADA administration patients vs. 33.77% of the late (>12 months) ($p < 0.001$); mucosal healing was observed in 53.75% of the early vs. 20.78% of the late ($p < 0.001$). Dose escalation was required for 30.00% of the early vs. 66.23% of the late (<0.01). In the early ADA administration group, 7.50% patients were considered non-responders at the end of the follow-up vs. 22.08% patients in the late administration group. These findings highlighted that early ADA administration (within 1 year of diagnosis) improves the clinical response and mucosal healing, and reduces the loss of response rate and need for dose escalation.

**Keywords: Crohn's disease, biological agents anti-TNF, Adalimumab, clinical outcome, clinical remission**

## INTRODUCTION

Crohn's disease (CD) is a severe, chronic and debilitating inflammatory disease involving the gastrointestinal wall of the entire digestive tract. The etiology of CD involves genetic and environmental factors, even if an immunological inflammatory component is always present. The presence of chronic active inflammation can induce the development of bowel damage, such as stenosis and fistula. Crohn's disease hallmarks include chronic diarrhea, abdominal pain, rectal bleeding, weight loss and, in affected children, growth may be impaired (1–3). The disease is characterized by recurring flare-ups alternating with periods of remission; both periods have a variable duration (2). In particular, over 50% of CD patients will develop penetrating lesions or stricture over long-term follow-up, dictating a need for surgical intervention (4). Previously, IBD

patients endured a lack of effective treatment options, and patients with moderate-to-severe CD were often relegated to prolonged systemic corticosteroid therapy and surgery as their only options. Resection of the lesions is a crucial strategy to manage fibrostenotic or medically refractory disease, which has a negative impact on patients' postoperative morbidity and mortality rates, as well as quality of life (5).

Since the CD etiology is unknown, currently there are no preventive strategies. Several treatments are nowadays available for inducing and/or maintaining remission in CD, but most patients need lifelong medication. The introduction of biological agents targeting tumor necrosis factor α (TNF) has dramatically changed the medical approach to CD. The first FDA approved anti-TNF for Crohn's disease was Infliximab (IFX) introduced in 1998, the second was Adalimumab (ADA) in 2007 and, more recently, Golimumab and Certolizumab became available. According to the most recent European and American Guidelines (European Crohn's and Colitis Organisation—ECCO—and the American Gastroenterological Association—GA), conventional treatments include anti-inflammatory drugs (corticosteroids, aminosalicylates, immunosuppressants such as thiopurines and methotrexate), antibiotics, nutritional therapy, and surgery. Biologic agents, such as tumor necrosis factor alpha inhibitors (anti-TNF agents), are recommended in CD cases that are refractory, dependent or intolerant to conventional treatments, in relapsing patients, or in the early stages of the disease in patients showing factors predictive of disease severity (6, 7).

Several recent studies suggested that TNF blocking agents are effective in Crohn's disease. In particular, a meta-analysis of 19 clinical trials compared the effectiveness and safety of TNF blocking agents (Infliximab, Adalimumab, and Certolizumab) in the treatment of CD, showing that anti-TNF therapy is safe and significantly more effective than placebo (8). Furthermore, a meta-analysis involving 12,586 CD patients reported that thiopurine administration resulted in a 40% decrease in the first intestinal resection (9). Similarly, subgroup analysis of the ACCENT II and CHARM studies demonstrated that IFX and ADA maintenance therapy reduced the need for both hospitalization and surgery (10, 11).

It is currently debated whether an earlier start on biologic drugs may curtail long-term complications, such as strictures and fistulae. In *post-hoc* analysis of the CHARM and ADHERE trials, the authors reported a significant improvement of the remission rates in CD patients who started ADA within the first 2 years from the diagnosis compared with those starting after 5 years (12). However, different open label cohort studies failed to confirm the same effect of early anti-TNF therapy. Our study aims to fill the knowledge gap about the link between administration timing and clinical outcome.

# MATERIALS AND METHODS

## Study Design

This single center case-series retrospectively evaluated Crohn's disease patients receiving Adalimumab between August 2008 and February 2016 at the Division of Gastroenterology and Digestive Endoscopy of the National Institute of Gastroenterology "S. de Bellis", Castellana Grotte, Bari, Italy.

## Patients Population

Ethics Statement: The investigation has been conducted in accordance with the ethical standards, the Declaration of Helsinki and international guidelines, and has been approved by the authors' institutional review board. All patients provided written informed consent.

The following criteria were used for patients' selection: CD diagnosis by either endoscopy, histology or radiology (MRI) (within the established date of Aug 2008). CD was classified according to the Montreal Classification (13). All data were analyzed to identify factors predictive of the clinical outcome.

All consecutively enrolled adult patients (between 18 and 71 years old) with active Crohn's disease, treated with Adalimumab, were included. Adalimumab monotherapy was administered at the dose of 160/80 mg for the induction regimen and 40 mg every other week for maintenance. Dose escalation was defined as increasing the frequency to weekly injections.

The primary endpoint was: Mucosal Healing (MH), defined according to the Simple Endoscopic Score for Crohn's Disease (SES-CD), a simple, reproducible, and easy-to-use endoscopic scoring system for Crohn's disease, based on ulcer size, ulcerated and affected surfaces and stenosis (a SES-CD score $<2$ means mucosal healing). The SES-CD score was assessed on each endoscopic evaluation from the first one to the end of the follow-up; -percentage of patients in deep remission calculated as concomitant clinical remission (HB score $< 5$), mucosal healing (SES-CD $< 2$) and C-reactive protein (CRP) in the reference range-safety (reported adverse events, laboratory tests) (14, 15). The secondary endpoints were: -clinical remission 52 weeks from the beginning of ADA administration defined according to the Harvey Bradshaw Index -HBI, a simple index of Crohn's disease activity based on the evaluation of general well-being, abdominal pain, number of liquid, or soft stools per day, abdominal mass and complications (an index score $< 5$ meaning remission); -steroid-free clinical remission 52 weeks from the start of the treatment and during the follow-up.

We also evaluated the clinical response (3 points or more from the baseline score HB) and the endoscopic improvements, defined as a reduction of the SES-CD score by more than 50% compared to baseline. Outcome analysis consisted of evaluating clinical and bio-humoral parameters every 3 months. The evaluation of clinical remission and mucosal healing, as well as of the secondary endpoints, was performed 12 months from the start of the therapy.

## Statistical Analysis

Continuous data were expressed as mean and standard deviation if normally distributed, as median and interquartile range (IQR) otherwise. Comparisons between values at the beginning and at the end of the study were performed with paired *t*-test for normally distributed variables, or Wilcoxon test for paired data.

Another aim was to evaluate predictors of a SES-CD. This score was classified as $<2$ and $\geq 2$ and its value at the end of the study was the dependent variable of a logistic regression model.

Predictors tested in the univariate model were: age class, gender, smoking habit, months from the diagnosis, dose escalation, steroid therapy, steroid dependency, steroid resistance, other therapies, type and site of disease, number of cycles and duration of steroid therapy, calprotectin at the beginning of the study, ferritin at the beginning, CRP at the beginning, albumin at the beginning.

A multivariate model was also built to evaluate predictors independently related to SES-CD; all variables were included in the model and then selected using the stepwise procedure. The final model included age class, sex and smoking habit as adjustment variables, together with those variables selected by the stepwise procedure.

In both models, to evaluate the effect of adjusting variables Type 3 analysis *p*-values were reported; to evaluate the statistical significance of the model the chi-square score was used, while the fitting of the models was assessed by considering the Hosmer and Lemeshow statistic (HL chi-square, a $p > 0.05$ suggests an adequate fitting).

A $p < 0.05$ was selected as statistically significant. All the analyses were performed with SAS 9.4 for PC.

# RESULTS

## Cohort Characteristics

One hundred fifty-seven patients (mean age 34.99 years, 68.15% males, 36.31% smokers) were enrolled in the study and followed up for a median time of 50 (6–102) months. Demographic and clinical characteristics of the patients enrolled are summarized in **Table 1**. The endoscopic evaluation was performed in all patients at a mean time of 12.5 months (range 10.8–16.4 months) from the beginning of the therapy. A second endoscopy was performed at a mean of 13.4 months from the first endoscopic evaluation (range 11.2–16.9 months). Disease distribution was 48 in the ileum (30.77%), 81 ileocolic (51.92%), and 27 colic (17.31%).

The disease phenotypes at diagnosis were inflammatory in 61 (38.85%) patients, stricturing in 47 (29.94%) and penetrating in 49 (31.21%) patients.

The majority of the observed patients 143 (91.01%) received at least one systemic steroids cycle before starting Adalimumab (with a median (IQR) duration equal to 20 weeks). However, not all patients responded to corticosteroid therapy. In our study, four (2.56%) of patients failed to respond to the initial treatment with steroids, while 110 (70.51%) of patients may be considered to be steroid-dependent (**Table 1**). Moreover, 75 patients received treatments other than steroids, including azathioprine 43 (27.3%), Infliximab 21 (13.3%), a combination of azathioprine and Infliximab 6 (3.8%), and antibiotics 5 (3.2%) (data not shown).

**TABLE 1** | Clinicpathologic features of enrolled patients.

| | ***n* = 157** |
|---|---|
| Follow-up (months) | |
| Median (range) | 50 (6–102) |
| Age (years) | |
| Mean (SD) | 34.99 (14.36) |
| Median (range) | 33.00 (12.00–74.00) |
| Gender (Male) (%) | 107 (68.15) |
| Smokers (%) | 57 (36.31) |
| Time from diagnosis to start ADA (months) | |
| Mean (SD) | 32.48 (43.30) |
| Median (range) | 12 (1–265) |
| Type of disease (%)–Montreal classification | |
| Inflammatory | 61 (38.85) |
| Stricturing | 47 (29.94) |
| Penetrating | 49 (31.21) |
| Location of disease *N* (%)–Montreal classification | |
| L1 Ileal | 48 (30.77) |
| L2 Colic | 27 (17.31) |
| L3 Ileocolic | 81 (51.92) |
| Steroid therapy (%) | 143 (91.08) |
| Steroid therapy, cycles | |
| Mean (SD) | 2.64 (2.62) |
| Median (range) | 2 (0–16) |
| Steroid therapy, total duration (weeks) | |
| Mean (SD) | 30.08 (29.48) |
| Median (Range) | 20 (0–170) |
| Steroid resistant patients (%) | 4 (2.56) |
| Steroid dependent patients (%) | 110 (70.51) |

## Adalimumab on Clinical Outcomes

The entire cohort of 157 patients described was treated using Adalinumab, the clinical outcomes was evaluated and analyzed. Of note, the administration of Adalimumab was withdrawn for a lack of response in four patients, and in one patient due to adverse events (severe psoriasis). Clinical remission was achieved in 79 (50.32%) patients at 12 months following the beginning of ADA administration, clinical response was observed in 55 (35.03%) patients (**Table 2**). Steroid-free remission was observed in 98 (62.42%) of the patients in clinical remission or clinical response. Mucosal healing was achieved in 59/157 patients (37.58%) treated with Adalimumab.

At 52 weeks 54 (34.39%) patients obtained a deep remission and, at the same time, endoscopic improvement was detected in 42 (26.75%) patients. Among patients with endoscopic improvement, 32/42 (76.19%) patients achieved clinical remission and 10/42 (23.80%) clinical response. Finally, only 23 (14.65%) patients were complete non-responders (**Table 2**).

At the end of the follow-up, among patients that obtained clinical response, 11/55 had clinical remission and 10/42 patients with endoscopic improvement had shifted to mucosal healing; in total 90/157 (57.32%) patients achieved in clinical remission and 69/157 (43.94%) patients were in mucosal healing. At the same time, 23/157 (14.65%) were complete non-responders, among them, 14/157 (8.92%) underwent intestinal resection (data not shown).

Dose escalation (defined as an increase in the selected ADA dose to 40 mg every week instead of every 2 weeks) was required in 75/157 (47.77%) cases (**Table 2**).

Clinical assessment, performed at the end of the follow-up for the whole cohort of 157 patients, showed significantly

**TABLE 2 |** Efficacy of ADA on clinical outcomes in patients with a disease duration of <12 months vs. more than 12 months.

| | | Administration ADA | | |
|---|---|---|---|---|
| | At 52 week $n = 157$ | <12 months $n = 80$ | ≥12 months $n$: 77 (%) | $p$-value* |
| Clinical remission (%) | 79 (50.32) | 53 (66.25) | 26 (33.77) | <0.001 |
| Clinical response (%) | 55 (35.03) | 21 (26.25) | 34 (44.16) | 0.02 |
| Deep remission (%) | 54 (34.39) | 39 (48.75) | 15 (19.48) | <0.001 |
| Endoscopic improvement (%) | 42 (26.75) | 29 (36.25) | 13 (16.88) | 0.006 |
| Mucosal healing (%) | 59 (37.58) | 43 (53.75) | 16 (20.78) | <0.001 |
| Dose escalation (%) | 75 (47.77) | 24 (30.00) | 51 (66.23) | <0.001 |
| Steroid-free remission (%) | 98 (62.42) | 71 (88.75) | 27 (35.06) | <0.001 |
| Non-responder (%) | 23 (14.65) | 6 (7.50) | 17 (22.08) | 0.01$ |

*Chi-square test; $ Fisher's exact test.*

**TABLE 3 |** Change of clinical outcome parameters from baseline to the last visit.

| Parameters | Baseline | Last observation | $p$-value* |
|---|---|---|---|
| CRP level < 5 g/L | | | <0.0001 |
| Mean (SD) | 39.80 (37.27) | 13.44 (23.93) | |
| Median (range) | 30 (2–175) | 4 (1–170) | |
| Ferritin level <30 mg/dL | | | <0.0001 |
| Mean (SD) | 17.22 (10.49) | 31.39 (14.78) | |
| Median (range) | 15 (2.1–55) | 31 (2.8–88) | |
| HBI score | | | <0.0001 |
| Mean (SD) | 13.59 (4.06) | 7.78 (5.18) | |
| Median (range) | 14 (6–28) | 6 (1–26) | |
| SES-CD | | | <0.0001 |
| Mean (SD) | 13.67 (5.79) | 7.00 (5.62) | |
| Median (range) | 13 (0–42) | 5 (0–23) | |
| Fecal Calprotectin μg/g | | | <0.0001 |
| Mean (SD) | 404.35 (220.55) | 228.56 (328.66) | |
| Median (range) | 376.50 (16–1,239) | 112 (22–3,313) | |
| Weight (Kg) | | | <0.0001 |
| Mean (SD) | 65.40 (13.00) | 69.15 (13.73) | |
| Median (range) | 65 (41–113) | 67 (46.136) | |

*Wilcoxon signed-rank test.*

**TABLE 4 |** Logistic regression model of clinical remission, and of mucosal healing on single factor.

| Variable | Odds Ratio | se(OR) | 95% CI | $p$-value |
|---|---|---|---|---|
| **Clinical remission** | | | | |
| Age | 0.97 | 0.01 | 0.95–0.99 | 0.02 |
| Number of cycles of steroids | 0.67 | 0.07 | 0.55–0.82 | <0.001 |
| Duration of steroid treatment | 0.96 | 0.01 | 0.95–0.98 | <0.001 |
| Dose escalation | 0.32 | 0.11 | 0.17–0.62 | 0.001 |
| Months from diagnosis | 0.98 | 0.005 | 0.97–0.99 | 0.001 |
| Ileocolic disease | 1.15 | 0.20 | 0.81–1.64 | 0.44 |
| Previous anti TNF | 0.21 | 0.12 | 0.06–0.65 | 0.007 |
| **Mucosal healing** | | | | |
| Number of cycles of steroids | 0.67 | 0.08 | 0.54–0.85 | 0.001 |
| Duration of steroid treatment | 0.97 | 0.01 | 0.95–0.99 | 0.001 |
| Dose escalation | 0.40 | 0.14 | 0.20–0.78 | 0.008 |
| **Multiple Logistic Regression Model of Clinical Remission and of Mucosal Healing on All Factors** | | | | |
| **Clinical remission** | | | | |
| Age | 0.98 | 0.01 | 0.95–1.00 | 0.10 |
| Number of cycles of steroids | 0.93 | 0.21 | 0.59–1.44 | 0.74 |
| Duration of steroid treatment | 0.98 | 0.02 | 0.94–1.02 | 0.27 |
| Dose escalation | 0.50 | 0.19 | 0.23–1.05 | 0.07 |
| Months from diagnosis | 1.00 | 0.01 | 0.98–1.01 | 0.82 |
| Ileocolic disease | 1.20 | 0.25 | 0.80–1.82 | 0.37 |
| Previous anti TNF | 0.44 | 0.29 | 0.12–1.57 | 0.21 |
| **Mucosal healing** | | | | |
| Number of cycles of steroids | 0.78 | 0.17 | 0.51–1.21 | 0.28 |
| Duration of steroid treatment | 0.99 | 0.02 | 0.95–1.03 | 0.55 |
| Dose escalation | 0.55 | 0.20 | 0.27–1.14 | 0.11 |

*OR, Odds Ratio; se(OR), standard error of OR.*

improved values compared to baseline, especially for those related to inflammation (CRP, HBI, SES-CD) (**Table 3**). Both the logistic regression model on single factor and the multiple logistic regression model on all factors identified the following factors as being significantly associated with unsuccessful clinical remission: age, number of cycles of steroid therapy, duration of steroid therapy, dose escalation, months from diagnosis, ileocolic disease, and previous anti-TNF therapy. Furthermore, factors significantly associated with unsuccessful mucosal healing were the number of cycles of steroid therapy, duration of steroid therapy, and dose escalation (**Table 4**).

## Early Disease Population

Short duration of the disease seems to be correlated to a better outcome, therefore we performed a sub-analysis comparing patients treated with ADA <12 months following the disease diagnosis (80/157) vs. more than 12 months (77/157). The main baseline characteristics of the patients enrolled are summarized in **Table 5**. Patients with a shorter disease duration were younger (31.60 years vs. 38.51 years, $p \leq 0{,}002$), and had taken lower doses of steroids or previous anti TNF (1.51 mean steroid cycle vs. 3.82 $p \leq 0.0001$ and 3.75% vs. 22.08% previous use of anti TNF $p \leq 0.001$), compared to patients with a disease duration >12 months. No differences were found regarding smoking habit, baseline disease activity, disease distribution, and behavior. Moreover, 80/157 (50.95%) patients started ADA treatment within 12 months (average time 6.17 months) and 77/157 (49.04%) patients after 12 months (average time 59.82 months) from diagnosis of CD. Besides, differences for CRP, ferritin, fecal calprotectin, albumin, SED-CD, and HBI levels between patients with disease duration <12 months and patients with disease duration >12 months are reported in **Table 5**.

Among all patients in deep remission (54/157), 39 (48.75%) were included in the group with disease duration <12 months vs. 15 (19.48%) with disease duration >12 months. The clinical remission rate was significantly superior for patients with disease duration <12 months (66.25%) vs. patients with disease duration

**TABLE 5 |** Baseline characteristics patients treated with ADA with a disease duration of <12 months vs. more than 12 months.

| | Administration ADA | | |
|---|---|---|---|
| | **<12 months (*n* = 80)** | **>12 months (*n* = 77)** | ***p*-value*** |
| Age (years) | | | 0.002 |
| Mean (SD) | 31.60 (13.42) | 38.51 (14.55) | |
| Median (range) | 28 (12–64) | 37 (15–74) | |
| Sex (Male) (%) | 54 (67.50) | 53 (68.83) | 0.86^ |
| Smokers (%) | 26 (32.50) | 31 (40.26) | 0.31 |
| Montreal classification–Behavior (%) | | | 0.44 |
| Inflammatory | 35 (43.75) | 26 (33.77) | |
| Stricturing | 22 (27.50) | 25 (32.47) | |
| Penetrating | 23 (28.75) | 26 (33.77) | |
| Montreal classification–Disease location (%) | | | 0.24 |
| L1 | 20 (25.32) | 28 (36.36) | |
| L2 | 13 (16.46) | 14 (18.18) | |
| L3 | 46 (58.23) | 35 (45.45) | |
| ADA started, months | | | <0.0001 |
| Mean (SD) | 6.17 (3.24) | 59.82 (48.49) | |
| Median (range) | 6 (1–12) | 46 (13–265) | |
| Steroid cycle | | | <0.0001 |
| Mean (SD) | 1.51 (0.95) | 3.82 (3.23) | |
| Median (range) | 1 (0–6) | 3 (0.16) | |
| Previous anti TNF (%) | 3 (3.75) | 17 (22.08) | 0.001^ |
| CRP, mean (SD) | | | 0.87 |
| Mean (SD) | 39.51 (36.48) | 40.09 (38.31) | |
| Median (range) | 32 (3–175) | 25 (2–165) | |
| Ferritin, mean (SD) | | | 0.21 |
| Mean (SD) | 16.15 (10.03) | 18.32 (10.90) | |
| Median (range) | 14.50 (2.30–45.00) | 17.00 (2.10–55.55) | |
| Fecal Calprotectin, mean (SD) | | | 0.03 |
| Mean (SD) | 437.99 (220.85) | 368.89 (216.06) | |
| Median (range) | 389 (16–1231) | 335 (45–1239) | |
| Albumin, mean (SD) | | | 0.17 |
| Mean (SD) | 2.90 (0.43) | 3.01 (0.46) | |
| Median (range) | 3 (1.7–3.8) | 3 (1.9–4.1) | |
| SES-CD, mean (SD) | | | 0.003 |
| Mean (SD) | 15.11 (6.22) | 12.17 (4.90) | |
| Median (range) | 15 (7–42) | 11 (0–25) | |
| HBI, mean (SD) | | | 0.53 |
| Mean (SD) | 13.77 (3.97) | 13.40 (4.17) | |
| Median (range) | 14 (7–28) | 13 (6–28) | |

**Wilcoxon rank-sum (Mann-Whitney) test; ^Chi-square test.*

>12 months (33.77%) ($p \leq 0{,}001$), and also better for the overall population (50.32%) (**Figure 1A**) (**Table 2**). Significant clinical response was observed in patients with disease duration >12 months ($p = 0.02$) to prove that adalimumab represents an effective and well-tolerated therapeutic option. Mucosal healing was significantly more frequent in patients with a disease duration <12 months, compared to patients with a disease duration >12 months ($p < 0.001$). At the end of the follow-up, almost all patients with a disease duration <12 months were characterized by endoscopic improvement 72/80 (43/80 MH and 29/80 endoscopic improvement) compared to patients with disease duration >12 months 29/77 (16/77 with MH and 13/77 with endoscopic improvement). Patients with a disease duration of <12 months achieved significant corticosteroid-free remission ($p < 0.001$) (**Figure 1B**). Dose escalation of ADA was obtained as increased frequency of weekly injections was successful in 75/157 (47.77%), 24/80 (30.00%) patients with a disease duration <12 months and 51/77 (66.23%) patients with a disease duration >12 months (**Table 2**). 2/80 patients (2.5%) treated with early ADA administration needed surgical resection at the end of the follow-up, compared to 12/77 (15.5%) of patients with late ADA treatment (data not shown).

## Anti TNF Naïve vs. Non-naïve

Of the 157 patients within the study, 137 (87.26 %) were naïve and 20 (12.73%) patients experienced anti-TNF treatment. Patients with disease duration <12 months were anti-TNF naïve 77/80 (96.25%) and 3/80 experienced (3.75%), patients with disease duration >12 months were anti-TNF naïve 60/77 (77.92%) and 17/77 experienced (22.07%). In particular, 68/157 ($51 < 12 + 24 > 12$) naïve patients achieved the clinical remission at 12 months compared with 4 ($2 < 12 + 2 > 12$) experienced patients. Therefore, we compared clinical remission, mucosal healing and dose escalation to determine the clinical outcome in both naive and (non-naïve) experience patients (**Table 6**). The rates of clinical remission were significantly higher in naïve patients with disease duration <12 months (66.23% vs. 40.00%, respectively; $p = 0.002$), while no significant difference was observed among experienced patients with duration disease <12 and >12 months ($p = 0.09$). A similar trend was observed for mucosal healing and dose escalation between naïve and experienced patients (**Table 6**).

# DISCUSSION

Since the introduction of biological agents, treatment strategies, able to induce and maintain remission and mucosal healing for CD patients, have dramatically improved (16). While there can be no doubts about their efficiency, an open discussion is still required to identify the most effective administration timing to achieve long term remission rates. The aim of our single-center retrospective analysis was to evaluate the real life efficacy of Adalimumab in patients with CD, to identify factors predictive of clinical outcome and to fill the knowledge gap regarding administration timing and clinical outcome. Although, there are several other similar real-life cohorts published, our study represents a real-life study of adalimumab in a single-center retrospective cohort of Italian patients with Crohn's disease.

In our study, 12 months after the beginning of ADA therapy, clinical remission was observed in more than half of the treated patients (79/157; 50,32%) with further improvement in the performance (90/157; 57,3%) at the end of the follow-up. This response rate is substantially equivalent to those reported by

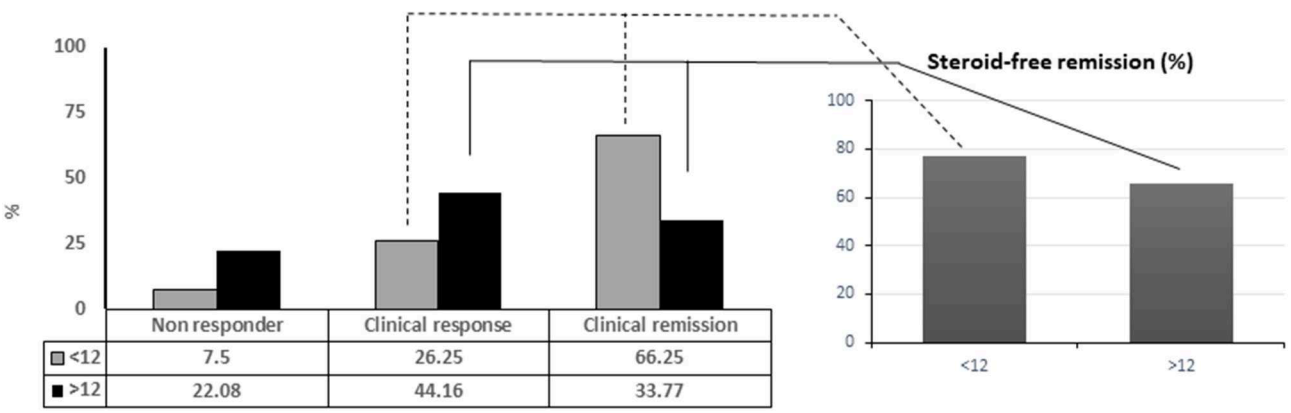


**FIGURE 1 |** Adalinumab treated patients achieving complete remission in relation with disease duration **(A)**. Percentage of patients under steroid free remission **(B)**.

**TABLE 6 |** Clinical outcomes at 12 mo–patients previously treated with anti-TNF.

| | Administration ADA | | | | | |
|---|---|---|---|---|---|---|
| | Naive | | | Experienced | | |
| | <12 months ($n = 77$) | >12 months ($n = 60$) | *p*-value | <12 months ($n = 3$) | >12months ($n = 17$) | *p*-value |
| Clinical remission | 51 (66.23) | 24 (40.00) | 0.002^ | 2 (66.67) | 2 (11.76) | 0.09§ |
| Mucosal healing | 42 (54.55) | 15 (25.00) | <0.001^ | 1 (33.33) | 1 (5.88) | 0.28§ |
| Dose escalation | 23 (29.87) | 38 (63.33) | <0.001^ | 1 (33.33) | 13 (76.47) | 0.20§ |

^*Chi-square test.*
§*Fisher's exact test.*

other authors (1, 3, 16–20). Furthermore, the percentage of patients with mucosal healing (37.58%) at 52 weeks raised to 43.9% at the end of the follow-up. The endoscopic data was obtained by performing at least two endoscopic evaluations during the follow-up, the first of them at 12.5 months from the start of the treatment. Our data offer a solid assessment of the treatment efficiency 12 months after the beginning of the Adalimumab administration.

It is crucial to underline that our data support the importance of an endoscopic evaluation at 52 weeks for a correct evaluation of the treatment efficiency.

Results previously published by Song et al. demonstrated the efficacy and safety of Adalimumab for Crohn's disease (21, 22). Furthermore, our data confirm what was previously reported about clinical remission and mucosal healing rates at 6, 12, and 24 months from the beginning of the treatment (18). Our multivariate analysis identified dose escalation, intervention timing later than 12 months from the diagnosis and previous treatment with an anti-TNF as prognostic factors negatively related to clinical remission achievement.

Unsurprisingly, an inflammatory phenotype and a short disease duration was associated with a higher mucosal healing rate (18). Adalimumab efficiency was negatively but significantly affected by longer disease duration and presence of strictures (23). These data, as recently published by Miyoshi et al. (24) suggested that treatment with this agent in the early stages of the disease may improve the clinical outcome, likely by preventing fibrosis development and, consequently, the need for surgeries (25–27). Although our understanding of fibrogenesis in CD continues to evolve, we believe that early administration of anti-TNF may block or attenuate the cascade of events leading to the fibrogenic process. Several TNF mediated mechanisms could occur, including epithelial tight junction disassembly, causing increased intestinal permeability and, consequently, an increased subepithelial exposure to bacterial antigens (25). Furthermore, fibroblasts, vascular endothelial growth factors, and endothelial permeability may have a pivotal role in the amplification of the inflammatory cascade (28). In light of our data, it is tempting to speculate that early control of gut inflammation is critical to prevent fibrostenotic intestinal injury previously described as a major factor leading to poor patient outcomes (29). Furthermore, the higher steroid-free remission (56.8%) and mucosal healing rates (43.9%) reported in the SONIC trial (30), enrolling biologic and immunosuppressant naïve CD patients with a short disease duration (median 2.3 years), contribute to indirectly support our evidence.

In routine clinical practice, a second anti-TNF drug is used when a first one has failed, regardless of whether patients are primary non-responders, secondary non-responders, or intolerant. Unsurprisingly, the meta-analysis results published in a systematic review by Gisbert et al. demonstrated that the efficacy of switching the anti-TNF agent in CD patients largely depends on the reason for switching (31). As the onset of fibrotic areas has an inverse correlation with the success rate of anti-TNF treatment, it seems clear that the administration timing should be among the most important factors for ADA-mediated clinical remission. Our multivariate analysis showed that dose escalation was a negative prognostic factor for both clinical remission and mucosal healing. Some clinical trials demonstrated that in Crohn's disease, Adalimumab dose escalation to 40 mg weekly was effective for managing secondary loss of response, allowing more patients to maintain clinical remission (32, 33). A recently published prospective reported that Adalimumab 80 mg administered weekly seems to be well-tolerated and may be effective in inducing clinical remission in CD patients previously treated with lower Adalimumab doses (34, 35). Our data for dose escalation for secondary loss of response during maintenance therapy (in patients with successful primary response) was 47.77%, in line with previous findings.

Although limited by potential bias due to the patients' prior treatment history, once enrolled in our clinical protocol, all patients were scored on identical biomarkers, radioscopic and endoscopic parameters. Furthermore, even if results were obtained through a retrospective study of early vs. late ADA administration, no differences were detected between the two groups of patients in terms of inflammation, calprotectin, endoscopic, and clinical index. Of note, the outcome was similar for non-smokers vs. smokers, suggesting that this factor was not relevant in the present study.

Patients in the early ADA-administration had a lower risk of dose escalation compared with late patients 12 months following biologic drug initiation (30.00% vs. 66.23%) and a lower risk of discontinuing or switching treatment, compared with 12 months after biologic initiation (6/80 vs. 17/77). Thus, a top-down approach to anti-TNF therapy may avert secondary loss of response in some patients.

Finally, remission rates were greater in naïve to anti TNF compared to non-naïve (experienced), but this may be mostly a consequence of an earlier intervention timing in naïve patients.

In conclusion, even considering the limitation of the present study consisting in a single-center retrospective cohort of Italian patients with Crohn's disease our data indicate that Adalimumab is a valid therapeutic option for the management of patients with moderate to severe Crohn's disease. Administration timing is a crucial factor, predictive of clinical outcome, indicating that ADA treatment should begin within the first year following the CD diagnosis. Although no acknowledged consensus has been reached in regard to the optimal timing for the administration of biological drugs in IBD, this study supports the view that introducing ADA treatment during the "window period," when structural alterations of the bowel are still not evident, may have a positive impact on the clinical history of the disease.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comitato Etico Istituto Tumori Giovanni Paolo II Bari–Prot. n. 42 del CE De Bellis 27/06/2017. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

MM, MCu, OB, RC, ES, RT, and PG: assembly of tissue and data, study design, and wrote the manuscript. MM and MCh: conceived of the study and editing of the manuscript. MLC: histological evaluation. MM, SD, EC, GS, PP, RD, and VG: data interpretation, evaluation. All authors critically reviewed the manuscript and gave final approval for publication.

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

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# Can We Predict the Toxicity and Response to Thiopurines in Inflammatory Bowel Diseases?

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Thiopurines are a cheap, effective treatment option in the management of inflammatory bowel disease (IBD). However, with the growing choice of targeted therapies available, as well as the well-documented toxicities of thiopurines, the role of thiopurines has been questioned. Nevertheless, given their inexpense in an era of spiraling healthcare costs, thiopurines remain an attractive option in the right patients. In the age of personalized medicine, being able to predict patients who will respond as well as those that will develop toxicity to a treatment is vital to tailoring therapy. This review will summarize the available literature with respect to predictors of response and toxicity to thiopurines in order to guide management in IBD. Specifically, toxicities addressed will include myelotoxicity, hepatotoxicity, pancreatitis, alopecia, gastrointestinal and flu-like symptoms, and complications associated with Epstein-Barr virus. While more work needs to be done to further our ability to predict both response to and side effects from therapies, pharmacogenomic research shows significant promise in its ability to personalize our use of thiopurines.

**Keywords: thiopurines, azathioprine, 6-mercaptopurine, inflammatory bowel disease, Crohn's disease, ulcerative colitis, toxicity, response**

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## INTRODUCTION

Thiopurines, including azathioprine, 6-mercaptopurine and tioguanine, are longstanding therapies within the ever-expanding inflammatory bowel disease (IBD) treatment armamentarium (1–5). They have shown themselves to be effective in the maintenance of remission in patients with IBD and have also resulted in reductions in the need for surgery, post-operative recurrence and IBD-related colorectal cancer risk. In addition, they improve pharmacokinetics of anti-tumor necrosis factor agents when used in combination with these therapies (6). Given their efficacy, oral delivery, and low cost they are frequently used as pre-biologic treatments in both Crohn's disease (CD) and ulcerative colitis (UC) and many clinicians have extensive experience with their use. However, up to 60% of patients will either respond inadequately or will develop toxicity to thiopurines (7), necessitating their cessation or treatment modification. With the continual advent of new targeted biologic therapies, the role of thiopurines in the current era is, therefore, being questioned (8).

The ability to use clinical and biologic characteristics of an individual to predict their disease course and to personalize their treatment pathway is the aim of precision medicine (9). An essential component of this goal is the ability to predict those who are more likely to respond or develop toxicity to a particular therapy, in order to improve the safety and efficacy of treatment choices. While some authorities suggest that treatment choices should not be influenced by cost, the compounding prevalence of IBD in conjunction with the increasing burden of drug costs mean

that such an approach is perhaps naïve within the context of finite resources. Thus, optimizing the use of inexpensive treatments like thiopurines could have significant financial advantages to health services.

While thiopurines are still felt to have a role in the current era (8, 10), our ability to tailor their use to a population which will both tolerate them and achieve the treatment goals that our new treatment paradigms demand will determine their use in the future. This article aims to summarize the available evidence with respect to clinical, genetic, and biological predictors of response and toxicity to thiopurines.

# PREDICTORS OF TOXICITY

Thiopurine use is undoubtedly hindered by the high incidence of adverse drug reactions which affect up to 25% of people who take them, resulting in drug discontinuation in 17% of patients (11). Side effects often occur in the first few months. Accordingly, the ability to predict which patients are likely to develop these potentially serious side effects would be of great use in clinical practice.

## Thiopurine-Induced Myelotoxicity

Thiopurine-induced myelotoxicity (TIM) is one of the most serious thiopurine-induced side effects and can occur at any time during treatment. In some patients this can lead to life threatening bone marrow suppression. Whilst leucopenia is the commonest hematological abnormality, thrombocytopenia, and pancytopenia can rarely occur. In a review of 66 studies, including more than 8,000 thiopurine-treated patients, the incidence rate of drug-induced myelotoxicity was 3% per patient year of treatment (12). In East Asian populations, however, the incidence of myelotoxicity can be as high as 15% (13).

The prodrug azathioprine is non-enzymatically converted to 6-mercaptopurine (6MP) and then through competing pathways is metabolized into thioguanine nucleotides (TGNs). TGNs exert their immunosuppressive effect by interfering with DNA replication of the most actively dividing cells, as well as by inducing apoptosis in activated and pre-activated T lymphocytes (14). Thiopurine-S-methyltransferase (TPMT) is an enzyme which catalyzes the methylation of 6-MP to 6-methylmercaptopurine (6-MMP), a non-therapeutic metabolite. Approximately 1 in 10 people have intermediate TPMT activity due to heterozygosity of TPMT, and 1 in 300 have TPMT deficiency, which is inherited in an autosomal recessive manner (15). In heterozygotes, TPMT*3A is the commonest mutant allele (85%), whilst TPMT*2 and TPMT*3C are rarer (15). TIM is strongly linked to low TPMT enzyme activity and high 6-TGN blood levels (16). Standard thiopurine dosing in heterozygous or TPMT-deficient patients leads to 6-TGN accumulation in the bone marrow and potentiates the risk of life-threatening bone marrow suppression.

TPMT phenotype testing is commonplace in clinical practice and is one of the most frequently used pharmacogenetic tests. TPMT enzyme assays can also be used alongside genotyping, which is used less commonly, to assess activity where rarer mutations may be missed on genotyping. Genetic testing is useful in patients with renal failure and reduced clearance of TPMT inhibitors, where enzyme activity can be falsely low. However, routine genotyping is not commonplace with some evidence suggesting that this may not be cost-effective compared with standard phenotyping (17, 18). TIM can also occur with normal TPMT activity necessitating regular full blood count monitoring in clinical practice to allow dose reduction or drug cessation in cases of TIM. A meta-analysis found that low TPMT is associated with TIM but not hepatotoxicity or pancreatitis (19).

Nudix hydrolase 15 (*NUDT15*) variants have also been linked to altered thiopurine metabolism and TIM (20). Mutations of *NUDT15*, which occur more frequently in the East Asian population, lead to reduced enzyme activity and TIM in a TGN-independent manner (20, 21). More recently, variants in *NUDT15* were found to be associated with increased risk of TIM among IBD patients of European ancestry (22). Furthermore, patients with mutations in both *TPMT* and *NUDT15* developed TIM faster (22). These findings highlight the importance of *NUDT15* genotyping, alongside *TPMT* phenotype, or genotype testing. This is supported by a recent systematic review and meta-analysis (23), and recently published guidelines provide suggested dosing regimens in patients with *TPMT* or *NUDT15* variants (24).

It may also be possible to predict early myelotoxicity by measuring thiopurine metabolites soon after treatment commencement. In a Dutch study, patients with 6-TGN levels of more than 213 pmol/8 $\times$ $10^8$ red blood cells (RBC) and 6-MMP levels higher than 3,525 pmol/8 $\times$ $10^8$ RBC measured after 1 week of thiopurine initiation were six times more likely to have early TIM (25).

## Thiopurine-Induced Hepatotoxicity

Thiopurine-induced hepatotoxicity (TIH) is an uncommon but important side effect of thiopurine use. Most commonly, this results in increased transaminase levels, which resolves with dose reduction or drug discontinuation. Less commonly, TIH manifests as idiosyncratic cholestasis or nodular regenerative hyperplasia. A systematic review, which included 3,485 patients, described an overall prevalence of 3.4% for TIH (26). In a pediatric cohort, TIH was found to be strongly correlated with 6-MMP levels with a 3-fold increased risk at levels >5,700 pmol/8 $\times$ $10^8$ RBC (16). In a Dutch cohort study of 270 adult patients, when TIH occurred it did so within 8 weeks in 85% of patients and was associated with elevated 6-MMP levels (27). Furthermore, in the same study, a predictive algorithm based on a week one 6-MMP level >3,615 pmol/$10^8$ RBC, older age, male gender and higher BMI yielded an area under the curve of 0.83 (95% CI: 0.75–0.91) for hepatotoxicity risk. Another study found elevated 6-MMP levels in those with TIH but sensitivity and specificity were poor (28). These studies highlight that whilst 6-MMP levels are associated with TIH, intervention should be reserved for those in whom the high 6-MMP levels are associated with abnormal liver function tests.

Nodular regenerative hyperplasia is a condition characterized by diffuse nodulation of the hepatic parenchyma, leading to portal hypertension. Although its natural history is not clearly understood, it can occur in patients treated with purine analogs,

particularly tioguanine (29). Studies have shown that this is more likely to occur in male patients with a stricturing small bowel disease phenotype (30–32). Although rare, regular monitoring of blood tests is necessary, particularly for the gradual onset of thrombocytopenia signaling portal hypertension.

Although there is no validated genetic predictor of TIH, there is a worldwide collaborative effort to achieve this aim. This includes the Helmsley IBD Exome Sequencing Program (33), and the Predicting Serious Side Effects in Gastroenterology (PRED4), conducted by the UK IBD Genetics Consortium.

## Thiopurine-Induced Pancreatitis

Pancreatitis occurs in <5% of patients treated with azathioprine or mercaptopurine and often occurs in the first month of treatment (11, 34, 35). Reinstating therapy upon recovery leads to recurrent pancreatitis, so indefinite drug withdrawal is required although a switch to tioguanine may be considered (34, 36). Thiopurine-induced pancreatitis (TIP) is an idiosyncratic drug reaction and the pathophysiology is unknown. Interestingly, and for unclear reasons, patients treated with thiopurines for IBD have a greater incidence of TIP compared to those treated for other diseases (37). However, TIP is almost always mild in IBD patients and generally responds rapidly to drug withdrawal (38). Smoking has been found to be a strong risk factor in TIP (39), along with having CD (11, 38).

Two genome wide association studies of patients with TIP found a link to the class II HLA region, with the most significant associations identified being at rs2647087 (40, 41). Patients heterozygous at rs2647087 have a 9% risk of developing TIP and homozygotes have a 17% risk (40) although tests to predict risk of TIP are not yet commonplace in clinical practice. Approximately 76 patients need to be genotyped for rs2647087 to prevent one case of pancreatitis, and given that most cases of TIP run a benign course, there is an argument that screening may not be a cost-effective strategy.

## Thiopurine-Induced Alopecia

Alopecia secondary to thiopurine use is a rare, dose-related adverse event, with an incidence of 1.5% in patients of Asian descent (13). Whilst clearly not life-threatening, alopecia can have profound psychological effects and increases the risk of non-compliance with therapy. Studies have shown that the *NUDT15* variants are associated with risk of thiopurine-induced alopecia (42, 43). Therefore, dose reduction in heterozygotes and thiopurine avoidance in homozygotes can mitigate and avoid both TIM and alopecia in this cohort.

## Gastrointestinal Toxicity and Flu-Like Illness

The most common but least serious adverse effects of thiopurines are gastrointestinal disturbances (nausea, vomiting, abdominal pain) and flu-like symptoms (malaise, fever, myalgia), which are responsible for drug discontinuation in many patients. The flu-like symptoms are likely to be immune-mediated and tend to occur shortly after starting treatment. It is not clear if the reactions are dose-dependent or idiosyncratic. A prospective evaluation of azathioprine-treated IBD patients found that *TPMT* heterozygosity strongly predicted GI adverse effects (37% heterozygous vs. 7% wild-type *TPMT*, $P < 0.001$) (44).

Switching treatment to 6MP may be one way to curb some of these side effects. An observational study and systematic review demonstrated 60% of patients intolerant of azathioprine were able to tolerate 6MP (45). In those ceasing 6MP due to further adverse effects, 59% experienced the same side effect as they had with azathioprine.

## Serious Complications Associated With Epstein-Barr Virus (EBV)

The association between thiopurine use and EBV-driven B-cell lymphoma has been understood for many years. A roughly four-fold increase in risk over background has been identified across several studies (46) and, thus, the greatest absolute risk is in those with the highest background risk, i.e., the elderly.

In addition, severe and potentially fatal EBV primary infections and post infectious lymphoproliferative disorders have also been associated with thiopurine use (46–48). This has prompted some to advocate for pre-treatment EBV serology testing and avoidance of thiopurines, if possible, in EBV seronegative individuals (48). In the CESAME study (Cancers Et Surrisque Associé aux Maladies inflammatoires intestinales En France), a low incidence of 0.1 per 1,000 patient years of postmononucleosis lymphomas was observed overall, rising to 3 per 1,000 patient years when considering young males, seronegative for EBV (46). In addition, in a pediatric population of 5,766 participants, there were 5 cases of haemophagocytic lymphohistiocytosis (HLH), all exposed to thiopurines, equating to an incidence of 0.2 per 1,000 patient years (49).

Despite the majority of pediatric patients being EBV seronegative at initiation of thiopurines (50), the incidence of HLH is low. Furthermore, as EBV is not the sole trigger of serious infectious complications like HLH (47), some argue against routine pre-thiopurine EBV testing (51, 52). Nevertheless, given the potentially severe, albeit rare, consequences of primary EBV infections or post infectious lymphoproliferative disorders in patients on thiopurines, coupled with the increasing availability of therapeutic alternatives, we carefully balance the risk and benefit of thiopurine use in EBV negative patients, but do not avoid its use completely.

# PREDICTORS OF RESPONSE TO THIOPURINES

The ability to predict who will respond to thiopurine therapy and to maximize likelihood of response earlier in the disease course would enable clinicians to tailor therapy sooner, with the aim of altering the natural history of the disease (53). Heterogeneity in definitions of response, as well as the tenuous relationship between clinical response and mucosal activity make interpretation of the literature challenging with respect to prediction of response. While thiopurine metabolite monitoring enables personalized dosing, it obviously relies on patients having already commenced the therapy; pre-treatment predictors are the ideal.

## Clinical Predictors

The relatively small numbers of patients in azathioprine efficacy studies has limited our ability to identify clinical predictive factors of response (3, 54). As such, clinical predictive factors have thus far not been incorporated into clinical practice in a significant way. A number of retrospective studies have identified factors that may predict response, or lack of it, although their results must be interpreted with caution.

Some of the largest studies addressing predictive factors of thiopurine response have yielded conflicting results in terms of disease type (CD vs. UC) and location. In a single center review of 622 patients, remission rates in those who completed 6 months of azathioprine were highest in UC patients compared to CD (87% vs. 64%, $p = 0.0001$) (55). In CD cases specifically, colonic distribution was associated with a higher rate of clinical remission compared to other distributions. This finding was mirrored in another study which found that azathioprine caused mucosal healing in 70% of patients with Crohn's colitis and 54% with ileitis (56). However, in a study of 139 IBD patients, rates of response to thiopurines were highest in patients with ileal CD (27 responders vs. 2 non-responders, $p = 0.003$) (57). No difference was found in response rate in other IBD subtypes, although numbers were small.

Body mass index (BMI) has been associated with response, surprisingly with opposite effects in UC and CD. In a large retrospective study ($n = 1176$), patients with UC with a BMI <25 had a lower flare rate after starting azathioprine than those with BMI >25, albeit only in those with disease duration <3 years (58). In CD, flare rates were similar between BMI groups, however upon azathioprine withdrawal, patients with a BMI <25 had higher flare rates than BMI >25 (58). It is theorized that adipocytes and fatty tissue may play an immunological role, involved in the physiologic and pathologic regulation of the immune system and inflammation (59). BMI, however, had no effect on thiopurine efficacy in a smaller study (57).

With regard to clinical disease activity, it has been reported that long term clinical response is improved in CD when azathioprine is commenced when patients are in remission (58). While this may reflect less severe disease, the same difference was not seen in UC. In a Korean study published in abstract form only, however, high Mayo score was associated with thiopurine treatment failure in patients with UC (HR 1.28, 95% CI 1.04–1.58, $p = 0.023$) (60). In addition, in a cohort of mixed IBD patients response was associated with shorter duration of disease at the time of commencing azathioprine than non-response ($47.4 \pm 6.6$ months among responders, vs. $85.4 \pm 14.6$ in non-responders, $p = 0.007$) (57), suggesting earlier introduction of thiopurines may improve response.

In contrast to these studies, a prospective double-blind trial of patients with a recent (<8 weeks) diagnosis of CD found that rates of corticosteroid-free clinical remission at week 76 were similar in azathioprine and placebo-treated patients (61). This finding was supported by an open-label French study, in which early (<6 months) administration of azathioprine was no more effective than conventional management (62). Whilst the findings of theses studies need careful interpretation (63, 64), the early introduction of thiopurines cannot, therefore, be recommended in all patients with CD.

Ethnicity may also play a role in thiopurine metabolism and response. In an observational study of Chinese patients with UC, standard dose thiopurine (>2 mg/kg/day) was compared to low dose thiopurine therapy (<2 mg/kg/day). Cumulative relapse-free survival rates were similar between groups, however a three-fold increased risk of leucopenia was seen with standard dosing (65). This may be reflective of variations in genotypes between ethnic groups such as has been seen with variants of *NUDT15*, associated with an increased risk of leucopenia, which are more commonly found in Asian patients (22, 66).

The effect of gender has been conflicting across studies, and likely plays no role. Female gender was found to be associated with thiopurine response by some (57, 67), with the opposite found in a pediatric population (68) and no difference in another larger adult population (55).

## Thiopurine Metabolites and TPMT

6-TGN levels have been shown to correlate with efficacy (69), and levels $\geq 235$ pmol/$10^8$ RBC are associated with clinical response and remission in thiopurine monotherapy (16). Meta-analysis data confirm this, showing a higher rate of clinical remission in patients with a 6-TGN above this threshold compared with below (62 vs. 36%, pooled OR 3.3, 95% CI 1.7–6.3; $p < 0.001$), as well as higher TGNs in patients in clinical remission vs. active disease (53, 70). However, it must be recognized that these data are based upon studies which are small, heterogeneous and generally retrospective (71) and a prospective multicenter study of thiopurine weight-based dosed IBD patients found a poor relationship between TGN and clinical response rate, with no useful TGN cut-off determinable (72).

While a threshold of 235 pmol/$10^8$ RBC may be sufficient for clinical remission, mucosal healing, increasingly recognized as a more robust, and potentially disease-modifying endpoint (73, 74), may require higher levels. A recent multicenter, international retrospective study showed that 6-TGN levels were associated with mucosal healing, and that a level of 397 pmol/$10^8$ RBC was 86.7% specific but only 35.3% sensitive for mucosal healing (75). However, higher 6-TGN levels are also associated with increased rates of early or late myelotoxicity (23), particularly above 450 pmol/$8 \times 10^8$ RBC (76), and so a fine balance exists between response and toxicity. Interestingly, while a lower thiopurine dose may be sufficient for Asian patients, as discussed above, it is the 6-TGN and not the dose that was associated with mucosal healing in a cohort including a large proportion of Chinese patients (75).

The optimal use of thiopurine metabolite levels (6-TGN and 6-MMP) to maximize response, however, is a controversial area, with practices varying across the world (77–79). Observational data support the use of TGN monitoring in non-responding patients, with TGN-directed dose optimization eliciting a response rate of 87–90% compared to 18–33% in which treatment was not TGN directed ($p<0.001$ for both studies) (77, 80). In one retrospective study of 169 patients undergoing thiopurine metabolite testing, the majority (52%) had subtherapeutic 6-TGN levels and testing resulted in a change in patient treatment in 68%

of patients overall and 86% of patients with active disease and sub-therapeutic levels (81).

However, prospective randomized trials of TGN monitoring vs. standard weight-based dosing in patients commencing on thiopurines for IBD have failed to show benefit (82, 83). In a study of 57 patients, rates of clinical remission in TGN-guided vs. standard weight-based dosing groups were similar at 16 and 24 weeks (82). However, it should be noted that mean 6-TGN levels in the standard group ranged between 216 and 266 pmol/$10^8$ RBC. This is in contrast to real world data suggesting 50% of thiopurine-treated patients are not receiving the appropriate weight-based dose, corresponding to 40–50% being underdosed on TGN criteria (80, 81). In another prospective study of 50 patients, clinical remission rates at week 16 were higher in the TGN-based vs. weight-based dosing. However, this failed to achieve statistical significance, possibly due to underpowering (40% vs. 16%, $p = 0.11$) (83).

Thiopurine metabolite testing is also helpful when preferential 6-MMP metabolism or "shunting" occurs. This phenomenon is associated with reduced efficacy and increased side effects (84). Fortunately, it can be overcome with a reduction in dose of the thiopurine and the introduction of allopurinol (85–87). Indeed, commencing low dose thiopurine-allopurinol combination therapy at thiopurine initiation, regardless of TGNs, may achieve higher response rates and reduced side effects (88).

While measuring 6-TGN may be useful for optimizing response, it may also be useful for predicting a lack of response. In patients with active disease on thiopurine therapy, persistence despite a therapeutic 6-TGN is unlikely to result in success (80), necessitating treatment alteration.

*TPMT* activity has also been assessed with regards to its role in predicting response. In a study of 39 patients with IBD, patients with *TMPT* activity <30.5 EU/mL were more likely to have a clinical response to thiopurines than those with higher *TPMT* activity (65 vs. 29%, $p = 0.05$), independent of TGN values. In patients with *TPMT* activity <30.5 EU/mL and a therapeutic 6-TGN, 100% responded compared to 25% with higher *TPMT* activity and low 6-TGN ($p = 0.01$) (89). Others, however, have found no relationship between TPMT activity and clinical response (72). Given biologic plausibility as well as evidence that low *TMPT* activity is associated with higher 6-TGN (16), clinical response is more likely to be related to TGN than the *TPMT* activity.

### Genetic Predictors

*TPMT* polymorphisms have not been associated with response (67, 90). In a prospective multicenter trial of 783 patients randomized to either *TPMT* polymorphism pre-screening and pre-emptive dose reduction vs. standard treatment, clinical response rates did not differ (90).

A recent small pharmacogenomic study assessed the role of polymorphisms of potential genes of relevance to azathioprine metabolism on clinical response and toxicity to azathioprine in IBD. *GSTM1* deletion, a polymorphism of the gene encoding Glutathione-S-transferase, the enzyme responsible for conversion of azathioprine to 6-mercaptopurine, was significantly associated with poor response to azathioprine on multivariate analysis albeit with a wide confidence interval (OR 9.22, 95% CI 1.081–78.62, $p = 0.042$) (67).

Published in abstract form only, a predictive model for achieving corticosteroid free remission with thiopurines at 26 weeks in a pediatric cohort of mixed IBD patients showed promise. Using novel pharmacogenetic genome-wide association study-identified loci, the previously identified IBD susceptibility locus HLA-DRB-1, and clinical features including pANCA positivity, disease duration, and diagnosis of UC as opposed to CD, the model had an area under the curve for corticosteroid-free remission of 0.985 (68).

## CONCLUSION

Thiopurines remain an effective treatment for IBD, with their relative cost, decades of use and the ability to measure and optimize metabolites maintaining their role in the biologic era. As we strive for an era of personalized medicine and gain further experience with our expanding therapeutic choices, our ability to predict thiopurine response and toxicity, and to tailor therapy accordingly, will determine its future role. While thiopurine metabolite monitoring shows utility in those already commenced on thiopurines, pharmacogenetic testing, which already plays a significant role in preventing toxicity, shows some promise in predicting response.

## AUTHOR CONTRIBUTIONS

RL, SH, and GC wrote and reviewed the manuscript. PI supervised and reviewed the manuscript.

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REVIEW
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# Tailoring Biologic or Immunomodulator Treatment Withdrawal in Inflammatory Bowel Disease

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There is currently no cure for inflammatory bowel disease. Most recent treatments and treatment strategies allow for healing intestinal lesions and maintaining steroid-free remission in a subset of patients. These patients and their doctors often ask themselves whether the treatment could be withdrawn. Several studies in both Crohn's disease and ulcerative colitis have demonstrated a risk of relapse, which varies between 20 and 50% at 1 year and between 50 and 80% beyond 5 years. These numbers clearly highlight that stopping therapy should not be a systematically proposed strategy in those remitting patients. Nevertheless, they also indicate that a minority of patients may not relapse over mid-term and that those who have relapsed may have benefited from a drug-free period before being treated again for a new cycle of treatment. In this context, it would be good to optimally select patients who can be candidates for a successful treatment withdrawal. The criteria impacting this decision are as follows: the risk of relapse (linked to factors like mucosal healing and biomarkers), the consequence of a potential relapse, the tolerance and potential side effects of therapy, patients' priorities and preferences, and the costs. Integration of these parameters allows for the proposal of a decisional algorithm that may help the patients and doctors to make an appropriate decision for their individual case.

**Keywords: Crohn's disease, ulcerative colitis, treatment withdrawal, prediction, relapse**

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## INTRODUCTION

The cure for a disease is logically considered as a main situation where a treatment withdrawal can be decided. However, there is currently no cure for inflammatory bowel disease (IBD). In our current conception, those are multifactorial polygenic diseases (1). Therefore, a cure is highly unlikely. What we could imagine is to be able to sufficiently modify the environment to be able to stop the ongoing immuno-inflammatory process (2). There are two limitations to this possibility: first, the self-perpetuation of inflammation would be installed and not be possible to stop even retrieving environmental triggers, and second, the cumulated tissue damage would generate symptoms. This second point should not be an obstacle to treatment withdrawal but would rather require complementary symptomatic treatments. Beyond this, a treatment withdrawal would also make sense when the benefit of the treatment is lower than its risk and/or cost. Most often, it is considered that cost here is a political health care system or a pragmatic insurance company decision that cannot be made at the level of individual patients. The situation where it could be decided on an individual patient basis is when the patient is not covered for his/her medical fees

and has to decide himself or herself how to spend money, including for health care. This situation is very rare in western Europe. Nevertheless, public or private health institutions have important decision to make in this field. For them, the benefit/cost ratio is certainly relevant and has to be taken into account. For the physician, it is thus usually the benefit/risk ratio that is dominant. Assessing this is not an easy task as the physician thus needs to integrate and compute at the same time the risk of ongoing drug therapy and the benefit of this therapy. Furthermore, the risk linked to treatment withdrawal is not limited to the risk of relapse. We also have to consider the probability of rapidly recovering remission after retreatment, and if the response to retreatment was not appropriate, the consequences of the disease flare, including the risk of surgical resection. It is even more complicated as the physician should also integrate the patient's preferences and priorities. Indeed, the acceptance of the risk of side effects and the risk of disease progression may vary from patient to patient.

The aims of this review article are to illustrate the most important factors to consider when contemplating treatment withdrawal in IBD and to propose a way to integrate these various factors. The benefit/risk and benefit/cost ratios of mesalazine has been recently reviewed and probably remains positive over time (3). Therefore, we will focus on biologic and immunomodulator withdrawal. As far as biologic therapy is concerned, there are currently essential data on anti-tumor necrosis factor (anti-TNF) and concerning an immunomodulator, essentially purines.

## THE RISK OF RELAPSE AFTER TREATMENT WITHDRAWAL IN IBD

The risk of relapse after treatment withdrawal is probably a point that has been best documented. Overall, both in Crohn's disease (CD) and in ulcerative colitis (UC), the risk of relapse after stopping anti-TNF is around 50% over 1–2 years (4, 5). It is probably increasing with time of follow-up and has been described around 70–80% in CD after 7–8 years (6). Withdrawal of immunomodulator seems to be associated with a slower relapse risk (7). It has been estimated to be around 20–30% after 1–2 years. However, here again it progresses with time and reaches >50% after 5 years (8). After retreatment, over the short term, most of the patients respond to resuming both anti-TNF or immunomodulator (4, 5, 9). For anti-TNF, a small proportion will lose response over time, but a substantial number of them are still effectively treated with the same drug more than 5 years later (6). In UC, up to 10% of withdrawn patients may have to undergo colectomy within 1 year after anti-TNF withdrawal (10), while this proportion seems lower in CD with also 10–15% but only over 7–8 years (6). These risks are too high to propose a treatment withdrawal in all patients reaching sustained steroid-free remission in IBD. This assertion is reinforced by patients' survey highlighting the fact that among them, the majority would only accept a maximum risk of relapse of 25% (11). According to this, we should try to identify a subpopulation with a risk of relapse lower than 25%. Predictors of relapse have been studied in many studies with anti-TNF and immunomodulators. No data are available for vedolizumab or ustekinumab. These predictors have recently been extensively reviewed, and results are heterogeneous (3–5, 12). This heterogeneity is explained by the heterogeneity of the study populations, including the differences between prospective trials and retrospective analyses of routine practice populations. In routine practice, the selection of the population for treatment withdrawal is more stringent, focusing on, for example, patients in endoscopic remission, while prospective trials may also have included patients still having endoscopic lesions. A certain heterogeneity can also be explained by predictors that have been studied, particularly in retrospective studies where only a limited amount of variables was available. Results were also different when considering the withdrawal of anti-TNF or immunomodulator and in CD or in UC. In general, predictors have been more difficult to disclose in UC than in CD. In the largest retrospective study so far, while a series of predictors could be found for CD, none was found for UC (12). Among the most prominent predictors are the direct or indirect signs of persisting disease activity: endoscopic lesions, elevated blood markers of inflammation (C-reactive protein), and elevated stool markers of inflammation (fecal calprotectin) (3–5). Other prominent predictors are linked to ongoing treatment: co-treatment with an immunomodulator and low or undetectable trough level of anti-TNF were associated with a lower risk of relapse when stopping this anti-TNF (3–5). According to this, persisting endoscopic lesions and trough level of the drug are often considered as key factors for clinicians to be assessed in clinical practice before considering drug withdrawal. Albeit important, they only represent part of the problem. Indeed, in the STORI cohort, even in patients with full endoscopic healing, the relapse rate after infliximab withdrawal was 30% over 1 year (as compared to 45% in the general population and 10% in the low-risk group). Likewise, a low or undetectable trough level of infliximab has been associated with a decreased risk of relapse upon withdrawal. This makes sense and probably corresponds to situations where infliximab has a minor impact on the maintenance of remission. It is, however, not so straightforward, as a low trough does not necessarily mean no effect of the drug. This drug may still generate relevant exposition linked to peak concentration and area under the curve of this concentration over 4–8 weeks. In the STORI cohort, the infliximab level was not associated with the risk of relapse in univariate analysis but was only selected in the multivariate model. Other factors have been proposed, but they either also indirectly reflect ongoing disease activity or current treatment or are more difficult to explain and need to be confirmed. Smoking, which has often been associated with bad outcome in CD, has only been found predictive of relapse after stopping anti-TNF in one study (3). Histologic remission, which is becoming an important outcome in UC and which is questioned in CD, has not been adequately studied as a predictor after treatment withdrawal in IBD. According to these results, the best candidates for anti-TNF withdrawal would be patients with clinically, biologically, and endoscopically inactive disease and with immunomodulator co-treatment and/or low-undetectable biologic drug level (**Table 1**). In the STORI cohort, it represented 15–20% of the patients recruited in the trial (9). This gives an estimation of the proportion of patients among those

**TABLE 1 |** Most important factors favoring treatment withdrawal in IBD.

| |
|---|
| **Factors associated with a lower risk of relapse** |
| Mucosal healing (mainly CD and anti-TNF) |
| Normal CRP (mainly CD) |
| Low fecal calprotectin (<250 μg/g) (mainly CD and anti-TNF) |
| Low or undetectable trough levels of biologic treatment (mainly CD and anti-TNF) |
| Immunomodulator co-treatment (mainly CD and anti-TNF) |
| **Factors associated with low cumulative intestinal tissue damage** |
| No complex perianal disease |
| No severe rectal disease |
| No intestinal or colonic stricture |
| No history of intra-abdominal abscess or fistula |
| Limited extent of the disease in the past |
| **Factors associated with increased risk of treatment side effects** |
| Older age (>65 years old) |
| Co-morbidities favoring infection or the risk of cancer |
| Side effects attributed to the treatment |
| **Patient's preference** |
| Pregnancy |
| High fear of treatment side effects |
| Low fear of surgery |
| Acceptance of relapse risk |
| **Cost** |
| Expensive medication |
| No/insufficient reimbursement |

*For the factors associated with a lower risk of relapse, the situations for which evidence is the strongest are put under brackets.*
*IBD, inflammatory bowel disease; CD, Crohn's disease; TNF, tumor necrosis factor; CRP, C-reactive protein.*

having longstanding steroid-free remission under combination therapy, with a low risk of relapse. However, as the retreatment upon relapse seems safe and effective and as a substantial number of patients may benefit from at least temporary drug withdrawal, the candidates for temporary withdrawal may be more numerous.

## THE CONSEQUENCES OF THE RELAPSE AFTER TREATMENT WITHDRAWAL IN IBD

Relapsing after biologic or immunomodulator treatment withdrawal would be a minor problem if a remission could rapidly be re-captured and without disease progression leading to the need of a surgical resection. The situation is obviously much different if the relapse is associated with the development of a complication, like a stricture, an abscess, or a fistula in CD, and an acute severe colitis in UC. For UC, the occurrence of such acute severe colitis remains unpredictable and does not help to tailor the decision (13). In some series, however, the colectomy rate was up to 20% of relapsing patients and is thus an important limitation for this strategy (10). CD patients already having a history of perianal fistulizing disease or intestinal strictures or fistula and abdominal abscess are at risk of recurrence (12, 14). Likewise, patients already operated on have a significant amount of intestinal tissue damage, and the clinician should be very careful not to increase it, particularly when there is a risk of short bowel or a risk of subtotal colectomy or stoma (15). In the published studies, the risk of relapse was particularly high in patients with previous fistulizing perianal disease (14). Probably explaining this, previous studies have illustrated that patients experiencing full clinical closure of their perianal fistulas under anti-TNF treatment usually keep signs of active inflammation in their fistulous tracks and that the full healing and disappearance of these fistulous tracks are very rare (16, 17). Likewise, previous studies have shown a possible increased risk of relapse in patients with a history of intestinal strictures or fistulas (12). In those studies, the risk of developing new strictures, fistulas, or abscess after anti-TNF of immunomudulator withdrawal was not clearly indicated, but in the long-term follow-up of the STORI cohort, with a median follow-up of 7 years, only 18% of the patients developed major complications including the need for surgical intestinal resection and new complex perianal fistulas. According to this, the best candidates for treatment withdrawal would be patients with no history of complex perianal disease; no significant and recent stricture, fistula, or intra-abdominal abscess; and no extensive surgical resection in CD (**Table 1**). Likewise, patients with left-sided UC or proctitis could be better candidates than those with pancolitis. Age is also important to take into account as young patients will have to live longer with their disease and are thus at increased risk of complications and cumulative intestinal tissue damage.

A key element in case of relapse after treatment withdrawal is the ability to re-capture the remission with the same drug. This may be jeopardized by drug immunogenicity for biologics and the development of anti-drug antibodies. These anti-drug antibodies have been associated with transient drug withdrawal, particularly with infliximab. This was particularly pronounced in early experience with infliximab when only induction treatment was given, followed by on-demand therapy. Scheduled treatment and immunomodulator co-treatment have clearly decreased immunogenicity, and in the STORI trial, only a few patients developed anti-drug antibodies and none experienced acute severe infusion reaction when resuming therapy (9). However, in the STORI trial, due to this theoretical risk of allergic reaction when restarting infliximab, a steroid infusion was given before resuming infliximab and the first infusions were performed at a slower pace, with a small amount of the drug infused during the first hour. This is still our practice today, although no controlled clinical trial validated this strategy. The risk of immunogenicity with more recent biologics in the context of transient drug withdrawal is less well-documented.

## THE RISK OF ONGOING TREATMENT IN IBD

The risk and tolerance of ongoing treatment is primarily influenced by age and comorbidities (18). The risk of severe infection under anti-TNF therapy has been shown to be significantly higher in patients older than 65 years (19). Likewise, anti-TNF and purine analogs are associated with

an increased risk of lymphoma (20). The relative risk has been estimated around 2 for anti-TNF and around 2–4 under purine analogs, while it culminated at 4–6 under combined therapy (20). However, this risk increases with age, leading to a substantial number (albeit still low in absolute numbers) of patients older than 65 years developing lymphoma under these drugs (21). Purines have also been associated with other forms of cancers, including skin cancers and urinary tract cancers (22, 23). For these reasons, most clinicians now try to decrease the use of purine analogs beyond 60–65 years of age. The impact of anti-TNF and other biologics on other cancers is not well-documented, apart from skin cancers and perhaps melanoma under anti-TNF (24). Nevertheless, due to the increased risk of cancers in aging people, drugs with a systemic immunosuppressive effect should be used with caution. Some comorbidities may also require attention. It includes chronic obstructive pulmonary disease, which is associated with an increased risk of bronchopulmonary superinfection (25). Again, this may be increased by drugs having a systemic immunosuppressive effect.

Another aspect is mild intolerance to the drug, like some skin manifestations under anti-TNF therapy (26). Most often, these manifestations are not sufficient *per se* to lead to treatment interruption if the benefit of the treatment remains significant (27). However, in some situations, it may represent one argument among many others that may influence the decision.

Therefore, from this point of view, the best candidates for treatment withdrawal would be patients with some degree of intolerance to the drug, or older patients (usually above 60–65 years of age) or those having comorbidities increasing the risk of infection or cancer (**Table 1**).

## THE COST OF ONGOING OR STOPPING TREATMENT STRATEGIES

The cost of ongoing treatment will vary very much depending on its nature: biologic therapy, biosimilar, or immunomodulator. Although recent studies have demonstrated that a growing part of the cost of management of IBD was linked to biologic therapy, this did not take into account the spared costs due to a decrease of hospitalizations or surgeries (28). In early studies with infliximab, the drug was considered as cost-effective in CD but only for one or a few years of therapy, the cost-effectiveness being not demonstrated beyond this duration (29). A more recent study specifically looked at the cost-effectiveness of a strategy of cycles of biologic therapies, including periods of withdrawals when the patients were in long-standing remission (30). This study showed that the cost-effectiveness of continuous therapy was favorable at some drug cost thresholds. Interestingly, with biosimilars, these thresholds have recently been reached in several European countries. The situation for biologics paid at the full price is different, and for those, the continuous treatment is generally less cost-effective than cycles of biologic treatment. The price of

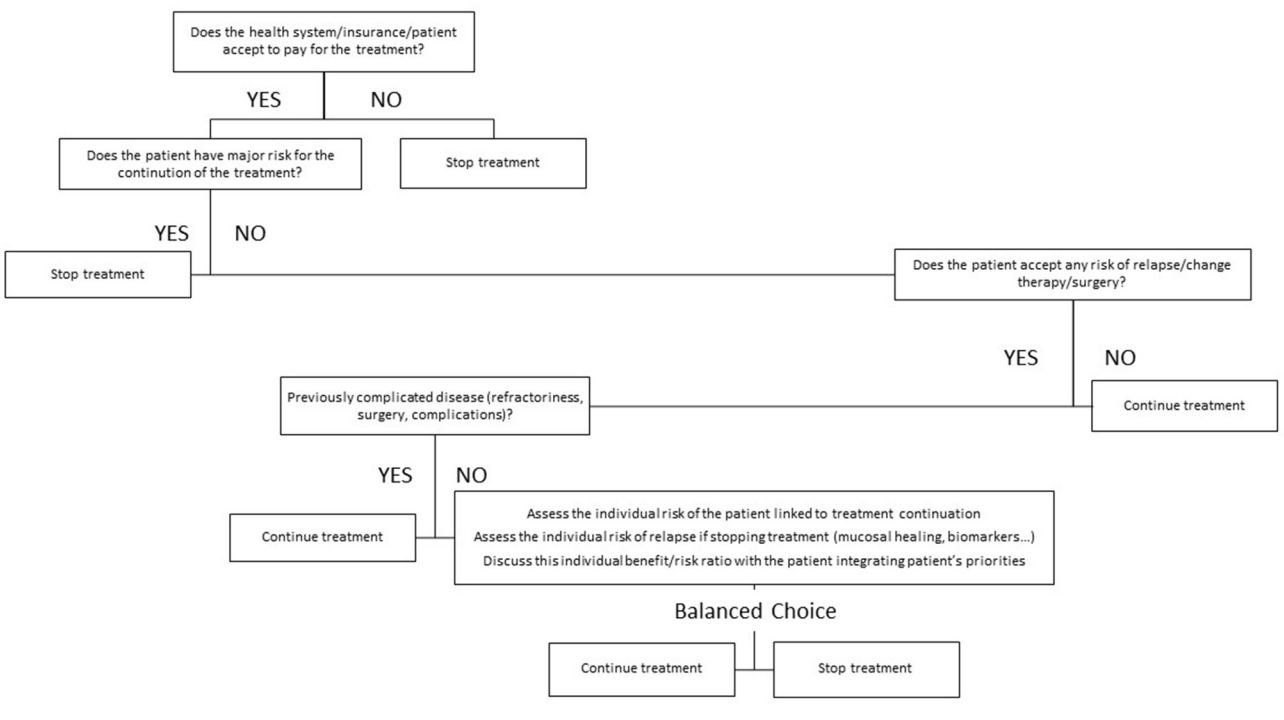


**FIGURE 1 |** Proposed algorithm for treatment withdrawal decision in inflammatory bowel disease (IBD). This algorithm may provide a hierarchy among the questions and factors that have to be assessed when contemplating treatment withdrawal in IBD. Some factors like the cost and the reimbursement may be specific to some health care systems and jurisdiction.

purine analogs is usually so low that continuous therapy is most often cost-effective.

## PATIENTS' PREFERENCES AND PRIORITIES

Due to personal views on the disease and its treatments, patients may be more keen to accept consequences or complications of the treatment or of the disease itself. The choice between medical therapy and surgical therapy (which may be a consequence of withdrawing therapy), for example, may vary among patients. In a dedicated patients' survey, it was shown that the risk of severe infection or lymphoma that the patient would accept to be in remission would vary very much but would be usually higher than the one accepted by their doctors (31). More specifically, concerning treatment withdrawal, it was shown that the patients would usually prefer to stop immunomodulator than biologic treatments and that the main reason for stopping therapy would be the fear of side effects and particularly cancer (11). As far as the risk of relapse that the patients would accept to be able to stop one of their treatment, the majority would accept up to 25% risk of relapse and up to 5% time with active disease to be able to stop one of their treatments (biologic or immunomodulator) (11). These numbers may serve as landmarks when considering treatment withdrawal. However, some patients would not accept any risk of relapse, while others would be ready to accept very high risk to decrease their therapy (11). These questions should be specifically asked to the patients before considering treatment withdrawal.

Pregnancy represents a particular situation in which treatment withdrawal is often contemplated or at least discussed. A pregnant patient is usually very keen to stop therapy even before the start of pregnancy. However, despite a relatively low amount of evidence, most guidelines consider that almost all treatments can be continued during pregnancy, except for methotrexate (32). The consensus is that the worst thing for a pregnancy both for the fetus and the mother is an uncontrolled disease and that everything should be made to keep remission during pregnancy.

From this point of view, the best candidates for treatment withdrawal would be the ones who, after a clear information and understanding of not only the risks linked to treatment withdrawal but also the consequences of continuing therapy, choose to stop this treatment (**Table 1**).

## INTEGRATIVE MODEL TO GUIDE TREATMENT WITHDRAWAL IN IBD

The decision to withdraw a treatment in IBD is not an easy one and is clearly multi-dimensional. There are several ways to try and integrate these different dimensions. Most sophisticated would include the development of a clinical decision support system (33). Such tools have been developed for other chronic diseases and can integrate several parameters and the positions of several actors involved in the decision process, including the patients. Artificial intelligence can be incorporated in those tools to optimize the decision. They have yet to be developed in the field of IBD. A simple tool could go through a rough and semi-quantitative weighing of the different factors and a graphical representation of the strength of arguments in favor of stopping or continuing therapy (4). This model has been proposed and illustrated in a previous publication dealing with treatment withdrawal in CD. Alternatively, and more simply, typical patients' profiles can be created in whom a decision of either treatment withdrawal or treatment continuation could be the optimal choice (34). Another relatively simple way to proceed would be to create an algorithm incorporating the different dimensions governing the treatment choice. This would require a hierarchy between the different dimensions allowing for building an algorithm driven by successive question. An example of such algorithm is presented in **Figure 1**.

## CONCLUSIONS

Systematic withdrawal of biologic therapy or immunomodulator when the treatment target has been reached is not evidence based and is not advisable. Nevertheless, for some subgroups of patients, it may represent an option associated with optimal benefit-risk and benefit-cost ratio. The decision to withdraw treatment in IBD patients in remission should thus be a tailored approach, mainly taking into account the past clinical history of the patient, the current disease state, the tolerance and risk of side effects as well as patients' preference and priorities. Optimal integration of all these aspects may require specific tools incorporating artificial intelligence. Simpler algorithms may also help the clinician in routine practice.

## AUTHOR CONTRIBUTIONS

EL conceived, wrote, and prepared the manuscript.

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REVIEW
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# Intestinal Organoids as a Tool for Inflammatory Bowel Disease Research

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Inflammatory Bowel Diseases (IBD) are difficult to model as freshly acquired tissues are short-lived, provide data as a snapshot in time, and are not always accessible. Many patients with IBD are non-responders to first-line treatments, and responders are prone to developing resistance to treatment over time—resulting in reduced patient quality of life, increased time to remission, and potential relapse. IBD is heterogenous and we are yet to fully understand the mechanisms of disease; thus, our ability to diagnose and prescribe optimal treatment remains ineffective. Intestinal organoids are derived from patient tissues expanded *in vitro*. Organoids offer unique insight into individual patient disease and are a potential route to personalized treatments. However, organoid models do not contain functional microbial and immune cell components. In this review, we discuss immune cell subsets in the context of IBD, and the requirement of immune cell and microbial components in organoid models for IBD research.

**Keywords: Inflammatory Bowel Disease, immunology, organoid, T cells, cytokines, regulation, diagnostics**

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## INTRODUCTION

Intestinal organoids are a three-dimensional *in vitro* model of the human intestinal epithelium that allow for robust, patient specific *in vitro* research of the development and properties of the intestinal epithelium. The prevalence of Inflammatory Bowel Diseases (IBD) is rapidly increasing across both developed and developing countries (1). IBD, such as Crohn's disease (CD) and Ulcerative colitis (UC), affects up to 0.5% of people in the Western world (1). Due to a lack of patient specificity and knowledge of disease mechanisms, successful treatment of these diseases remains difficult. Frontline IBD treatments have limited efficacy in large groups of patients. For example, Infliximab, a biologic anti-tumor necrosis factor (TNF) antibody treatment, is only effective in 60–87% of patients, 23–46% of whom become secondary non-responders within 5 years (2). Mechanisms of IBD are yet to be elucidated and are difficult to pinpoint in individual patients.

In this review, we explore the potential benefits and limitations of intestinal organoid cultures for immunological research in IBD. The terminology for organoids is complex and is used interchangeably. In this review, we refer to an intestinal "organoid" as a self-organizing, self-renewing, multicell-complex nominally derived from intestinal crypt Leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5)+ stem cells excised from primary tissue, human or murine (3). This definition is distinct from organoids derived from induced pluripotent stem cells (iPSCs), which contain both an epithelial and mesenchymal component (4). Primary intestinal crypt stem cells, when grown in suitable matrix and media, organize themselves into three-dimensional epithelial structures, exhibiting genetic and physiological similarities to their organ of origin.

Intestinal organoid models are the result of stem cell research and still lack standardized methods to include the intestinal microbiota and immune cells of the lamina propria. IBD is the result of a complex interplay between the intestinal epithelial barrier (IEB), the immune system, and the microbiota (5). The addition of a viable immune system to the organoid model, as well as a microbiota, may allow for mechanistic studies of IBD. Here, we discuss intestinal organoid models and their relevance and requirement for the development of a biologically accurate *in vitro* model of intestinal inflammatory diseases, focusing on intestinal immune cells.

## THE IMMUNE SYSTEM IN IBD

IBD is potentially only an umbrella term for different diseases, most of which have not yet been accurately described. Identified mechanisms that can lead to IBD include: loss of immune tolerance to commensal bacteria, inflammatory and suppressive immune cell defects, polymorphisms in pattern-recognition receptor genes (e.g., *NOD2*), defects in autophagy, and tight junction dysregulation and defects (6, 7). Each mechanism is different; however, all have one trait in common—each result in varying degrees of disruption of the IEB, their associated tight junction proteins, and the overlaying mucus layer. On the basolateral side of the IEB is the lamina propria, a tightly regulated region of the intestine, containing a large immune population on the basolateral border of the IEB. This immune population is located in the lamina propria to detect and clear viral, fungal, and bacterial migrants from the lumen.

Cells of the IEB contain Toll-like receptors (TLRs) that can induce production of inflammatory or anti-inflammatory responses to foreign materials (8). For example, binding of TLR9—a membrane-bound protein complex, stimulated an anti-inflammatory tolerogenic response upon binding of bacterial unmethylated CpG dinucleotides on the apical surface (lumen) of enterocytes, whereas binding of CpG by basolateral (lamina propria) TLR9 stimulated a pro-inflammatory response (8). In a homeostatic scenario, the polarized nature of the TLR response of the epithelial cells allows protection against invaders that breach the barrier, without an excessive response to the luminal microbiota; however, the disruption of the IEB in IBD patients allows greater migration of luminal contents into the lamina propria (9). This is one example of a pathway that can result in the establishment of a positive feedback loop of epithelial inflammation. Immune cells detect the unwanted foreign presence in the lamina propria and mount an immune response. Inflammatory cytokines, such as IL-6, IL-17A, IL-17F, interferon (IFN)γ, and TNF are produced by resident immune cells (10). While this immune response can contribute to increased epithelial turnover and pathogen clearance, it can also generate off-target cellular damage, further increasing IEB permeability.

An in-depth analysis on every immune cell type and their associations with disease is beyond the scope of this review. However, we will discuss some immune cell subsets frequently associated with disease status in IBD patients.

### Antigen Presenting Cells (APCs)

Antigen presenting cells, such as dendritic cells (DCs) and macrophages, are important sentinels of gut microbial and dietary antigens. DCs are situated in the lamina propria in gut-associated lymphoid tissues, such as Peyer's patches and intestinal draining lymph nodes. DCs have long dendrite projections that can sample luminal antigens via paracellular spaces. DCs provide T cells with pro- or anti-inflammatory signals. There are two major types of DC found in the gut, CD103+ and CD103– DCs. CD103 is a marker of immune cell residency; its ligand, E-cadherin, is a surface protein found on intestinal epithelial cells. CD103+ DCs, broadly speaking, have a suppressive immune capacity, presenting antigen to lamina propria T cells and driving regulatory T cell (Treg) differentiation. CD103– DCs in the gut can prime Th1 and Th17 T cells but are less commonly associated with gut tissues due to the absence of the CD103 integrin (11). DCs in the gut respond to the presence of retinoic acid and transforming growth factor-β (TGF-β), secreted by intestinal epithelial cell interactions with luminal microbes (12). This DC-epithelial crosstalk drives intestinal tolerance by inducing a suppressive DC phenotype. Patients with IBD have been reported to have lower frequencies of CD103+ DCs in both inflamed and non-inflamed tissues and have reduced ability to induce Treg differentiation, compared to healthy controls (13).

### Regulatory T Cells (Tregs)

Tregs are critical in the regulation of the intestinal environment. Peripheral Tregs express T cell receptors (TCRs) specific for self-antigens, in contrast to intestinal Tregs, which can express TCRs specific for microbial antigens. Tregs are abundant in the intestinal mucosa and are also modulated directly by bacterial antigens. For example, *Faecalibacterium prausnitzii* and *Bacteroides fragilis*, both common gut commensals, induced Treg activation and differentiation via the surface proteins, microbial anti-inflammatory molecule (MAM) and polysaccharide A (PSA), respectively (14, 15). Both *F. prausnitzii* and *B. fragilis* are often missing or present at low abundancy in patients with IBD (16). Both MAM and PSA prevented dextran sulfate sodium (DSS)- and 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis in murine models, reducing Th1, Th17, and Th2 immune responses, and promoting Treg production of IL-10 and TGF-β (14, 15). TGF-β and IL-10 are suppressive cytokines, primarily produced by Tregs and tolerogenic DCs.

In the context of intestinal epithelial cells, TGF-β is an inducer of epithelial-mesenchymal transition (EMT). EMT is a process in which cells of the epithelial barrier lose their cellular polarity and transition into a mesenchymal phenotype (17). This process is a natural part of wound healing. In an immune context, TGF-β is recognized by TGFβ-receptors I and II, which initiate downstream signaling through SMAD1/3/4/6/7, resulting in suppression of inflammatory responses and induction of CD4+ T cell differentiation into Tregs. Murine models deficient in TGF-β or specifically blocked in T cell-TGF-β signaling, developed spontaneous autoimmune disease (18, 19). IL-10 suppression is mediated via interaction with the IL-10 receptor, expressed on hematopoietic cells, resulting in STAT3 phosphorylation and subsequent activation of a broad range of anti-inflammatory

genes. Deficiency in IL-10 leads to spontaneous development of aggressive autoimmune disease in adoptive transfer models of murine colitis. IL-10R polymorphisms have been associated with early-onset UC and impaired TGF-β signaling in IBD patients (20).

## Effector T Cell Subsets

T cells are a major source of pro-inflammatory cytokines in IBD. Gut resident T cells exist in a tolerogenic environment. However, patients with IBD have excessive intestinal T cell activation (21). The cause of this T cell dysregulation is largely unknown. T cells that produce IFNγ have long been implicated in onset of IBD. IFNγ is a pro-inflammatory cytokine vital for immune responses and has been linked to IBD severity in mice and humans (22). IFNγ recruits immune cells to sites of infection and improves inflammatory responses by inducing major histocompatibility complex (MHC) class I and II expression (23). IFNγ directly increases intestinal epithelial permeability by reducing expression of tight junction proteins and perturbing apical actin organization (24). Th17 cells produce IL-17, a pro-inflammatory cytokine with functional roles in many autoimmune diseases, such as rheumatoid arthritis and IBD. T cell populations that express either IFNγ and/or IL-17 are often found at higher frequencies in human inflamed tissues of IBD patients (24, 25). Excessive T cell activation in IBD can be caused by numerous pathways: (1) Inflammatory T cell subsets are resistant to Treg suppression (26); (2) T cells may be specific for commensal bacterial species (27); (3) unregulated activation of APCs in the gut (28); (4) compromised intestinal barrier integrity, leading to recurring bacterial insult (29). It is likely that IBD is just a term for manifestation of gut inflammation, and the mechanisms of disease are much more complex than our current categorization of CD and UC allows. It is also likely, that in many cases of IBD, T cells are mediators of gut inflammation, but not the initial cause of disease. Nonetheless, adoptive transfer murine models of colitis have shown that T cells alone can cause IBD-like pathology, and suppression of T cell pathways can abrogate intestinal inflammation (30).

## Immune Cell Regulation and Function

Polymorphisms in *NOD2* and *ATG16L1* loci have been highly associated with IBD (31). Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) is a pattern recognition receptor expressed by a range of cells, including monocytes, dendritic cells, macrophages, and enterocytes. NOD2 recognizes muramyl dipeptide (MDP), a cell wall protein expressed by both gram-positive and gram-negative bacteria. Recognition of MDP by NOD2 induces a signaling cascade that leads to phosphorylation of IκB, which activates NF-κB, an inducer of inflammatory cytokine responses (32, 33). In healthy people, activation of the NOD2 pathway leads to immune cell activation, generation of pro-inflammatory cytokines, and eventual bacterial clearance (34). Compared to wild-type (WT) mice, *NOD2*-knockout mice have reduced bacterial clearance, reduced numbers of goblet cells, reduced protective mucins and anti-microbial molecules, and increased abundance of non-commensal bacteria, such as *Bacteroides vulgatus*—each of these abnormalities can contribute to intestinal dysbiosis and onset of inflammation (35). Human genome wide association studies (GWAS) have shown between 30 and 50% of Crohn's disease patients have *NOD2* polymorphisms, suggesting NOD2 polymorphisms are a risk factor for disease, but alone, not sufficient or necessary for disease (32, 36). Intestinal organoids offer a valuable model to investigate mechanisms of disease, as the model itself is highly manipulatable, biologically relevant, and suitable for genetic manipulation (3).

The *Atg16l1* gene is highly associated with incidence of Crohn's disease. *Atg16l1* encodes a core structural protein of immune cell autophagosomes (37). Bacteria, bacterial antigens, and cellular components are packaged within intracellular phagosomes for degradation. Phagosomes, loaded with products destined for degradation, fuse with degradative enzyme-containing lysosomes—forming an autophagolysosome (37). In a healthy system, the autophagolysosome facilitates degradation of cellular components into reusable products—macroautophagy. *Atg16l1* polymorphisms have been shown to reduce the formation of phagosomes. Murthy et al. (38) showed that in a CD variant (T316A), murine and primary human macrophages had enhanced degradation of ATG16L1 proteins by Caspase 3, resulting in defective bacterial clearance compared to controls (38). The inability to degrade and recycle cellular and bacterial components can lead to their accumulation. Not only does this starve the immune cell of a vital source of recycled cellular components, accumulation of these products can have potent inflammatory effects upon cell death. High concentrations of cellular debris, both host-derived and foreign, can be recognized as damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), respectively. PAMPs can be recognized by a variety of Pattern Recognition Receptors, and the release of high concentrations of PAMPs from autophagy-deficient cells can be recognized as an infection—generating immune responses by other immune cells. DAMPs are potent "danger-signals" released from damaged tissues and can be recognized by a variety of immune cells, eliciting a potent pro-inflammatory response, leading to pathology and further cell death. Expression of TLR2, TLR4, and TLR9 is higher in inflamed tissue from IBD patients, which could exacerbate inflammatory responses to DAMP and PAMP accumulation (39, 40).

# THE INTESTINAL ORGANOID MODEL

The gastrointestinal tract is a complex organ that is constantly exposed to foreign materials and organisms. The intestinal epithelium is a single layer of cells, with entire cell turnover every 2–6 days (41). The stem cells responsible for this continual turnover are positioned within the base of crypts, tube-like invaginations that facilitate the protection of the stem cells from constituents of the luminal environment. The stem cells, which are identified by their expression of the Wnt-target surface protein, Lgr5, persist in a niche defined by the secretions of neighboring cells within the crypts and underlying mesenchymal cells. Intestinal crypt stem cells can

be cultured with deep-crypt cell factors in specialized media to mimic the *in vivo* environment. Wnt, produced by both the underlying mesenchymal cells and flanking Paneth cells in the small intestine, is an inducer of Ascl2, a master regulator of stem cell phenotype. Lgr5+ stem cells proliferate and generate transit-amplifying (TA) cells. TA cells are highly proliferative cells that divide a finite number of times before differentiating into gut specialist cells (42). As these cells are generated, they move toward the lumen, distal to the crypt. As Paneth cells are found deep within crypts, the Wnt concentration lessens as TA cells ascend toward the lumen, allowing TA cells to differentiate into their mature cell phenotypes. The large intestine does not contain Wnt-producing Paneth cells, but receives Wnt from the underlying mesenchyme and potentially specialized epithelial cells (43).

Organoid cultures can be established either from individual Lgr5+ stem cells or isolated stem cell-containing crypts. These are seeded into a supporting matrix, such as Matrigel, which provides the high laminin levels characteristic of the stem cell niche (44). Newly established large intestine organoid cultures do not contain functional Wnt-producing cells; therefore, exogenous Wnt is added. In small intestinal organoids, the development of Paneth cells allows a reduction in exogenous Wnt levels, but the absence of mesenchymal and Paneth cells in colonic organoids requires the continued presence of relatively high levels of exogenous Wnt in the media to maintain the cultures. Cell fate is determined by exposure to different niche factors produced by sub-epithelial fibroblasts at the serosal surface of the crypts. Factors such as Noggin and Gremlin inhibit bone morphogenetic protein (BMP), inducing cellular differentiation (45, 46). Differentiation of crypt cells occur in response to the inverse gradient relationship between Wnt and BMP. Wnt concentrations are highest in the base of the crypt, as cells ascend toward the lumen, BMP concentration rises as Wnt concentration falls, inducing differentiation in ascending cells. This crypt design, coupled with mesenchymal and flanking cell chemical influences, allows the IEB to produce cells in a stochastic manner. This complex cell-to-cell signaling enables neutral drift mechanics, ensuring a specific differentiated cell is generated when required and old cells are shed (47).

Intestinal organoids are derived from the isolation and culture of primary stem cells of intestinal crypts or iPSCs. Intact intestinal crypts can be isolated from colonoscopy biopsy samples by $Ca^{2+}$ chelation (48). While there are a number of different protocols for the isolation of the crypts (3, 49) the common feature is the incubation of the biopsy samples in $Ca^{2+}$-free Ringer solution containing ethylenediaminetetraacetic acid and dithiothreitol, followed by mechanical agitation. This results in the isolation of a relatively pure population of intact intestinal crypts containing Lgr5+ stem cells. When cultured in a suitable matrix, such as Matrigel, stem cells within the intestinal crypts survive, avoid anoikis, and proliferate. The crypts then close, form, and develop into a sphere-shaped organoid structure (spheroid). Over the next several days, the cells that comprise the spheroids proliferate and differentiate into gut specialist cells. Lgr5+ stem cells migrate to different areas of the spheroid and undergo crypt-fission events, generating multiple sites of proliferation, thus expanding the culture (3). Mature organoids can then be mechanically disrupted and passaged over months, and maintain genetic similarity to their *in vivo* cell counterparts.

## ORGANOIDS AS A TOOL FOR IMMUNOLOGICAL RESEARCH

The human intestine is a complex organ with strict organization of structural domains. Current intestinal epithelial models, such as Caco-2 cell-lines (an immortalized cell-line derived from human epithelial colorectal adenocarcinoma cells) (50), are useful for drug absorption screening but do not adequately replicate intestinal structure. Tian et al. (51) demonstrated that mouse-derived organoids could validate pathways of microRNA (MIR31) expression. MIR31 was induced by the presence of either TNF or IL-6, similar to results from a colorectal cancer cell line. These data suggest that organoids can be used in conjunction with cell lines to provide a biologically relevant point of comparison *in vitro*. Organoids allow for in-depth analysis of pathogen-host cell interactions and investigation and validation of human cell mechanisms and pathways. However, in general, current organoid systems do not contain functioning immune cells, nor do they contain microfold (M) cells, a critical cell for intestinal antigen sampling. The introduction of a functional immune system to organoid models could change the way intestinal disease is modeled and investigated.

The human immune system is difficult to study, as samples taken from donors are only a snapshot in time. The ability to culture patient tissues and co-culture with the same patient's immune cells is, in theory, the gold standard for immunological research. In an organoid model, the researcher has control of the cells added to culture, providing the ability to systematically observe the effect each cell type might have on a specific epithelium. For instance, IBD patients often have low frequencies of intestinal CD103+ DCs, and CD103+ DCs promote intestinal tolerance. If patient DCs are isolated, expanded, and induced to a tolerogenic phenotype: can epithelial integrity be restored *in vitro*? How does the addition of specific immune subsets affect organoid permeability and growth? Does a beneficial effect require the addition of a combination of immune subsets? Can the addition of recombinant proteins improve model readouts? How does the addition of a microbiota affect these changes? Can immune cell subsets or cytokines of interest be identified to elucidate the most relevant treatment option for an individual? The organoid model provides a tool to answer these questions by allowing a systematic approach to an overly complex immunological system.

3D organoids contain no stromal tissues or lamina propria. Recently, groups have generated 2D monolayers from 3D organoid culture systems (52). This is an interesting model concept, sacrificing crypt-physiology, but gaining the ability to further manipulate the model system. Mechanically disrupted 3D organoids are seeded on Transwell® membrane plates, generating a polarized gut cell lining that acts as a selectively-permeable barrier, separating the apical and basal compartments of the well. This effectively mimics *in vivo* physiology, providing

a lumen (apical compartment) and a lamina propria (basal compartment). This model system has been shown to be effective for the investigation of pathogen-epithelial-immune cell interactions. Noel et al. generated this system using human small intestine organoids co-cultured with macrophages and pathogenic *Escherichia coli* (52). Macrophages were observed extending dendrites through the monolayer to interact with *E. coli* in the "lumen." Co-culture with macrophages also provided resistance to pathogenic *E. coli*-induced permeability. These data suggest that the organoid model is highly adaptable to experimental requirements, and relevant for the investigation of the immune system in the gut.

M cells endocytose luminal antigens for presentation to immune cells in the lamina propria in a highly controlled manner (53). M cells are positioned in mucosa-associated lymphoid tissues (MALT), areas of dense immune cell presence, found in the submucosal regions of the gastrointestinal tract. M cells sample antigens, enclose them in vesicles, then deliver the vesicles to immune cells stationed directly adjacent to the M cell basolateral membrane. A tolerogenic environment is logical for an environment exposed to high levels of foreign material; however, as a consequence of tolerance, the intestine is susceptible to pathogenic insult. M cells allow for immune surveillance of the lumen without the requirement of a luminal immune presence. M cells are thus vital to immune preparation and stimulation in the gut. In an adoptive transfer model in which mice lacked Spi-B, a transcription factor critical for the development of M cells ($Spib^{-/-}$), $Spib^{-/-}$ mice had reduced bacterial uptake and sampling in Peyer's patches, and, as a consequence, had a reduced immune response compared to WT mice (54). It is possible to generate M cells in murine organoid models using recombinant RANKL protein, an NF-κB ligand that induces the expression of SpiB transcription factor and drives M cell differentiation (55). The induction of functional M cells in human organoid models could improve model biological relevance and provide deeper insights into antigen presentation at the intestinal barrier.

## ORGANOIDS AS A TOOL TO INVESTIGATE IBD-INDUCED INTESTINAL FIBROSIS

Sites of inflammation in the intestine are at high risk of developing fibrosis, an excessive buildup of connective tissues. During and after an inflammatory response, damaged intestinal tissues are primarily repaired by intestinal myofibroblasts, among other mesenchymal cells (56). Mesenchymal cells promote the deposition of extracellular matrix (ECM), a network of glycoproteins and collagen, which provide structure and anchoring support to surrounding cells. In IBD, disruption of the fibrogenic process can lead to improper repair of the intestinal barrier, leading to the formation of ulcers or fistulas (57, 58). In contrast, uncontrolled overproduction of ECM can cause a buildup of connective tissues, narrowing the intestine (strictures) (56). The generation of ECM is promoted by immune-mesenchyme crosstalk. Inflammatory cytokines, such as TNF, produced by activated macrophages, can promote or inhibit myofibroblast production of various ECM-degrading metalloproteinases (MMP) (59, 60). In contrast, myofibroblast-derived TGF-β1, also induced by TNF, induces the production of myofibroblast-derived tissue inhibitors of metalloproteinases (TIMP), which inhibit MMP-mediated degradation of ECM (59). Thus, a complex crosstalk of immune and mesenchymal cells is required to maintain healthy restoration on inflammation-induced epithelial damage.

Most current organoid models do not include a mesenchymal component. 3D organoids are a collection of intestinal cells in a Matrigel suspension, usually a single layer thick. Organoid monolayers are typically derived from mechanically disrupted organoids, seeded directly onto a plate or membrane. Rodansky et al. (61), cultured 3D embryonic stem cells which were differentiated into human intestinal organoids. Mature organoids with high mesenchymal cell numbers (compared to other organoids) were selected and used to evaluate the efficacy of the anti-fibrotic drug, spironolactone, *in vitro*. Myofibroblasts in their model were activated by TGF-β1 (as indicated by an increase in the mRNA of pro-fibrotic genes), and subsequently inhibited by the addition of spironolactone. Rodansky et al. demonstrated the clinical and research potential of intestinal organoids as a future model of fibrosis, a model which has been thus far been limited to less biologically relevant models and animal models.

## DEVELOPMENT OF TOOLS FOR PERSONALIZED DIAGNOSIS AND TREATMENT ASSAYS

Organoid systems could be designed to support co-culture with any immune cell type. While Noel et al. showed that macrophages were able to function in a 2D organoid system, it is unclear if other immune cells would be successful without antigen presentation via the M cell pathway (52). Regardless, these findings provided evidence that an organoid model can facilitate a functional immune cell component that can both interact with and influence the organoid epithelial barrier.

### Throughput

The design of a high throughput personalized screening system could reduce the impact of non-optimal therapy prescription. Theoretically, a high-throughput system could be designed, in which patient organoids are grown in a 96-well plate and tested with different treatments, immune cell compositions, and microbial species. This model design could lead to the optimization of patient treatments before application, reducing chance of treatment failure. Recently, rectal organoids derived from patients with cystic fibrosis were successfully used to predict patient response to treatment (62). This was particularly important as cystic fibrosis patients with rare genetic mutations are not examined in clinical trials. Modern IBD treatments are designed to target multiple immune mediators, highlighting the value of high-throughput *in vitro* personalized screening for identification of promising treatment targets, in an individual.

Organoid cultures could also be useful in understanding the mechanisms of IBD, before onset of inflammation. In some cases of IBD, Th17 cells are responsible for excessive mucosal inflammation via the release of IL-17A, IL-17F, and the heterodimer IL-17A/F (63). If healthy control or IBD patient organoids are co-cultured with IBD patient Th17 cells, it may be possible to identify whether these cells have deleterious effects on organoid growth, proliferation, or permeability. In the same way, Tregs could be investigated for their ability to suppress other immune cells. This could be further investigated with the addition of a functional microbiota in the apical compartment of the system. The current consensus on IBD implicates a loss of tolerance to gut commensals could be the cause of chronic inflammation; however, the field still lacks the evidence required to define which mechanisms of disease are the cause or a consequence of disease. The organoid model provides a unique opportunity to investigate IBD *in vitro*, potentially providing answers to fundamental unknowns of IBD.

## The Intestinal Microbiota

Multiple groups have introduced bacteria to organoids. For example, microinjection of *Salmonella typhimurium* into the lumen of intestinal organoids induced transcriptional changes relating to cytokine expression patterns (64, 65). After injection into the lumen, *S. typhimurium* penetrated host cell membranes and resided within host-cell vacuoles. These experiments suggest that the organoid model is capable of not only hosting microbial lifecycles, but also responding to their presence. Researchers could introduce a large fraction of the species found within an individual's microbiota via microinjection of microbes isolated from patient-specific fecal material. However, fecal material will only provide live-facultative anaerobes, and the oxygen required for organoid culture will not facilitate obligate anaerobe survival. The addition of antibiotics, pH changes, antivirals, antifungals, recombinant cytokines, probiotics, or immune subsets, could provide valuable insight into responses to the microbiota and improve understanding of intestinal homeostasis.

How the microbiota interacts with immune cells of the intestine remains largely unknown. Gut-resident immune cell subsets maintain gut homeostasis, but little is known about the mechanisms of control. Short chain fatty acid production by gut microbes is important for intestinal barrier homeostasis (66). In a study of IBD patients treated with anti-TNF, patients with remitting IBD, but not non-remitting IBD, had a microbiome more similar to that of healthy people (67). Non-remitting IBD patients had lower expression of intestinal metabolites than remitting IBD patients and healthy controls; butyrate expression was restored in remitting IBD patients.

Probiotics may be an important emerging field for initiating intestinal homeostasis for patients with intestinal diseases; however, due to a lack of knowledge on microbe-barrier-immune cell interactions, probiotics remain ineffective as treatments (68). Theoretically, a high-throughput intestinal organoid system could provide insight into these interactions, allowing the production or selection of biologically relevant probiotics that not only reduce dysbiosis, but promote immune tolerance and homeostasis. To achieve this, researchers could use co-culture experiments in which microbes are introduced to organoid systems containing a functional immune system. Positive readouts could include improvement of epithelial integrity, production of tolerogenic cytokines, immune cell differentiation into tolerogenic phenotypes, or a reduction of pro-inflammatory cytokines.

## Genetic Manipulation of The Organoid Model

Organoid models may provide a powerful model for the use of genetic manipulation tools, which could provide answers to fundamental questions posed by current disease research. Tools such as CRISPR-Cas, transposon mutagenesis, and siRNAs could be used to research the effect of known genetic variants identified by GWAS studies. Mutations in the *NOD2* locus are associated with disease in patients with CD; however, mutations in this locus do not manifest in disease in all individuals with *NOD2* mutations. Using genetic modification tools, mutations could be introduced in a stepwise manner, allowing observation of specific genetic variants and their effects on the system. Genetic tools, such as CRISPR-Cas have already proven to be valuable for the observation of the effects of genetic suppression and over-expression. Organoid models could provide a useful model for the validation of GWAS analyses in human tissues.

Zuo et al. (69), derived intestinal organoids from WT and peroxisome proliferator-activated receptor delta (PPARD)-expressing mice, under control of a villin promotor. This approach generated mice that expressed *ppard* in villin-positive gastric progenitor epithelial cells, and subsequent culture of PPARD-positive murine gastric organoids. PPARD, a nuclear hormone receptor, is upregulated in a variety of cancers, including gastric, breast, and lung cancers. Organoids derived from PPARD-positive mice resulted in tumor growth when injected into immunocompetent mice, whereas WT mouse organoids did not. PPARD-positive mice had higher infiltration of CD45+ immune cells into the gastric tissues, and organoids derived from these tissues secreted more CCL20 and CXCL1, than WT mouse organoids. Thus, Zuo et al. generated an organoid model capable of recapitulating the effects of PPARD, *in vitro*. These models allow for a high degree of experimental manipulation which could be used to investigate numerous other markers of interest for cancer and IBD.

# CLINICAL RELEVANCE

In recent years, a large variety of treatment options for patients with IBD have become available. However, IBD heterogeneity and treatment variety makes it difficult to identify which treatment option is the optimal choice for an individual. This leads to the administration of treatments on a trial and error basis. Biologics, such as immune response targeting monoclonal antibodies (mAb), have paved the way for more precise manipulation of the immune system.

Anti-TNF biologics, such as Infliximab, Adalimumab, and Golimumab, target and block TNF, a potent inducer of

inflammation (70–72). Macrophages are a major source of TNF, however, other cells, such as T cells, also produce TNF (10). Blockade of TNF has been successful in reducing severity of a number of diseases, such as rheumatoid arthritis, ankylosing spondylitis, and psoriasis. However, not all patients with IBD respond to anti-TNF treatment, and 23–46% of responders develop resistance to treatment over time, often due to the development of treatment-targeting antibodies (2, 73). More concerning is the number of primary responders, whom in the absence of treatment-targeting antibodies, still have ineffective responses to treatment over time, suggesting different treatment mechanisms are a requirement in individual patients.

Anti-integrin biologics, such as Natalizumab (α4-integrin) and Vedolizumab (α4β7-integrin) aim to prevent the migration of immune cells to the intestinal mucosa (74, 75). DCs present antigen to T cells in gut MALT, which induces the expression of α4β7-integrin on the T cell surface (76). As α4β7+ T cells circulate in the blood, they can be bound by the cell surface-α4β7 ligand, mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1). MAdCAM-1-expressing cells are highly localized to intestinal high endothelial venules and facilitate capture and trafficking of circulating α4β7+ to the intestinal mucosa (77). Thus, anti-integrin biologic treatments broadly reduce gut inflammation by reducing intestinal mucosa immune cell presence.

Ustekinumab is an mAb that targets the p40 subunit of IL-12 and IL-23 (78). IL-23 is a pro-inflammatory cytokine that induces the differentiation and survival of Th17 cells. Patients with IBD often have high concentrations of IL-23 in blood and intestinal mucosa (79). Ustekinumab treatment has been effective for patients with high numbers of Th17 cells in the intestinal mucosa and for non-responders to anti-TNF therapy. Recently developed treatment options are Filgotinib and Tofacitinib. Filgotinib and Tofacitinib are Janus Kinase (JAK) inhibitors, which inhibit JAK-Signal transducer and activator of transcription (STAT) signaling pathways.

Each of these treatments comes with variable efficacy and a range of side-effects that reduce patient quality of life. Currently, identification of which treatment is appropriate for an individual is conducted step-wise—increasing time-to-remission, reducing patient quality of life, and increasing cost of treatment and care.

## CONCLUSIONS

The gold standard of a scientific model is one that is: amenable to manipulation, robust, biologically relevant, and sustainable. Intestinal diseases and intestinal cell-cell interactions are limited by the accessibility of fresh human tissue. Human colonic and small intestine tissues are difficult to obtain and have a short life span. Organoid systems offer an elegant solution to this problem via the generation of functional organoids from fresh intestinal adult stem cells. The ability to not only maintain, but expand colonic tissue *in vitro* is a revolutionary breakthrough for intestinal research. The organoid model has the potential to become the gold standard of intestinal and immunological research. The ability to observe patient tissue *in vitro* provides a unique opportunity to observe patient tissues before the onset of disease. However, the model requires more data before this type of investigation can be fully realized. Addition of a functional immune system, a complete microbial influence, and the generation of M cells remain to be optimized. Furthermore, the generation of a universal protocol and mainstream organoid media will make the model more accessible for laboratories and clinics looking to adopt the model and provide more accurate comparison of data between laboratories.

## AUTHOR CONTRIBUTIONS

HA conceived the idea and wrote the manuscript. AB, MS, and RK provided specific input on organoids, gastroenterology, and immunology, respectively. All authors edited the manuscript.

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REVIEW
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# A Personalized Approach to Managing Patients With an Ileal Pouch-Anal Anastomosis

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Quality of life after ileal pouch-anal anastomosis (IPAA) surgery is generally good. However, patients can be troubled by pouch-related symptoms and pouch disorders that can be inflammatory, mechanical/surgical, and functional. Management of patients with IPAA begins with measures to maintain a healthy pouch such as optimizing pouch function, providing tailored advice on a healthy diet and lifestyle, screening for and addressing metabolic complications of IPAA, pouch surveillance, and risk stratification for risk of pouchitis and pouch failure. Pouchitis is the most common inflammatory disorder. Primary pouchitis is a spectrum currently classified into three progressive phases—an antibiotic-responsive, an antibiotic-dependent, and an antibiotic-refractory phase. It is predominately microbially mediated in acute antibiotic-responsive pouchitis and predominately immune mediated in chronic antibiotic-refractory pouchitis (CARP). Secondary prophylaxis is recommended for recurrent antibiotic-responsive and for antibiotic-dependent pouchitis. Secondary causes of antibiotic-refractory pouchitis should be ruled out before a diagnosis of CARP is made. CARP is best classified as primary sclerosing cholangitis associated, immunoglobulin G4-associated, and autoimmune. Primary sclerosing cholangitis-associated CARP can be treated with budesonide or oral vancomycin. Early recognition of immunoglobulin G4-associated pouchitis minimizes ineffective antibiotic use. Autoimmune CARP can be managed in a manner similar to UC. The current place of immunosuppressives in the treatment algorithm depends on availability and early access to biological agents. Vedolizumab and ustekinumab are the preferred first- and second-line biologics for autoimmune CARP owing to their efficacy, better side effect profile, and low immunogenicity and need for concomitant immunomodulatory therapy. Antitumor necrosis factor should be reserved for autoimmune CARP failing the above and for CD of the pouch. There are no guidelines for the surveillance of pouches for dysplasia. Incidence varies based on a patient's risk. Since incidence is low, a risk-stratified approach is recommended.

**Keywords: IPAA, carp, pouchitis, prophylaxis, ileoanal pouch, probiotic, prebiotic, surveillance**

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## INTRODUCTION

Restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) is the preferred surgical treatment for most patients with ulcerative colitis (UC) and familial adenomatous polyposis (FAP). Quality of life (QOL) after colectomy and IPAA is generally good (1, 2). However, patients with IPAA are at risk of pouch-related symptoms of increased frequency, dietary intolerances,

urgency, and incontinence (1). Furthermore, patients are at risk of inflammatory, surgical or mechanical, and functional pouch-related disorders. Management of patients with an IPAA begins soon after pouch creation and ileostomy closure with measures to optimize pouch function, maintain a healthy pouch, risk stratify patients to guide primary and early secondary prophylaxis for pouchitis, and ensure routine screening and monitoring for metabolic complications of the pouch. Furthermore, it is essential to have a thorough personalized approach for the various inflammatory, surgical/mechanical, and functional pouch-related disorders. Finally, some patients with IPAA are at risk of dysplasia and adenocarcinoma of the pouch. Knowing which patients are at risk and how best and how frequently to survey them is important. To ensure these various aspects of care are adequately delivered, it is recommended that IPAA patients continue to be managed in high-volume centers with multidisciplinary inflammatory bowel disease (IBD) or pouch clinics. Our IBD clinic manages ∼1,250 IBD patients, which includes a growing number of IPAA patients at a rate of 10 IPAAs performed annually. This review outlines the principles of diagnosing and managing IPAA patients, with a focus on how to risk stratify and personalize management decisions in individual patients.

## ANATOMY OF THE ILEOANAL POUCH

An ileoanal pouch is created from 2(J), 3(S), or 4(W) limbs of the small intestine. Of these three pouch designs, the J pouch is the most popular owing to the ease of its creation and reliability of its function. S pouch has the advantage of an additional 2–3 cm of small bowel that can be connected to the anorectal transition zone, reducing anastomotic tension, and improving blood supply in those with a short mesentery. However, suboptimal evacuation and more challenging construction have led to it largely being replaced by the J pouch. The W pouch has largely been abandoned.

## OPTIMIZING, MAINTAINING, AND MONITORING THE "HEALTHY" POUCH

### Optimizing Pouch Function and Maintaining a "Healthy" Pouch

Quality of life following IPAA surgery is generally good and, in some studies, reported to approach that of the general population at 12 months (3). Indeed, pouch function with reduced frequency and increased consistency continues to improve over the first 6–12 months as the pouch adapts. At 1 year, the accepted normal average bowel frequency is five to six during the day and one to two overnight. It is important that patients are educated about this adaptation period and the new "normal average bowel function," particularly if the underlying indication for surgery was FAP or colitis-associated neoplasia where no or minimal symptoms existed before IPAA surgery. Furthermore, patients should be educated about dietary and pharmacological measures that can help improve pouch frequency and consistency.

Antidiarrheal medications such as loperamide, diphenoxylate/atropine, and codeine can be used to help reduce pouch frequency. Evidence supporting their efficacy is sparse. Loperamide is most widely used and, at a dose of 8 mg/day, has been shown to reduce pouch frequency and total stool weight (3).

Supplemental fibers like psyllium husk are frequently prescribed by colorectal surgeons to reduce frequency and improve stool consistency. Psyllium husk is a water-soluble fiber that is minimally fermentable. The reduced frequency and increased stool consistency can be explained by its effects on slowing upper gastrointestinal transit and increased stool bulk through water trapping. However, tolerability and efficacy of supplemental and dietary fibers are not universal among patients with some paradoxically developing loose stools and bloating. This could be related to the amount of fiber and associated small intestinal bacterial overgrowth (SIBO) (4). We recommend a trial of water-soluble minimally fermentable supplemental fibers such as psyllium husk in symptomatic patients who have an adequate intake of dietary fibers, starting at the smallest dose, increasing it in those who show partial response, and stopping in those who develop paradoxical worsening of symptoms or diarrhea.

There is currently no standardized dietary advice for IPAA patients. Observational studies suggest that most IPAA patients have at least one intolerable dietary substance negatively impacting pouch function (5, 6). However, there seems to be significant intersubject variability in what food type is intolerable (5). Therefore, a generalized dietary recommendation is not easy. One of the few products consistently shown to increase pouch frequency are caffeine-containing products (7, 8). A useful recommendation is not to exceed a cup or 250 g of a caffeine-containing product a day. Beyond such a recommendation, it is difficult to generalize dietary advice. Most patients end up following an individualized dietary habit through trial and error. A physician's main role is to ensure that the patient's diet has an adequate nutritional content, can optimize pouch function, and promotes a healthy pouch microbial community. Most diets adapted have adequate nutritional intake. Helping patients follow a diet that optimizes pouch function and promotes a healthy pouch microbial community can be challenging. Diet is the predominate factor that shapes the microbiota structure and function. This effect is mainly via dietary fibers and poorly digestible carbohydrates available for bacterial fermentation. A diet adequate in fermentable fibers is therefore central to achieving a healthy microbiota spectrum. However, readily fermentable fibers as fructooligosaccharides, inulin, and soluble non-starch polysaccharides, found in vegetables and fruits, induce an increase in pouch microbial mass and gas production, both of these factors contributing to increased stool bulk, reduced consistency, and increased frequency. Furthermore, the higher incidence of small intestine bacterial overgrowth in IPAA patients leads to more bacterial fermentation in the small bowel and release of gas causing bloating (4). Therefore, a more pragmatic approach is to try and achieve a balanced intake of fibers.

In addition to meal contents, meal volume, frequency, and timing influence pouch frequency. One study demonstrated a positive correlation between meal volume, meal frequency, and

late night meals and pouch frequency, recommending no more than three meals a day with the last at least 2 h before bedtime (5).

## Preventing, Screening for, and Diagnosing Metabolic Complications of IPAA

Patients with healthy and inflamed IPAAs have a higher risk of iron deficiency anemia (IDA). Other causes of anemia include B12 deficiency, which has been reported in up to 25% of pouch patients (9). Patients with IPAA also have a higher incidence of low vitamin D and serum calcium independent of pouch inflammation (10). Vitamin D deficiency has been reported in 10–68% of patients (11). Bone loss is common in IPAA. Risk factors include old age, low BMI and pouchitis, primary sclerosing cholangitis (PSC), pouch villous atrophy, and lack of calcium supplementation (12). We recommend a baseline bone mineral densitometry in all patients. We also recommend calcium and vitamin supplementation in those with low levels of vitamin D or calcium, risk factors for, or confirmed, osteopenia.

## Risk Stratification and Prophylaxis for Pouchitis

The risk of developing pouchitis, the most common disorder of IPAA, varies among patients. Numerous risk factors have been identified. Assessing for the presence or absence of these risk factors can help guide the need for primary and secondary prophylaxis for pouchitis and manage patient expectations. Some risk factors such as the NOD2/CARD15 mutation (13) and certain Toll-like receptor genotypes (14) are costly, not widely available, and not routinely performed. We instead recommend focusing on risk factors that can be routinely assessed in clinic, providing a pragmatic risk stratification strategy.

I. Primary Sclerosing Cholangitis (PSC): A positive association between pouchitis and PSC has been reported in numerous studies. The cumulative incidence of acute pouchitis at 10 years has been reported to be 70–80% (15, 16). Most studies have reported a higher incidence of chronic pouchitis among PSC ranging between 50 and 60% (15, 17, 18).

II. Extraintestinal manifestations (EIMs): EIMs are a risk factor for acute and chronic pouchitis (19, 20). In one study, patients with precolectomy EIMs had a higher incidence of pouchitis compared to those with no EIM (39 vs. 26%, $P < 0.01$) (19). *De novo* EIMs post-IPAA are associated with an even higher risk of pouchitis (19). EIMs are also associated with a risk for chronic pouchitis with an odds ratio of 2.69; $P = 0.047$ (20).

III. Concomitant autoimmune disorders: Unsurprisingly, "the presence of at least one autoimmune disorder is associated with a 2-fold risk of chronic antibiotic-refractory pouchitis (CARP)" (21). Immunoglobulin G4 (IgG4), a biomarker of autoimmune disorders, is associated with CARP. Antineutrophil cytoplasmic antibody is another serologic marker positively associated with chronic pouchitis with an odds ratio of 1.76; $P < 0.01$ in one study (22).

IV. Extensive colitis and backwash ileitis: The association of extent of colitis and back wash ileitis and acute and chronic pouchitis is unclear. Some studies have found extensive colitis to be a risk for acute and chronic pouchitis (23, 24). Others have found no association (25, 26). Backwash ileitis was shown in one study to be associated with increased pouch mucosal permeability (26). This is supported inconsistently by studies showing a positive association between backwash ileitis and acute and chronic pouchitis (27, 28). The discrepancy in these results can partly be explained by the difference in sample size, median follow-up, and difference in definition of pouchitis. We consider back wash ileitis as a useful adjunctive risk factor to the overall risk of pouchitis, rather than an independent risk factor.

V. Corticosteroid exposure before proctocolectomy: Steroid dependence and high monthly steroid dose (defined as $\geq$ 500 mg/month before colectomy) have been associated with acute and chronic pouchitis, respectively, possibly reflecting more aggressive underlying autoimmune disease (29, 30).

VI. Periproctocolectomy thrombocytosis: In a prospective study evaluating the clinical factors for the development of pouchitis perioperative thrombocytosis, defined as a platelet count of $>450 \times 10^9$/L, it was found on multivariate analysis to be an independent risk factor for chronic pouchitis (odds ratio, 3.1; $P = 0.03$) (29).

VII. Young age: A few studies have reported and association between younger age at UC diagnosis or IPAA surgery and acute and chronic pouchitis as well as severity of pouchitis. In one study, patients who developed pouchitis had an earlier onset of UC ($22.6 \pm 1.3$ years of age) compared with those who did not develop pouchitis ($27.9 \pm 1.1$ years of age; $P < 0.005$) (31). In a Japanese study, chronic pouchitis was positively associated with age at the onset of UC of <26 years (32). In the Cleveland Clinic Ileal Pouch Center, chronic pouchitis is diagnosed more in pediatric patients than in their adult counterparts (33).

VIII. Sex: Male sex is associated with acute and chronic pouchitis (33). A shorter male mesentery does theoretically risk-reduced pouch perfusion. While this can explain the increased incidence of ischemic pouchitis in men, how this affects the pouch microbial community and mucosal immune response is not clear.

IX. Type of ileal pouch: Although harder to construct and with inferior pouch function, S pouches are significantly less likely to be complicated with CARP than J pouches ($P < 0.001$) (34).

X. Postoperative non-steroidal anti-inflammatory drug use: Defined as more than 1 week of regular NSAIDs postoperatively, NSAID use has been associated with chronic pouchitis (20).

XI. Smoking status: The association of smoking and acute and chronic pouchitis is interesting. Smoking is known to have a protective effect in UC and a detrimental effect on the natural course of Crohn's disease (CD). The protective effect in UC is unclear, but smoking or nicotine reduces gut mucosal permeability and hence the antigen load triggering a mucosal immune response (35). Chronic antibiotic-refractory pouchitis is predominately immune mediated and is often compared to UC. Indeed, smoking

has been negatively associated with CARP (29). The effect of smoking on acute antibiotic-responsive pouchitis is less clear. In two studies, a never-smoker status was a risk factor for all pouchitis (36). In another study, active smoking was positively associated with acute pouchitis (29). One possible explanation for the increased prevalence of acute antibiotic-responsive pouchitis in smokers is the effect of smoking on the microbiome, which is known to be crucial for mediating acute pouchitis (37).

Chronic antibiotic-refractory pouchitis, which is immune mediated, has several shared etiopathological risk factors. Patients with these risk factors have a primed immune system with a lower threshold for initiating and maintaining an abnormal mucosal immune response. Therefore, patients harboring one or more of these risk factors should be counseled about primary prophylaxis of pouchitis, as is discussed below. Similarly, those harboring one or more risk factors who have acute antibiotic-responsive pouchitis (<4 episodes of acute pouchitis a year) can also be counseled about early commencement of secondary prophylaxis.

## Primary Prophylaxis of Pouchitis

### Probiotics

Probiotics are live microorganisms belonging to the gut flora that can be safely ingested to exert health benefits. Probiotics have been tried for primary prophylaxis of pouchitis in at-risk patients. The probiotic agent VSL#3 (*Lactobacillus* spp., *Bifidobacterium* spp., *Streptococcus salivarius* spp., and *Thermophilus* spp.) at a dose of 3 g/day was found in one randomized placebo-controlled trial of 40 patients to be associated with a lower pouchitis rate at 12 months (10%) compared with placebo (40%), $P = 0.04$ (38). In a separate randomized trial of 31 patients, there was no difference in the rate of pouchitis between those randomized to VSL#3 vs. placebo (39). *Clostridium butyricum* MIYAIRI, in a separate randomized trial of 17 patients, showed a trend toward less acute pouchitis compared to placebo (11 vs. 50% $P = 0.14$) over a period of 24 months (40). Although often of interest to patients, we acknowledge that the evidence base to support the use of probiotics for primary prophylaxis of pouchitis is not strong.

### Antibiotics

There is paucity of research on the safety and efficacy of antibiotics for primary prophylaxis. In a small placebo-controlled randomized trial of 38 patients, tinidazole at a dose of 500 mg daily was associated with a lower rate of pouchitis at 12 months 19 vs. 58% in the placebo group, although it did not reach statistical significance ($P = 0.21$) (41).

### 5-Aminosalicylates

There are no data on the efficacy of mesalazine in primary prophylaxis. The efficacy of sulfasalazine as a primary prophylactic agent was assessed in a retrospective case series where only 15% of the 20 patients on sulfasalazine (2,000 mg/day) developed pouchitis compared with 65% of the 31 controls at a median follow-up of 68 months (10–104) (42).

In conclusion, in patients with one or more risk factors for pouchitis, we recommend primary prophylaxis using probiotics. Since VSL#3 has the strongest available data, we recommend VSL#3 at a dose of 3 g daily. Other probiotics can be tried if VSL#3 is unavailable or costly. Alternatively, sulfasalazine can be used as the 5ASA of choice. We do not recommend using oral antibiotics as primary prophylaxis. This should be combined with dietary advice aimed at achieving a diet balanced in fermentable fibers to ensure a favorable microbial community.

## Secondary Prophylaxis of Pouchitis

The indications for and measures used in secondary pouchitis prophylaxis are discussed below.

# EVALUATION AND MANAGEMENT OF POUCH-ASSOCIATED DISORDERS

Pouch disorders can be classified as inflammatory, surgical/mechanical, and functional. Inflammatory disorders include pouchitis, cuffitis, and CD of the pouch. Surgical and mechanical disorders can be broadly divided into obstructive complications and leakage and fistula-related complications. Functional disorders include irritable pouch syndrome (IPS) and pelvic dyssynergia.

# EVALUATION OF THE ILEOANAL POUCH

While laboratory tests are needed to investigate most pouch-related disorders, the most appropriate diagnostic test depends on the presenting signs and symptoms in an individual patient, as outlined in **Figure 1**.

A. Diarrhea, cramps, urgency, and incontinence symptoms: pouchitis, cuffitis, CD, and IPS—best investigated with pouchoscopy and biopsy.
B. Dyschezia, incomplete evacuation, bloating, obstructive symptoms: stricture, floppy pouch complex, and pelvic dyssynergia—best investigated with anopouch manometry and barium defecography.
C. Fever, night sweats, coccygeal pain, leukocytosis: pathogens cytomegalovirus (CMV)/*Clostridioides difficile*, abscess, sinus fistula. CARP, cuffitis, and CD of the pouch rarely present with these symptoms—best investigated with fecal microscopy, culture, and sensitivity/*C. difficile* toxin and MRI of the pelvis.

## Diagnostic Tests Used to Evaluate IPAAs

A. Pouchoscopy: Can be performed with a gastroscope or a colonoscope although we prefer the former. The three areas to examine include the prepouch ileum, the pouch body, and the cuff. A normal J pouch has an owl-eye appearance. Retroflexion is useful to assess the rectal cuff and essential if fistula is suspected. Biopsies should be taken from the three examined areas, biopsying away from suture lines.
B. Imaging: The utility of cross-sectional imaging such as MRI or CT scan of the pelvis is mainly to investigate early and late mechanical or surgical complications as well as suspected perianal or peripouch complications of CD. Barium defecography is useful when investigating obstructive pouch-related disorders.

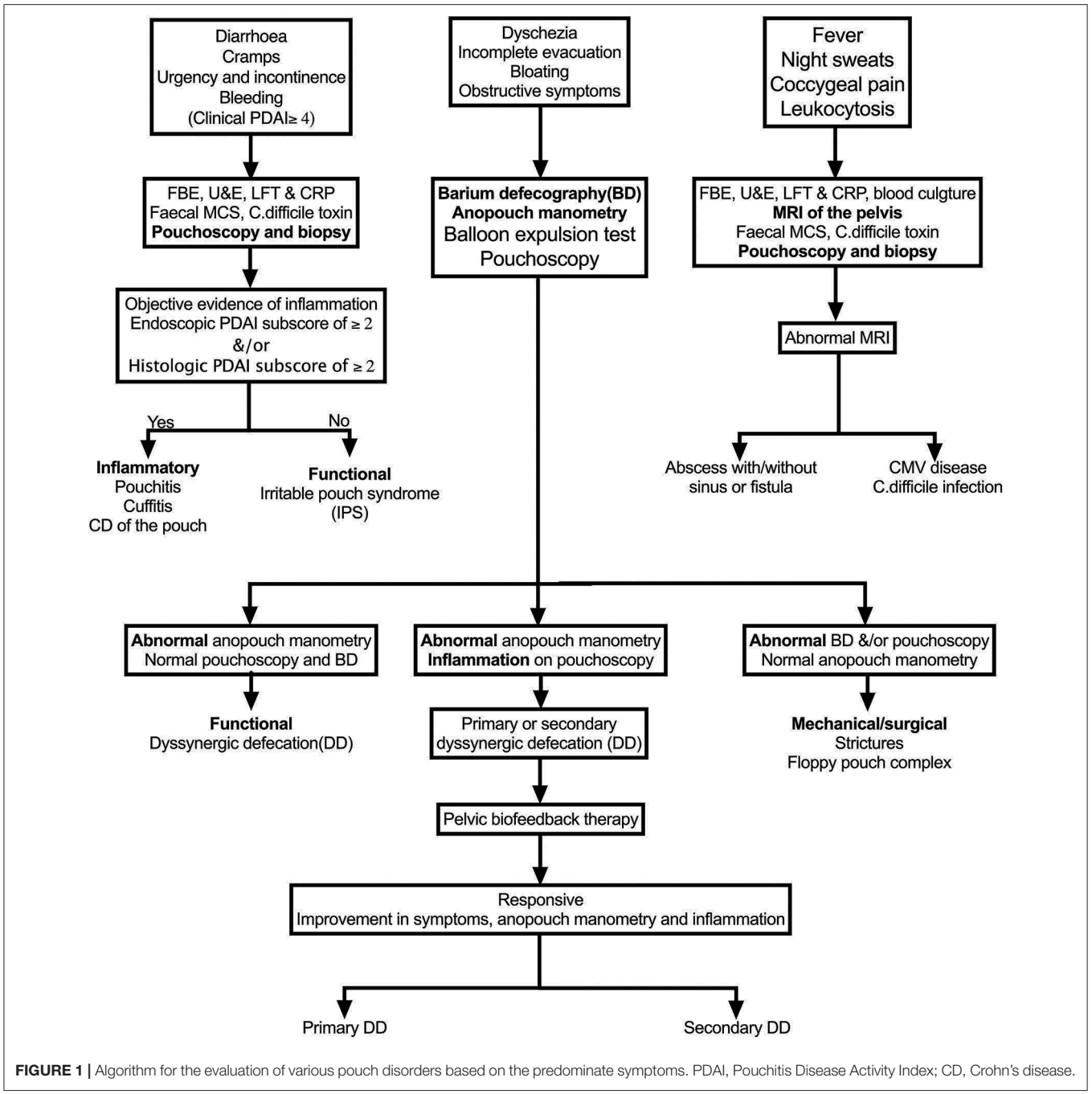


**FIGURE 1 |** Algorithm for the evaluation of various pouch disorders based on the predominate symptoms. PDAI, Pouchitis Disease Activity Index; CD, Crohn's disease.

C. Laboratory investigations:

1. Bloods: Useful laboratory tests include full blood evaluation, urea and electrolytes, liver function tests, C-reactive protein. Patients with anemia should be further evaluated for underlying causes, especially iron deficiency anemia and B12 deficiency.
2. Stool.

- *Clostridioides difficile* toxin is particularly important in patients exhibiting fever or who are refractory to antibiotics.
- Fecal calprotectin: There are limited data on the utility of fecal calprotectin as a non-invasive diagnostic tool for pouchitis. In a study of 54 patients with IPAA (46 UC and 8 FAP) who presented for routine pouchoscopy surveillance, fecal calprotectin was statistically significantly higher in patients with active pouchitis compared to those with inactive pouchitis. Receiver operating characteristic analysis demonstrated that a fecal calprotectin threshold of 92.5 μg/g was 80% sensitive and a 76.5 specific for the diagnosis of pouchitis [Pouchitis Disease Activity

Index (PDAI) $\geq$ 7] (43). In another study of 60 patients with IPAA-UC, in the 10 patients (17%) who developed pouchitis, the median calprotectin was 112 μg/g. Importantly, calprotectin at a cut-off of 56 μg/g 2 months before patients became symptomatic of pouchitis had a 100% sensitivity and 84% specificity in predicting the episode of pouchitis (44). In a cross-sectional study of 32 UC patients who had had their IPAA created at the age of 12 ± 4 years, mean fecal calprotectin was 71 ± 50 μg/g among patients who have never had pouchitis ($n = 10$), 290 ± 131 μg/g among patients who have had at least one episode of pouchitis ($n = 15$), and 832 ± 422 μg/g among patients who have recurrent episodes of pouchitis ($\geq 4$ episodes/year) (45). We can conclude from these studies that fecal calprotectin is a practical and non-invasive investigation for symptomatic IPPA patients; however, the optimal threshold to diagnose pouchitis remains to be determined.

D. Functional investigations: Anopouch manometry, balloon expulsion test, barium, or MR defecography are all investigations used to investigate patients with chronic dyschezia and are detailed below.

# MANAGEMENT OF POUCH-RELATED DISORDERS

## Inflammatory Disorders

### Pouchitis

Inflammation of the pouch is the most common pouch-related disorder with around 50–60% of UC patients and 20% of FAP patients suffering at least one episode at 10 years, a fifth of whom go on to develop chronic pouchitis (1, 46). A useful way to classify pouchitis is to divide it into primary and secondary pouchitis.

#### *Primary pouchitis*

This is defined as idiopathic inflammation of the pouch. Although etiopathogenesis is not completely understood, it is believed to be an abnormal immune response to some aspect of the pouch microbiome. It appears that early on, inflammation is largely microbially mediated as evident by the efficacy of antibiotics. Over time, inflammation can become predominately immune mediated, necessitating the addition of immunosuppressants. The pathogenesis of pouchitis and its subtypes are outlined in **Figure 2**. This could also explain the reduced frequency, delayed onset, and milder form of pouchitis in patients with FAP, whose immune system is not as "primed" as those with underlying UC (46). Primary pouchitis can further be classified according to the number of episodes of pouchitis and response to antibiotics into acute antibiotic responsive (<4 episodes a year), chronic antibiotic-dependent (4 or more antibiotic-responsive episodes or need for ongoing antibiotic use), and CARP, which is largely immune mediated. There are several diagnostic indices to assess inflammation of the pouch. The most widely used is the 18-point PDAI, which consists of symptom (0–6 points), endoscopy (0–6 points), and histology

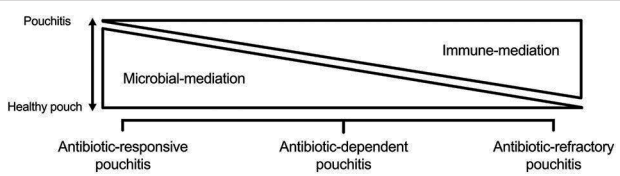


**FIGURE 2 |** Primary that is predominately microbially mediated in antibiotic-responsive pouchitis and predominately immune mediated in chronic antibiotic-refractory pouchitis. Antibiotic-dependent pouchitis is somewhere in between.

(0–6 points) subscores, as is outlined in **Table 1**. A total PDAI score of $\geq 7$ points is considered diagnostic for pouchitis (47). A modified score, the modified pouchitis disease activity index (mPDAI), which omits histology, was suggested as an equally accurate alternative for the diagnosis of pouchitis with a score of $\geq 5$ (48). The PDAI endoscopy score has six components (edema, granularity, loss of vasculature, friability, mucus exudate, and ulceration). One randomized controlled trial showed that the six components equally "contributed" to the total endoscopic score (48). However, recently, the appropriateness and reliability of each of the individual endoscopic components of the PDAI and other available diagnostic instruments, like the Heidelberg pouchitis disease activity index, was reassessed. Subsequently, the authors proposed removing edema, granularity, loss of vascularity, and mucus exudates as they were believed to be either inappropriate endoscopic features or of uncertain appropriateness with moderate interrater reliability. Ulceration, erosions, and bleeding were considered appropriate, with only ulcerations reaching substantial interrater reliability (49). Until these newly proposed criteria are verified, we suggest using the total PDAI score, including histological components. The histological subscore is composed of acute inflammatory changes such as neutrophil infiltration, crypt abscesses, and ulceration, seen on a background of chronic inflammation characterized by some degree of villous atrophy. A histological subscore of at least 2 is needed for a diagnosis of pouchitis. The PDAI is outlined in **Table 1** (50).

#### *Secondary pouchitis*

Around 25% of chronic pouchitis are secondary to underlying conditions that need to be investigated and ruled out before a diagnosis of CARP is made. These include the following:

- Ischemia: Ischemia is one of the most common causes of secondary chronic pouchitis. It is characterized by asymmetric inflammation of the pouch involving the distal half, the afferent limb, or staple line (51). Risk factors include male gender and weight gain, as the proposed etiology is mesenteric tension. Ischemic pouchitis can be very challenging to treat. A trial of hyperbaric oxygen can be tried if available. In those with morbid obesity, bariatric surgery with consequent weight loss can reduce mesenteric tension and improve blood supply (52). Biological agents such as vedolizumab are recommended by The Cleveland Clinic Pouch Center, but outcomes have not been published.

**TABLE 1 |** Pouchitis disease activity index (PDAI)[a].

| | Score |
|---|---|
| **CLINICAL** | |
| **Stool frequency** | |
| Usual postoperative stool frequency | 0 |
| 1–2 stools/day > postoperative usual | 1 |
| 3 or more stools/day > postoperative usual | 2 |
| **Rectal bleeding** | |
| None or rare | 0 |
| Present daily | 1 |
| **Fecal urgency or abdominal cramps** | |
| None | 0 |
| Occasional | 1 |
| Usual | 2 |
| **Fever (temperature > 37.8°C)** | |
| Absent | 0 |
| Present | 1 |
| **CLINICAL SCORE** | /6 |
| **Endoscopic inflammation** | |
| Edema | 1 |
| Granularity | 1 |
| Loss of vasculature | 1 |
| Mucopurulent exudate | 1 |
| Friability | 1 |
| Ulceration | 1 |
| Endoscopic score | /6 |
| Acute histological inflammation | |
| **Polymorphonuclear inflammatory infiltrate** | 0 |
| Mild | 1 |
| Moderate + crypt abscesses | 2 |
| Severe + crypt abscesses | 3 |
| **Ulcers per lower power filed (%)** | 0 |
| <25 | 1 |
| 25–50 | 2 |
| >50 | 3 |
| Maximal acute histological inflammation | /6 |

[a] *Sandborn et al. (47).*

- Crohn's disease of the pouch: The actual incidence of CD of the pouch is not known. In one study, 48 of 164 (28%) of patients initially diagnosed as having UC were diagnosed with CD upon reviewing their colectomy specimen before creating an IPAA (53). A two- or three-stage IPAA allows examination of the colectomy specimen for transmural inflammation or granulomas before an IPAA is created. However, CD of the pouch can occur *de novo*. The risk of *de novo* CD of the pouch in patients diagnosed with UC preoperatively is ∼6% (54) and in those diagnosed with indeterminate colitis preoperatively is 15–20% (55). Known risk factors include a young age at diagnosis of UC (<20 years) and young age of surgery, indeterminate colitis, patchy colitis on colectomy specimen, active smoking, family history with CD, and seropositive anti-*Saccharomyces cerevisiae*-IgA (36, 56, 57). CD of the pouch can manifest as one of three predominate phenotypes, inflammatory, fibrostenotic, and fistulizing.
  a) Inflammatory CD of the pouch results in chronic pouch inflammation that may be associated with prepouch ileitis (PI) and deep ulcers in the pouch that is refractory to combination antibiotics for 4 weeks.
  b) Fibrostenotic CD results in ulcerated strictures anywhere in the jejunum, ileum, pouch inlet, or mid-pouch, associated with inflammation and/or ulcers of the afferent limb in the absence of NSAID use.
  c) Fistulae attributed to CD are non-anastomotic, developing at least 6 months after ileostomy closure in the absence of postoperative complications such as pelvic sepsis, leaks, or sinuses.

  The diagnosis of CD currently rests on a combination of clinical, endoscopic, histological, and radiological features. Fibrostenotic and fistulizing CD presenting in the fashion described above can usually be diagnosed endoscopically and radiologically. Crohn's disease presenting with chronic pouchitis can be harder to diagnose and distinguish from primary CARP, especially given that granulomas are only seen in 12–13% of cases, and transmural inflammation on radiological assessment is seen in both CD and CARP (1). The importance of distinguishing CARP from CD of the pouch lies in guiding the choice of biologic as antitumor necrosis factors (anti-TNFs) are more effective in those with CD (58) of the pouch compared to CARP patients who show better response to vedolizumab and ustekinumab (59, 60).

- Infections: CMV and *C. difficile* infection. The presence of fever should raise the suspicion of CMV and *C. difficile* infections.
  a) *C. difficile* infection (CDI) is a common cause of secondary pouchitis reported in as many as 18% of patients (61). Oral vancomycin should be considered first line in the management of pouch CDI. Recommended dose of oral vancomycin is 500–1,000 mg/day for 2–4 weeks. In patients with mild acute CDI who are metronidazole naive, oral metronidazole 500 mg twice daily for 2 weeks may be used as an alternative first line. Oral fidaxomicin 400 mg/day for 10–14 days or fecal microbiota transplantation are reserved for refractory or recurrent CDI (52, 62).
  b) CMV infection: CMV infection is rarely associated with pouchitis. The main risk factor is immunosuppression. On pouchoscopy, there is pouchitis and often ulcerating PI (63). Diagnosis should be based on the presence of CMV inclusion bodies or positive immune histochemistry. The presence of CMV PCR alone does not constitute a diagnosis of CMV pouchitis or require treatment. In one study, a positive CMV PCR was found in 41% of patients with antibiotic-responsive pouchitis that responded to conventional oral antibiotics (63). When therapy is considered, intravenous ganciclovir at a dose of 5 mg/kg every 12 h is the initial treatment of choice. In patients responding to IV ganciclovir, we recommend switching to an equivalent dose of oral valganciclovir—900 mg twice daily—2 days later to complete the 2- to 3-week course (63).

- Nonsteroidal anti-inflammatory drugs (NSAIDs): Regular use of NSAIDs postoperatively, defined as daily use of more than 1-week post-IPAA, has been found to be associated with acute and chronic pouchitis (36). Furthermore, patients on regular NSAIDs and pouch-related disorders benefit from complete discontinuation of these drugs, emphasizing the importance of inquiring about and stopping such agents in IPAA patients (64).
- Celiac disease: Celiac disease can develop *de novo* in patients with IPAA (65). Even if serology tests for coeliac were previously done and normal they should be repeated, and if positive, a duodenal biopsy should be performed to confirm the diagnosis.
- Once secondary pouchitis is ruled out a diagnosis of CARP, also referred to as immune-mediated pouchitis, is made. It is useful to classify CARP into PSC-associated CARP, IgG4-associated CARP, and autoimmune CARP; the management of each somewhat differs. The diagnosis of PSC is based on a magnetic resonance cholangiopancreatography, with or without a liver biopsy. The diagnosis of IgG4-associated pouchitis is confirmed by an elevated serum IgG4 with or without pouch and prepouch ileal infiltration with IgG4-positive plasma cells. Autoimmune CARP is simply CARP not associated with PSC or IgG4.

## Management of Primary Pouchitis

### Acute Antibiotic-Responsive Pouchitis

First-line therapy includes a 2-week course of metronidazole (15–20 mg/kg/day) or ciprofloxacin (1,000 mg/day) (66). Ciprofloxacin appears to be more effective than metronidazole in treating active pouchitis, with fewer adverse effects (67). Tinidazole (1,000 mg/day or 15 mg/kg/day for 14 days) can be used as an alternative in those intolerant or failing the above and is considered one of the most potent agents here (52). In pregnant patients with pouchitis, amoxicillin-clavulanic acid may be safely used (52). Rifaximin 500 mg twice daily is also effective, but due to its cost and low side effect profile, it is best reserved for chronic antibiotic-dependent pouchitis requiring ongoing antibiotics (68). The efficacy of antibiotics suggests that some aspect of the pouch microbiome is injurious to the mucosa or triggers an immune response; therefore, attempts have been made to alter the microbiome or its metabolic output without the use of antibiotics.

Probiotics have been tried in acute pouchitis. High-dose VSL#3 at a dose of 3 g twice daily was found to be effective in a 4-week open-label trial (69), but a randomized controlled trial of 33 patients using a different probiotic showed no clinical, biochemical, or endoscopic response (70). Until there is further evidence to support their efficacy, probiotic agents are not recommended for the treatment of acute antibiotic-responsive pouchitis.

Dietary intervention is another potential alternative. In patients with IPAA, there is emerging evidence implicating the relative and absolute concentration of the microbial metabolites hydrogen sulfide (H2S) and butyrate, a short-chain fatty acid (SCFA), in the pathogenesis of pouchitis. Studies have shown an association between H2S production and the number and severity of pouchitis episodes (71). Reduced fecal butyrate has also been associated with pouchitis in a number of studies (72, 73). Since they are by-products of bacterial metabolism, H2S and SCFA production depends on the availability of dietary substrates. A diet which aims at increasing SCFA and reducing H2S can theoretically target the potential pathogenesis of pouchitis, but there exists no data supporting its tolerability or efficacy to date.

Patients failing to respond to 2 weeks of one of the antibiotics can be treated with the other agent for 2–4 weeks. Patients failing metronidazole should be treated with ciprofloxacin. Patients failing ciprofloxacin can be treated with metronidazole, although we prefer using tinidazole, as it appears to be better tolerated and more efficacious against potentially resistant microbes (74). Patients failing 4 weeks of monotherapy should be treated with 4 weeks of combination therapy. Combination therapy of ciprofloxacin and metronidazole for 4 weeks achieved remission in 82% of patients in an open-label study (75). Those intolerant to metronidazole can be treated with a 4-week course of ciprofloxacin and tinidazole (74) or a 2-week course of ciprofloxacin and rifaximin (76). Patients failing 4 weeks of combination therapy are considered to have CARP and need to be investigated for secondary causes of pouchitis. Those who do respond would benefit from the same measures used in patients with antibiotic-dependent pouchitis discussed below. In patients readily responding to first line antibiotics, secondary prophylaxis to prevent future episodes can be considered. This is particularly useful in those with the aforementioned risk factors, when episodes are recurrent or when approaching important life milestones such as marriage, having children, commencing a new job, or planning a vacation.

### Chronic or Recurrent (Four or More Episodes) Antibiotic-Dependent Pouchitis

The etiopathogenesis of idiopathic pouchitis is better thought of as a spectrum, whereby it is predominately microbially mediated in antibiotic-responsive pouchitis and predominately immune mediated in CARP. Antibiotic-dependent pouchitis is somewhere in between (see **Figure 2**), with treatment measures aimed at the microbiome, with or without the addition of measures aimed at suppressing the mucosal immune response.

### Addressing the Microbial Component

Any of the antibiotics used for the treatment of antibiotic-responsive pouchitis can be used at the lowest needed dose to maintain remission in antibiotic-dependent pouchitis. However, prolonged use of metronidazole and ciprofloxacin is associated with potential adverse effects such as peripheral neuropathy and tendinopathy, respectively. In a study that followed 39 patients with antibiotic-dependent pouchitis on metronidazole or ciprofloxacin for 1 year, adverse effects were reported in 11 (28%) patients, and antibiotic resistance was found in at least one stool sample of 28 (78%) patients (77). Rifaximin is an oral, broad-spectrum, minimally absorbed GI-specific antibiotic with no clinically significant bacterial resistance (78). In an open-label study of 51 patients with antibiotic-dependent pouchitis, rifaximin at a dose of 200–1,800 mg/day was used to maintain

remission following a 2-week course with ciprofloxacin or metronidazole. At 3 months, 33 (65%) patients remained in remission. Of the 33, 19 (58%) remained in remission for 12 months (79).

Alternative approaches that bypass the need for antibiotics in antibiotic-dependent pouchitis through attaining and maintaining a healthy microbiota spectrum or microbiota function include (i) the use of probiotics, (ii) the use of the potentially healthy products of microbiota fermentation such as butyrate, or (iii) the use compounds that bind or inactivate the potentially harmful products of microbiota metabolism such as bismuth that binds H2S.

### *Probiotics*

Probiotics have been tried for secondary prophylaxis in patients with antibiotic-responsive and chronic antibiotic-dependent pouchitis. Two early placebo-controlled randomized studies of 40 and 36 patients investigated the efficacy of VSL#3 at a dose of 6 g/day. In both, 85% of the treatment group maintained remission at 9 months compared to 0% in the placebo group (80, 81). However, postmarketing open-label studies and more recent randomized trials have been disappointing (82). The cause of these contradictory outcomes is uncertain. Various factors could potentially play a role in patients' response to probiotics such as host genetic or mucosal immunological factors, microbiota profiles, or probiotic composition or dose. In addition, it is not known whether patients' different dietary habits played a role in the different responses. A better understanding of how probiotics work could help choose the right probiotic composition and dose for the right host. One of the most common proposed mechanisms of probiotic benefits is suppression of resident pathogenic bacteria; however, in the randomized trial of VSL#3 use for primary prophylaxis that measured fecal cultures, VSL#3 was not associated with decreased fecal concentrations of *Bacteroides*, coliforms, *Clostridioides*, enterococci, or total aerobes and anaerobes in responders despite the increased fecal concentration of all eight strains of ingested bacteria, suggesting that protection was not mediated by "suppression of endogenous luminal bacteria" (80). Bifidobacterium are primarily acetate producers but also are primary degraders of fibers providing intermediates to most other saccharolytic bacteria. An increase in SCFA production following ingestion of *Bifidobacterium*-containing probiotic has not been assessed. Finally, an intriguing proposed mechanism is the induction of host-protective immune responses. Lactobacilli have been found to stimulate secretory immunoglobulin A, mucosal interleukin-10, and systemic Th2 responses (83). Understanding these mechanisms of action, the patient's microbiota structural and functional profile and what members of the bacterial community are responsible for the constant antigenic drive leading to Th2 cellular activation may, allow an individualized approach of targeted probiotic therapy.

### *Prebiotics*

Fibers are preferentially fermented over protein by gut microbiota, increasing SCFA and reducing H2S, potentially reducing or preventing inflammation. Inulin was tried in a 3-week crossover randomized double-blinded placebo-controlled trial. It resulted in a statistical reduction in endoscopic and histological PDAI subscores (84). There were no differences in pouch microbiota on fecal cultures. In another crossover placebo-controlled study, 14.3 g of fructans (fructooligosaccharides) increased fecal butyrate and reduced protein fermentation while slightly increasing stool frequency from six to seven bowel actions a day. An equal amount of resistant starch increased fecal butyrate without changing protein fermentation, stool frequency, or weight (85). The combination of fibers and probiotics has also been tried. In a pilot study published only in abstract form, the combination of probiotic (*Lactobacillus* GG) and prebiotic (fructooligosaccharides) capsules resulted in complete resolution of symptoms and reversal of endoscopic and histological features in 10 patients with chronic antibiotic-dependent and antibiotic-refractory pouchitis (86). Furthermore, The Cleveland Clinic Pouch Center found that combining over-the-counter probiotics, as dietary supplements, with fibers in the form of tablets, capsules, suppositories, enemas, and foams resulted in a 3-fold rise in SCFA production in the ileal pouch, although tolerability and clinical efficacy was not reported (87). The efficacy of topical SCFAs in the ileal pouch by administering SCFA enemas has been tried in small uncontrolled studies. They have shown an overall minimal clinical response rate (88–90).

### *Bismuth*

One approach that has been proven effective at reducing fecal H2S is the use of bismuth that binds sulfide in a dose-dependent manner (91). In healthy volunteers, a dose of 524 mg of bismuth subsalicylate (Pepto-Bismol®) four times daily (qid) resulted in 100-fold reductions in H2S release (92). The efficacy of bismuth has been tried in patients with CARP. In an open-label study, bismuth-citrate carbomer enemas were shown to be effective with 83% of patients entering remission. However, in a randomized trial of CARP patients, bismuth carbomer foam enemas nightly for 3 weeks were found to be ineffective (93). Oral bismuth subsalicylate at a dose of 250 mg three times daily or qid) was found to be safe, tolerable, and effective at improving symptoms in 85% of patients with CARP, allowing half of them (45%) to discontinue antibiotics after 4 weeks (94).

In conclusion, we have a number of measures available to address the microbial component in patients with antibiotic-dependent pouchitis. We recommend starting with probiotics recommending VSL#3 at a dose of 6 g daily given its reported efficacy and safety in earlier studies. If it is costly or unavailable, we would recommend trying an alternative probiotic containing Lactobacilli and Bifidobacteria. We recommend using oral antibiotics as a second-line maintenance agent due to their potential side effects, possible reduced long-term efficacy and cost. Rifaximin is our antibiotic of choice due to its safe side-effect profile. We use a dose of 500 mg daily, although any dose between 200 and 1,800 mg can be used. This strategy can by limited by rifaximin's high cost. We reserve ciprofloxacin (250–500 mg/day) or tinidazole (250 mg/day) for those who cannot obtain rifaximin or when it has failed. Owing to the side effects from long-term use, we would not recommend using ciprofloxacin or tinidazole continuously for more than 1 year. Oral bismuth subsalicylate at a dose of 250 mg (three times daily or qid) is used as third line

following oral antibiotics. Until we have more robust data on the dose, efficacy, and tolerability of fibers and SCFA, we recommend combining all these measures with dietary advice on a diet rich in fermentable fibers, individually adjusting the quantity and type of fibers according to the patient's tolerability. Finally, since antibiotic-dependent pouchitis is both microbially and immune mediated, it is reasonable to add measures addressing the immune response to any of the above in an attempt to help patients remain in remission while discontinuing medications with potential side effects such as ciprofloxacin.

## Addressing the Mucosal Immune Response

Patients partially responding to measures targeting the pouch microbiome or those on long-term antibiotics wanting to reduce or discontinue them can be treated with measures aimed at suppressing the mucosal immune response.

### *5ASA*

Topical and oral mesalazines have been tried in patients with CARP showing a 50% remission rate (74). Sulfasalazine at a dose of 2 g/day was investigated as a primary prophylaxis agent. Given their safety and tolerability profile, topical or oral 5ASAs can be tried in patients with antibiotic-dependent pouchitis to see if they can help reduce or discontinue antibiotic use (42).

### *Corticosteroids*

Budesonide enemas at a dose of 2 mg/100 ml a day for 6 weeks were found to be non-inferior and more tolerable than metronidazole for the management of acute pouchitis (24). Oral budesonide was assessed in 14 patients with acute pouchitis ($n = 6$) and chronic pouchitis ($n = 8$) associated with PSC. Patients were treated with 9 mg/day of budesonide for 1–3 months and maintained on 3–6 mg/day for 9 months. At 1 year, 75% maintained remission including all of those with acute pouchitis and six of eight of those with chronic pouchitis. An 8-week course of oral budesonide controlled ileal release (9 mg/day) was also successful in inducing remission in 75% of patients with autoimmune CARP. The use of budesonide can therefore be tried, although more data on long-term efficacy and safety are needed before this is a standard recommendation.

## Chronic Antibiotic-Refractory Pouchitis

In CARP, pouch microbiota may still play a role in driving inflammation, as evident by some response to antibiotics, but the disease is predominately immune mediated and is sometimes referred to as immune-mediated pouchitis. Therefore, it is best managed with medications that address the mucosal immune response. The classification of CARP into PSC-associated CARP, IgG4-associated CARP, and autoimmune CARP helps guide management.

a) PSC-Associated CARP

   I. Budesonide: As detailed above, budesonide has been shown to be effective in inducing and maintaining remission in PSC-associated CARP (95), but the dose needed for long-term maintenance and its long-term efficacy and safety are yet to be determined.

   II. Vancomycin: Oral vancomycin (500–1,000 mg/day) is successfully used to achieve and maintain remission in PSC-associated pouchitis/enteritis at the Cleveland Clinic Center for Ileal Pouch Disorders (52). We have had similar success inducing remission with oral vancomycin at a dose of 250 mg qid. Furthermore, vancomycin may provide an added benefit of improving liver function tests (96–98). There are no published data on the long-term efficacy or safety of oral vancomycin in IPAA patients. Most of the available data are from patients with recurrent CDI. These studies have not shown an increased risk of adverse events; however, they are limited by short duration of follow-up and lack of prospective, standardized follow-up to detect safety-related outcomes (99). Oral vancomycin has been shown to reduce bacterial richness and diversity and to increase the risk of vancomycin-resistant enterococcus colonization in patients with recurrent CDI. In PSC patients, oral vancomycin has been well-tolerated (97, 98). Therefore, the efficacy and potential hepatoprotective effect of oral vancomycin and the long-term efficacy and side effects of other immune suppressants should be weighed against the potential adverse effects of long-term vancomycin use. We recommend a trial of oral vancomycin to induce remission at a dose of 250 mg qid for 4–8 weeks followed by an attempt to maintain remission with a dose of 125 mg−250 mg qid. This can be tried before or after other immune suppressants used for non-PSC-associated pouchitis.

b) IgG4-Associated Pouchitis

   IgG4-associated pouchitis was first described by Shen et al. (100). This is an immune-mediated pouchitis often associated with a long segment of PI (101). Early recognition may help minimize antibiotic use and direct treatment to measures addressing the mucosal immune response early on. There are limited data on treatment options. There are no data on the efficacy of 5ASA or immunomodulators such as thiopurines and methotrexate. Corticosteroids such as budesonide have been reported to improve inflammation in case series (102). Patients failing budesonide should be considered for biological therapy. Unlike autoimmune CARP, the efficacy of different biological agents is not published. There are case reports of IgG4-mediated diseases (pancolitis and ocular adnexal disorder) responsive to adalimumab and infliximab (103, 104). Rituximab, a monoclonal antibody against CD20-positive lymphocytes, is used successfully in other IgG4-mediated diseases (105). We recommend using oral budesonide as first-line treatment starting with a dose of 9 mg for 8 weeks, then weaning it down to a maintenance dose of 3–6 mg daily. The next step is not clear. A step-up approach similar to that of autoimmune CARP can be followed, although vedolizumab and ustekinumab are not necessarily preferred over anti-TNFs. Rituximab can be considered in those failing other biologics and before pouch excision or diversion.

c) Autoimmune CARP

   The management of autoimmune CARP shares a great deal of similarity to that of UC.

I. 5ASAs: Topical and oral mesalazines Canasa® suppositories (1,200 mg/day), Rowasa® enemas (4,000–8,000 mg/day), and oral Pentasa® (2,400–4,800 mg/day) have been tried in patients with CARP, demonstrating remission rates of 50% (74). Owing to their safety profile, oral or topical mesalamine agents are the preferred first-line drugs for autoimmune CARP.

II. Budesonide: As detailed above, topical budesonide enema 2 mg/100 ml and oral budesonide-controlled release 9 m/day have been shown to effective in inducing remission in acute and autoimmune CARP (95, 106). Budesonide enemas can be tried in those intolerant or failing 5ASAs. While oral budesonide may be useful in inducing remission particularly in those with associated PI, the dose needed to maintain remission and the long-term efficacy is not yet known. As such, ongoing use should be weighed against the long-term efficacy and safety of immunomodulators and biological agents.

III. Immunomodulators: Historically, immunomodulators including azathioprine (50–100 mg/day), 6-mercaptopurine (50–100 mg/day), and oral or subcutaneous methotrexate (7.5–25 mg/week) have been used as second-line therapy for autoimmune CARP, particularly in those with extra intestinal manifestations. There is, however, a paucity of data on the use of immunomodulator monotherapy for pouchitis (107). In contrast, there are more data supporting the efficacy of biological agents, particularly vedolizumab and ustekinumab, in the treatment of autoimmune CARP (59, 60). The current place of immunomodulators in the treatment algorithm, therefore, depends on availability and early access to biological agents.

IV. Biological agents: To this date, no randomized controlled studies assessing the effectiveness of biological therapy for CARP exist. Most available data come from small observational studies.

   a) Vedolizumab: In the largest observational study, 20 patients with chronic, antibiotic-dependent, or refractory pouchitis were treated with vedolizumab using the standard IBD dose in 10 centers in Germany. At 14 weeks, the overall reported response rate (defined as a PDAI fall of 3 points or more) was 64% with a drop of median PDAI from 10 to 3 and discontinuation of antibiotics in 17 out of 19 patients. In addition, no serious side effects or intolerances were reported (59). Other case series have reported similar efficacy (108, 109).

   b) Ustekinumab: In the largest observational study, 24 patients with CARP (including 2 with PSC-associated CARP) were treated with ustekinumab using standard CD dosing. There was a 50% clinical and endoscopic response. The clinical response demonstrated was an improvement in median pouch frequency from 8 to 6 ($P = 0.002$). The endoscopic response was a decrease in ulcerated surface from >10 to <10% (60).

   c) Anti-TNF: In a systematic review, the short- and long-term efficacy of anti-TNF therapy (infliximab and adalimumab) in CARP were analyzed. Short-term efficacy was defined as clinical remission at week 8. Long-term efficacy was defined as clinical remission at the end of year. Short-term efficacy was 10%, and long-term efficacy was 37%. There was significant heterogeneity among the studies. For example, one study assessing the short- and long-term efficacy of infliximab on 24 CARP patients showed an 88% clinical response rate (14 partial, 8 complete) at week 10 with 56% maintaining this response at a 20-month median follow-up. In a more recent study, not included in the meta-analysis, the efficacy and tolerability of infliximab ($n = 12$) and adalimumab ($n = 3$) were assessed. At week 14, clinically relevant remission, defined as a mPDAI <5 and a reduction of mPDAI $\geq$ 2 points from baseline, was achieved in 43.5% of the infliximab group and 38.5% of the adalimumab group. In the long term, 40.7% discontinued anti-TNF therapy due to intolerance or drug reaction (109).

   We recommend using vedolizumab as the first-line biological therapy followed by ustekinumab owing to their efficacy, better side-effect profile, and low immunogenicity, and need for concomitant immunomodulatory therapy. We recommend reserving the use of anti-TNFs to those failing vedolizumab and ustekinumab. The management algorithm for pouchitis is seen in **Figure 3**.

V. Surgery: Surgery may be considered as a last resort for patients with CARP refractory to all medical therapy. The procedure of choice is an end ileostomy, with or without pouch excision. This should be reserved for patients with ongoing symptoms significantly impacting on QOL as stoma complication rates can be as high as 35–40% (110). Furthermore, the decision to remove the pouch or leave it *in situ* includes balancing a 35–40% of pouch stump sinus with pouch excision vs. a 50–60% of diversion pouchitis and other complications including pouch stricture dysplasia (110).

## Cuffitis

Cuffitis is defined as residual inflammation of the rectal cuff which will appear on pouchoscopy as 360° circumferential inflammation of the rectal cuff with histological findings consistent with UC proctitis. Patients are at a higher risk if there is a long-retained cuff > 2 cm. The treatment of cuffitis is similar to that of proctitis, starting with topical therapy with 5ASAs, corticosteroids, and escalating treatment to oral 5ASAs, immunomodulators, and eventually biological agents. As with proctitis, refractory cases can be treated with tacrolimus suppositories (111). Importantly, medically refractory cuffitis should raise suspicion for CD-associated cuffitis or pericuff fistula, sinus, or abscess. This can be further investigated with a pelvic MRI, contrast pouchogram, and examination under anesthesia. Furthermore, a foreign body, such as a retained suture in the anterior wall of the cuff, can cause local ulceration with significant urgency not responding to topical treatment. Diagnosis can be made on pouchoscopy with local ulceration

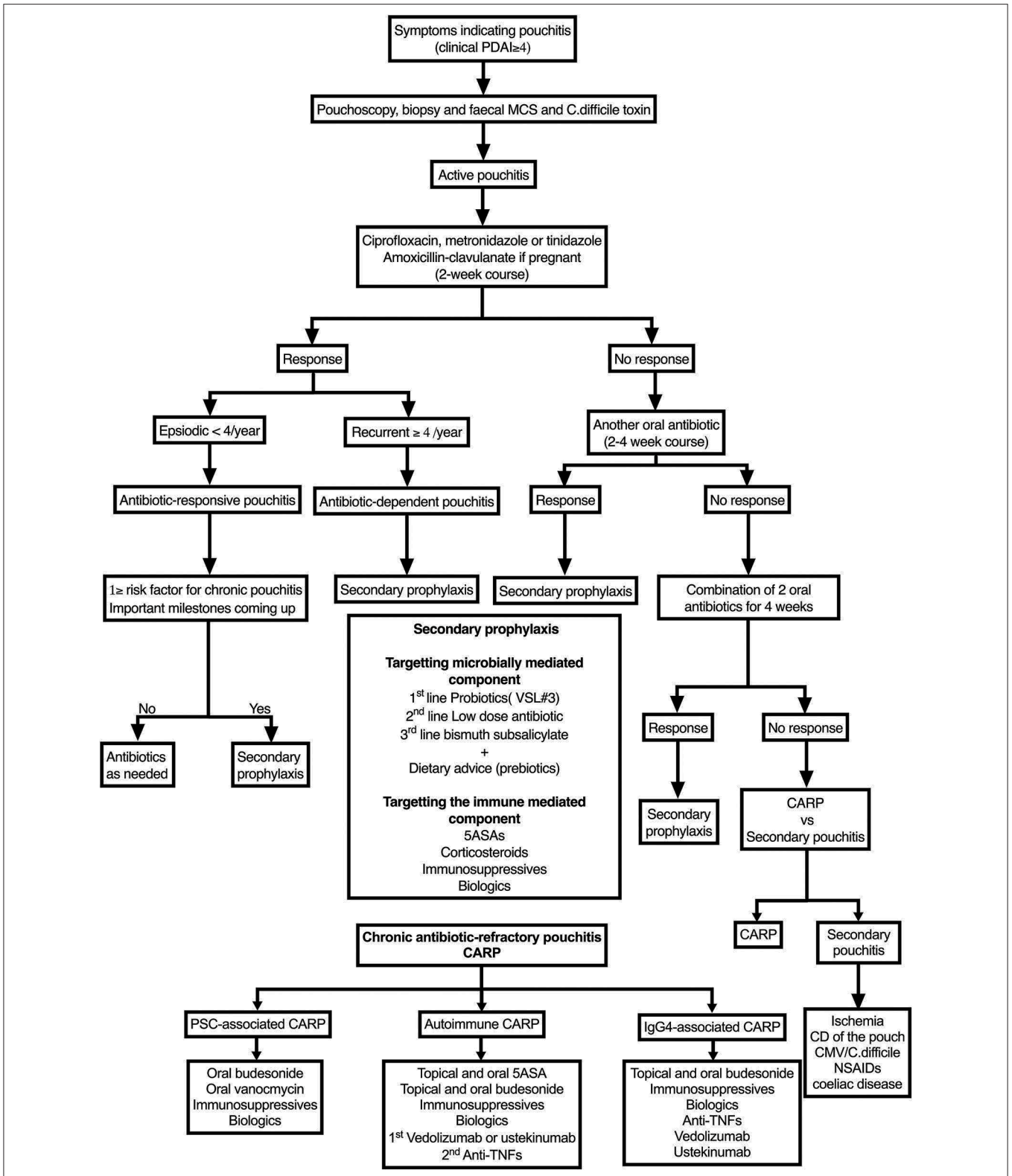


**FIGURE 3 |** Algorithm for managing pouchitis. PDAI, Pouchitis Disease Activity Index; PSC, primary sclerosing cholangitis; NSAIDs, non-steroidal anti-inflammatory drugs; CARP, chronic antibiotic-refractory pouchitis.

at 3–5 o'clock with an underlying foreign body; treatment is surgical. Finally, idiopathic medically refractory cuffitis can be treated surgically. Redo IPAA is possible if there is a long-retained cuff. Another surgical treatment is cuff mucosectomy and pouch advancement (112).

### Crohn's Disease of the Pouch

At present, there are no guidelines on the best treatment approach for patients who develop CD of the pouch, and the risk of pouch failure with diversion or excision remains high at 17–57% depending on the series (55). The efficacy of various treatments are discussed comprehensively by Lightner et al., concluding that different treatment regimens may be effective based on phenotypic stratification, with fistulizing disease requiring the most aggressive treatment (55). Debilitating CD of the pouch refractory to all medical therapy should be discussed in a multidisciplinary team at a high volume center, as it may need surgical intervention in the form of permeant diversion or pouch excision. These procedures are associated with higher risks of complications and can be as challenging as IPAA surgery. The complication rate of permanent diversion or secondary ileostomy is 35–40% in addition to the risk of diversion pouchitis and pouch strictures (50–60%) precluding dysplasia surveillance. Pouch excision, on the other hand, is not without risks with a reported 35–40% risk of pouch stump sinus. Therefore, surgery should be reserved to CD patients failing all other treatment options (113).

### Prepouch Ileitis

Prepouch ileitis (PI) is defined as acute or chronic inflammation of the prepouch ileum extending in a contiguous fashion from the pouch inlet beyond 2 cm and up to as much as 30, and in one study 50 cm (114, 115). It manifests endoscopically as erosions, ulcers, erythema, friability, and strictures. It is usually associated with pouchitis (115, 116). The importance of recognizing and distinguishing PI from pouchitis alone is that PI appears to be an immune-mediated process not seen in FAP, is less responsive to antibiotic therapy, and is associated with a more severe course than pouchitis alone. Furthermore, PI needs to be distinguished from CD, which has a much higher rate of pouch failure. A diagnosis of CD can only be made when disease is more proximal, is segmental, includes deep fissures, includes fistulas, is associated with perianal disease, and the finding of "transmural lymphoid aggregates and epithelioid granulomas" (114). Moreover, CD manifesting as PI is less likely to be associated with pouchitis. Importantly, in IPAA patients with a history of indeterminate colitis, the diagnosis of PI strongly suggests CD or CD-like behavior with high pouch failure rate, and, therefore, a need for early aggressive medical and surgical therapy. Finally, the presence or absence of PSC or IgG4 should be determined as the PSC- and IgG4-associated PI should be managed like PSC- and IgG4-associated CARP, respectively. The management of PI associated with IPAA-UC follows the guidelines of managing idiopathic pouchitis. Since antibiotics have a 50% failure rate and the disease is predominately immune mediated, we recommend either commencing treatment with immunosuppressives or biologics or escalating rapidly to them. Immunosuppressive and biological treatments are the same as that used for autoimmune CARP. There is a small number of published studies on the efficacy of immunosuppressives and biologics, and they are largely observational and retrospective. Most data are with infliximab, with response rates ranging from 25 to 56% (114, 117). Accordingly, the decision on which immunomodulator or biologic to use should be individualized taking into consideration the patient's prior biological exposure, age, and infection and cancer risk.

## SURGICAL AND MECHANICAL POUCH DISORDERS

A basic understanding of surgical and mechanical complications is useful when managing symptomatic pouch patients. This helps facilitate the most appropriate diagnostic test and the best effective treatment, be it medical or surgical. It is useful to broadly divide these disorders into obstructive and leakage-related septic complications. The complications, their risk factors, best diagnostic investigation, and recommended treatment are outlined in **Table 2**.

## FUNCTIONAL POUCH DISORDERS

### Irritable Pouch Syndrome

Around a third of patients with symptoms of frequency and urgency persisting beyond the 6- to 12-month adaptation period post-IPAA creation have no evidence of inflammation on laboratory tests or pouchoscopy (120). Using the total PDAI, these patients would have a score of <7 with a 0–1 pouchoscopy subscore. A diagnosis of IPS has been coined for these patients (120). There are no Rome criteria for the diagnosis of IPS; therefore, not all patients with symptoms of urgency and frequency may have IPS. Such a diagnosis, although not necessary, may offer reassurance and may guide management, as IPS therapy resembles that of IBS, starting with dietary modifications and then including use of antidiarrheals, antispasmodics, and even antidepressants (e.g., amitriptyline). Since there is significant intersubject variability on what food type causes symptoms, dietary modifications need to be personalized following a detailed review of the patient's dietary habits using a food frequency questionnaire and, if possible, a food dairy. Meal volume and frequency have also been shown to correlate with stool output; hence, meal frequency and volume should also be determined. Lactose intolerance can develop *de novo* after IPAA in some of patients. Poorly absorbed carbohydrates and fibers can be fermented by bacteria releasing gas and increasing stool bulk, exacerbating bloating, and pouch frequency. Indeed, most patients do report improved pouch symptoms of frequency urgency and bloating with a diet low in carbohydrates and fibers and high in meat. Interestingly, supplemental fibers like psyllium husk, frequently prescribed by colorectal surgeons, can reduce frequency and improve stool consistency in pouch patients when used in small amounts. These poorly fermentable

**TABLE 2 |** Surgical and mechanical disorders of IPAA.

| Disorder | | Risk factors | Incidence (%) | Presentation | Diagnosis | Treatment |
|---|---|---|---|---|---|---|
| **OBSTRUCTIVE** | | | | | | |
| Stricture | Stoma site<br>Inlet<br>Anastomosis | End to end anastomosis<br>Ischemia<br>Anastomosis dehiscence<br>Pelvic sepsis<br>De-functioning ileostomy | 5–11 | Obstructive symptoms (abdominal pain, bloating, distention, incomplete evacuation) | Pouchoscopy<br>MRE<br>CTE | 1st line: balloon dilatation. Needle knife for anastomotic structures in women.<br>2nd line: surgical stricturoplasty |
| Floppy pouch complex[a] | Pouch prolapses | Low BMI** female sex | 0.3 | Obstructed defecation | Pouchoscopy (Collapse)<br>BD* (bulging) of the anterior pouch wall | Endoscopic banding.<br>Surgery is ineffective. |
| | Pouch folding | Low BMI female sex | Unknown | Obstructed defecation | Pouchoscopy: pouch angulation.<br>BD: C-shaped pouch | Surgical treatment |
| | Afferent limb syndrome | Low BMI female sex | | Obstructed defecation<br>Acute small bowel obstruction | BD: minimum contrast enters afferent limb | Surgical treatment |
| | Efferent limb syndrome | Long S-pouch efferent limb<br>J-pouch with Long retained cuff (>7 cm) | | Obstructed defecation<br>Acute small bowel obstruction | Pouchoscopy: long cuff or efferent limb and angulation at body<br>BD: similar findings | Surgical treatment<br>Endoscopic balloon dilation of pouch inlet if surgery not possible or fails |
| **SEPTIC DISORDERS** | | | | | | |
| Anastomotic[b] leakage | Pelvic sepsis | Preoperative corticosteroid use<br>Anastomotic tension<br>Intra and post-operative blood transfusion<br>Male sex<br>BMI > 30 | 6–37 | Postoperative sepsis | Laboratory blood tests<br>Imaging: CT abdomen and pelvis<br>BD | Antibiotics, percutaneous drainage, and surgical treatment |
| | Presacral sinus | Male sex<br>Pelvic sepsis | 5 | Night sweats, fevers, tail bone pain, and weight loss | Pouchoscopy<br>MRI of the pelvis<br>BD | Endoscopic sinusotomy<br>Pouch redo surgery |
| | Anastomotic fistula (Within 6 months post IPAA) | Pelvic sepsis<br>1 or 2 stage IPAA<br>Female. sex: risks vaginal fistula | 7 | Draining fistula<br>Pain and pelvic sepsis from an abscess | Pouchoscopy<br>MRI of the pelvis<br>EUA+ | Surgical treatment |

*BD: Barium defecography.*
**BMI: Body mass index.*
*+EUA: Examination under anesthesia.*
[a]*Khan and Shen (118).*
[b]*Li et al. (119).*

fibers can slow the gastrointestinal transit and increase stool bulk through water-trapping effects. Therefore, use of poorly fermentable fibers can be tried particularly if bloating is not a predominate symptom. Some foods such as bananas, potatoes, pasta, and bread have been reported to decrease stool consistency or "thicken stools" and therefore may be tried to see if this helps reduce frequency (121, 122). Finally, one study found meal volume and frequency and late-night meals to correlate with pouch frequency, recommending no more than three meals with the last at least 2 h before bedtime (5).

Patients who have ongoing symptoms despite simple dietary modifications and a trial of fiber may benefit from a trial of the low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols diet. This was found in a 6-week trial in 12 patients to improve median pouch frequency from eight to four in symptomatic patients with no pouchitis (123). Those whose symptoms persists despite dietary modifications and a low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols diet can try antidiarrheal agents like loperamide or codeine or antispasmodics like hyoscyamine. If bloating is the predominate symptom, and since SIBO is common in patients with IPAA (4), a diagnostic and therapeutic trial of antibiotics used in SIBO can be tried. Finally, IPS is characterized by visceral hypersensitivity (23). Therefore, like IBS, neuropathic medications like amitriptyline can be tried at the dose used for IBS at 10–50 mg nightly.

It is important to note that some IPAA patients report no increased frequency or urgency and no obstructive symptoms. Instead, they are profoundly troubled by other symptoms such as seepage, nocturnal incontinence, daytime incontinence, and intense perianal burning. General advice provided to reduce seepage includes a small meal at least 3 h before bedtime, emptying the pouch at bedtime, and taking 4 mg of loperamide. The latter has been the only measure associated with improved

sphincter continence (124). As sphincter strength decreases over time, daytime incontinence can affect up to 40–50% of patients after 20–30 years, causing significant distress and impacting on social life and QOL (125). The dietary measures discussed for frequency and urgency can be tried here, especially food types found to "thicken stools." Fiber supplements can increase stool bulk, and loperamide can reduce frequency and strengthen anal sphincter (124). Perianal burning is usually triggered by known foods, such as spices and citrus fruits; such known triggers can be restricted or avoided. There is no specific treatment for burning, but barrier ointment can provide symptomatic relief.

Dyssynergic defecation (DD) or non-relaxing pelvic floor dysfunction is an underdiagnosed pouch disorder (15, 17). It is defined as "the paradoxical contraction and/or impaired relaxation of pelvic floor and anal muscles during defecation" (126). DD can coexist with mechanical and inflammatory pouch disorders. Therefore, it is not unreasonable to assess all IPAA patients presenting with dyschezia for DD, even if initial workup reveals a structural or inflammatory cause (17). When coexisting with inflammatory or mechanical pouch complications, DD can be divided into primary and secondary DD. When DD is the initial trigger leading to fecal stasis and potentially long-standing inflammation as in chronic pouchitis, DD is considered primary. Here, biofeedback therapy targeting DD can improve symptoms, anopouch manometric values, and inflammation. When DD is secondary to chronic pouchitis or pouch outlet stricture or prolapse, it is classified as secondary DD. Here, treating the inflammation or the mechanical disorder can improve symptoms, anopouch manometric values, and inflammation.

There is, at present, no standard criteria for the diagnosis of non-relaxing pelvic floor dysfunction in IPAA patients. Although not validated for IPAA, the same tests used for the diagnosis of DD in patients with an intact colon have been used in IPAA patients using the same normal reference ranges based on healthy controls. These tests include anorectal manometry (ARM) or anopouch manometry, the balloon expulsion test, and barium or magnetic resonance defecography. Abnormal ARM, defined as paradoxical contractions, and failed balloon expulsion were found in one study in 50–60% of patients with functional pouch disorders presenting with dyschezia (15). In another study, a positive balloon expulsion test, defined as >200 g of weight added in the left lateral position or >60 s before balloon expulsion in the seated position, was found in 78% of patients. In contrast, positive ARM, defined as a total of two abnormal ARM values of elevated mean resting anal pressure, reduced pouch–anal gradient, reduced rectal (pouch) pressure, anal relaxation <20%, or an elevated residual anal pressure, was present in only 21% of those with DD. Barium or magnetic resonance defecography can be a useful additional test when balloon expulsion test and ARM are inconclusive, with the added benefit of ruling out pouch outlet obstruction. Finally, since DD that coexists with inflammatory or mechanical pouch complications can be primary or secondary and since there is no simple way of differentiating between the two, assessing response to a trial of biofeedback therapy has been proposed as a non-invasive means of distinguishing the two (19). Primary DD would show manometric and symptomatic response to biofeedback (17). Conversely, those with secondary DD would show symptomatic and manometric response to treating the inflammation with a course of antibiotics or anti-inflammatory or treating the mechanical complication such as stricture (19).

# POUCH DYSPLASIA AND CANCER

## Incidence

The exact incidence of pouch dysplasia and pouch cancer is not clear. In the two largest cohort studies, at 20 years, the incidence of pouch dysplasia was 2.2% in the Cleveland Clinic cohort and 6.9% in the Dutch cohort (127, 128). There are even fewer publications on pouch cancer. The cohort study from The Cleveland Clinic reported a cumulative incidence of cancer of 4.2% at 20 years (127), whereas the Dutch cohort reported a cumulative incidence of 3.2% at 20 years (128). The primary site of dysplasia and cancer is the ATZ or cuff (129).

## Risk Factors for Dysplasia

The single most important risk factor for pouch dysplasia and cancer is colitis-associated neoplasia before colectomy. In The Cleveland Clinic cohort, colitis-associated neoplasia was associated with pouch dysplasia and pouch cancer with hazard ratios of 3.62 (95% CI, 1.59–8.23) and 13.43 (95% CI, 3.96–45.54), respectively. In the Dutch study cohort, colitis-associated neoplasia was similarly associated with dysplasia and cancer of the pouch with hazard ratios of 3.76 (95% CI, 1.39–10.19) and 24.69 (95% CI, 9.61–63.42), respectively (127, 128).

Other risk factors have included concurrent PSC, chronic inflammation of the cuff or the pouch, and mucosal villous atrophy (129). Interestingly, in the Cleveland Clinic cohort, PSC was not shown to be a risk factor, but this might have been due to type II error (127).

## Diagnosis

Pouchoscopy with biopsy is the test of choice for pouch neoplasia surveillance. Neoplastic lesions may appear as depressed, slightly raised lesions or, if advanced, appear mass-like. However, they can also be flat and invisible on endoscopy. In a retrospective study of 11 patients with pouch cancer, 3 (27.3%) had no endoscopically visible lesions at the time of cancer diagnosis (129). The use of narrow band imaging or conventional chromoendoscopy for early detection of pouch neoplasia has not been studied, although their utility in improving polyp detection and colitis-associated neoplasia suggests a potential benefit in at risk patients. Lesions, however, may be endoscopically visible. Until more data are published, we recommend taking at least four or quadrant biopsies from the cuff even if it is normally appearing on white light and chromoendoscopy. As with colitis-associated dysplasia, specimens are best reviewed by an expert gastrointestinal pathologist and any dysplasia confirmed by a second expert pathologist.

## Surveillance

There are no unifying consensus recommendations for pouch neoplasia surveillance. Pouch cancer carries a high mortality (123). Pouchoscopy surveillance can diagnose dysplasia allowing

early intervention. Since pouch dysplasia and cancer incidence is low and pouchoscopy and biopsy is somewhat, we support a risk-stratified approach into high, medium, and low risk (129).

### High Risk

Includes patients with previous colitis-associated neoplasia before or at colectomy, history of indefinite dysplasia of pouch or focal low-grade dysplasia of the pouch. Pouchoscopy is recommended every year.

### Intermediate Risk

Includes patients with chronic pouchitis, cuffitis, severe mucosal atrophy, previous biopsies showing hyperplastic or serrated changes in the cuff or pouch, concurrent PSC, and family history of colorectal cancer. Pouchoscopy is recommended every 1–2 years.

### Low Risk

None of the above. Can undergo pouchoscopy every 3 years commencing 10 years after IPAA surgery.

## Treatment

The management of pouch adenocarcinoma is surgical and includes abdominoperineal resection with permanent ileostomy. The need for neo or adjuvant chemotherapy remains unclear due to the rarity of the disease. Because pouch high-grade dysplasia is considered a marker for concurrent or subsequent pouch carcinoma, once confirmed, the recommended treatment is pouch excision (130). Endoscopically resectable pouch low-grade dysplasia should be performed by an experienced endoscopist and followed up closely. If endoscopically invisible or unresectable, pouch low-grade dysplasia should be treated with pouch excision.

## SUMMARY

Quality of life after IPAA surgery is generally good. However, patients can be troubled by pouch-related symptoms and pouch disorders that can be inflammatory, mechanical/surgical, and functional. Maintaining a healthy pouch includes optimizing pouch function, providing advice on a healthy diet and lifestyle, screening for and addressing metabolic complications of IPAA, pouch surveillance, and risk stratification for risk of pouchitis and pouch failure. Patients harboring one or more risk factors for pouchitis can be offered primary prophylaxis. Pouchitis is the most common inflammatory disorder. Primary pouchitis is best classified according to antibiotic response into antibiotic responsive, antibiotic dependent, and antibiotic refractory. This is a spectrum of the same disease. It is predominately microbially mediated early on in acute antibiotic-responsive pouchitis and ends up becoming predominately immune mediated in CARP. Secondary prophylaxis is recommended for recurrent antibiotic-responsive and for antibiotic-dependent pouchitis. Probiotics are first-line secondary as prophylactic agents, followed by the antibiotic rifaximin and then bismuth. Prebiotics such as fibers are best combined with any of the above and delivered in the form of a healthy diet that can be individualized based on patients' tolerance of fermentable fibers. Secondary causes of antibiotic-refractory pouchitis should be ruled out before a diagnosis of CARP is made. Ischemic pouchitis is one of the most common causes. Infections such as CMV and *C. difficile* are associated with fever and night sweats. Other secondary causes include celiac disease, NSAID, and CD of the pouch. Crohn's disease of the pouch can be inflammatory, fibrostenosing, and fistulizing. CARP is best classified as PSC associated, IgG4 associated, and autoimmune. The former two are often associated with PI. PSC-associated CARP and PI can be treated with budesonide or oral vancomycin. Early recognition of IgG4-associated pouchitis minimizes antibiotic use. Budesonide seems to improve inflammation and should be used as first line. Step-up therapy includes immunosuppressive and biologics including anti-TNFs, vedolizumab, and ustekinumab. Autoimmune CARP can be managed in a manner similar to UC. First line includes topical and oral 5ASAs, followed by oral or topical budesonide. There are limited data on the efficacy of immunosuppressives. The current place of immunosuppressives in the treatment algorithm depends on availability and early access to biological agents. Vedolizumab and ustekinumab are the preferred first- and second-line biologics for autoimmune CARP owing to their efficacy, better side effect profile, and low immunogenicity, and need for concomitant immunomodulatory therapy. Anti-TNF should be reserved for autoimmune CARP failing the above and for CD of the pouch. There are no guidelines for the surveillance of pouches for dysplasia. Incidence varies based on a patient's risk. Pouch cancer carries a high mortality. Pouchoscopy surveillance can diagnose dysplasia allowing early intervention. Since incidence is low, however, a risk-stratified approach is recommended.

## AUTHOR CONTRIBUTIONS

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

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REVIEW
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# Predicting Response to Vedolizumab in Inflammatory Bowel Disease

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Vedolizumab is known to be safe, well-tolerated, and effective. However, as personalization becomes an increasingly important aspect of IBD care and in lieu of guidelines to inform clinicians on positioning of biologics, there is a need to reliably predict response to inform patient preferences and shared decision-making. Recent data from clinical trials and real-world evidence have elucidated predictors of clinical and endoscopic response while providing the framework to establish predictive models. Current models are able to predict that those patients with less severe disease, without prior biologic exposure and who demonstrate early response to VDZ have the highest rates of durable clinical and endoscopic response and remission. When incorporating these models into clinical practice, clinicians will be able to identify those patients who are likely to respond before drug initiation as well as early non-responders and response latency after initiation of vedolizumab. In a shift toward personalization of medicine in IBD, the ability of predictive models for vedolizumab to aid pre-biologic and early management will inform both clinician and patient. Ideally this will provide both a personalized and more cost-effective approach, though further studies in cost-analysis in this framework are needed. Though current models are comprehensive of existing data, future research on microbial and translational biomarkers will be additive and necessary to provide full personalization of treatment.

**Keywords: vedolizumab, biologic, response, prediction model, IBD**

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## KEY CONCEPTS

- Vedolizumab is safe, well-tolerated, and effective.
- UC and CD patients with less severe disease, without prior biologic exposure, and who demonstrate early response to VDZ are most likely to respond to therapy.
- The CDST from Dulai et al. can be used before initiation of VDZ to determine those most likely to respond and those who may be more likely to benefit from early consideration of dose escalation or alternative therapy.
- The CDST from Dulai et al. was able to predict drug exposure, rapidity of onset, and clinical outcomes including clinical and steroid-free remission.

## INTRODUCTION

Vedolizumab (VDZ) is a humanized monoclonal anti-integrin biologic approved for moderate to severe Crohn's Disease (CD) and Ulcerative Colitis (UC). Vedolizumab selectively inhibits leukocyte extravasation into the gut, and few other less clinically relevant tissues, via disruption of alpha4beta7 integrin on leukocytes and adhesion molecules on the vascular endothelium.

Phase 3 clinical trials confirmed the efficacy of VDZ in CD and UC and observational cohorts have confirmed its real-world effectiveness and safety. Despite the favorable safety profile and effectiveness of VDZ there are no guidelines to aid clinicians with its positioning among biologics. The ushering in of the biologic era brought with it the luxury of greater choice. With multiple options available for therapy in moderate to severe Inflammatory Bowel Diseases (IBD), many of which appear to be equivalent in effectiveness and safety, there has been a necessary push to improve shared-decision making around treatment choices. Hierarchical preferences of providers and patients could bring traditionally second-line therapies to the forefront. With personalization of therapy to these preferences and without formal guidelines or robust comparative clinical trials, it will be increasingly important for clinicians to critically evaluate existing data for many treatment-related factors, including predicting response. In this article we will review current literature from clinical trials, their *post-hoc* analyses, and real-world data that elucidate predictors of primary response to VDZ in CD and UC.

# PREDICTORS OF CLINICAL RESPONSE AND REMISSION

## Baseline Disease Activity

Subgroup analyses of the GEMINI 1 and 2 trials evaluated demographic and baseline characteristics associated with response and/or remission at 6 and 52 weeks. Less severe clinical disease scores, CDAI score $\leq$ 330 and Mayo score $<$ 9, were associated with higher likelihood of remission compared to placebo at 6 and 52 weeks in CD and UC (1, 2). Real-world observational cohorts have supported this finding. The US VICTORY consortium found that those patients with baseline clinically severe CD or active perianal disease were less likely to obtain clinical remission (3). The French GETAID cohort found that patients with more severe baseline UC or CD were less likely to achieve clinical remission at 14 and 54 weeks (4, 5). An Israeli cohort reported that mild clinical disease activity was associated with increased clinical remission in CD at 14 weeks, with no predictors in UC (6). A German cohort of 97 CD patients found that a low Harvey-Bradshaw Index (HBI) score and no hospitalizations in the preceding year predicted clinical remission at 14 weeks (7). In the largest cohort assessed, Chaparro et al. found that higher baseline HBI in CD to be a negative predictor and mild disease in UC to be a positive predictor of clinical remission at 14 weeks (8) (**Table 2**).

## TNF Antagonist Exposure

It's known that efficacy of TNF antagonists is lower with a second agent after loss of response to a first, and it could be expected that this would be seen with other biologics following TNF antagonist therapy (9, 10). In a pooled *post-hoc* analysis of GEMINI 2 and 3, TNF antagonist naïve patients who had responded to VDZ at 6 weeks were more likely to achieve or maintain remission at week 52 as compared to TNF antagonist failure patients (11) (**Table 1**). Sands et al. found that patients with CD who had failed TNF antagonist therapy were more likely to be in clinical remission at 10 weeks but not 6 weeks as compared to placebo (26.6 vs. 12.1% [$p = 0.001$] and 15.2% vs. 12.1% [$p = 0.433$]) (14). The VICTORY consortium observed that prior TNF antagonist exposure was associated with lower rates of remission and mucosal healing in CD and decreased rates of response and remission in UC, and this observation remained irrespective of the statistical approach applied to the data (15). Similarly, results from Stallmach et al. demonstrated that TNF antagonist exposed UC patients were less likely to achieve clinical remission (16). An Israeli cohort in contrast found that prior TNF antagonist exposure had no effect on outcomes of UC or CD at 52 weeks, though limited by low numbers of TNF-naïve patients (8%) (17) (**Table 2**).

## Concomitant Immunosuppressive Therapy

The GEMINI trials were not powered to assess combination therapy, however, sub-group analyses did not observe a difference between VDZ monotherapy and combination therapy on rates of response or remission (1, 2). Real-world cohorts observed that steroid use was associated with lower rates of response in CD (5, 6) and UC (4, 16), possibly a confounding due to indication as steroids are more often used in patients with more severe disease, but immunomodulator addition after induction was associated with increased response and remission in CD (16). These data did not bear out in remaining real-world cohorts. For example, no differences were noted with any concomitant therapy in Israeli cohorts or the VICTORY or Cross Penine cohorts (3, 17–19). Regardless of these results, it is important to remember that the appeal of the relative safety for VDZ is decreased with combination therapy with corticosteroids and/or immunomodulators (20), and there also does not appear to be the same risk of immunogenicity or benefit of increased trough levels with concomitant immunomodulators for VDZ (21, 22) (**Table 2**).

## Biomarkers

GEMINI 1 and 2 demonstrated that elevated inflammatory markers were associated with lower rates of clinical response and remission (1, 2) and data from real-world cohorts support this finding. A 172 cohort of UC and CD patients from a pair of Boston academic centers observed that rates of remission were lower with elevated CRP (23). The French GETAID cohort shared this finding for patients with UC (5). Stallmach et al. found that an early (week 14) reduction in CRP or fecal calprotectin was associated with higher rates of remission at 54 weeks (16).

However, biomarkers assessed in current trials and real-world cohorts are nonspecific and related to overall disease activity. Battat et al. reviewed novel biomarkers which were postulated to be associated with VDZ response in UC due to their potential relationship to the α4β7 and adhesion molecule interaction that is inhibited by VDZ (24). At induction, lower soluble TNF was associated with achieving remission. During maintenance, lower soluble VCAM-1 and higher soluble α4β7 were associated with achieving remission. These results are promising and suggest that novel biomarkers could be incorporated into future studies and prediction models to improve VDZ-specific response prediction (**Table 2**).

## Microbiome

The gut microbiome is known to be associated with mucosal inflammation in IBD. Ananthakrishnan et al. recruited a

**TABLE 1 |** *Post-hoc* analysis of GEMINI trials.

| References | Cohort | Outcomes |
|---|---|---|
| Feagan et al. (12) | *Post-hoc* analysis of GEMINI 1 | **Week 6 Clinical Remission (TNF-naïve):**<br>VDZ 23.1% vs. Placebo 6.6% (RR = 3.2; 95% CI 1.3–7.9)<br>**Week 6 Clinical Remission (TNF-failure):**<br>VDZ 9.8% vs. Placebo 3.2% (RR = 3.2; 95% CI 0.7–14.5)<br>**Week 6 Mucosal Healing (All Patients):**<br>VDZ 40.9% vs. 24.8% (RR = 1.6; 95% CI 1.2–2.3)<br>**Week 52 Clinical Remission (TNF-naïve):**<br>VDZ 53.1% vs. Placebo 26.2% (RR = 2; 95% CI 1.3–3)<br>**Week 52 Clinical Remission (TNF-failure):**<br>VDZ 36.1% vs. Placebo 5.3% (RR = 6.6; 95% CI 1.7–26.5)<br>**Week 52 Mucosal Healing (All Patients):**<br>VDZ 53.8% vs. Placebo 19.8% (RR = 2.7; 95% CI 1.9–4) |
| Sands et al. (11) | *Post-hoc* analysis of GEMINI 2 and 3 | **Week 6 Clinical Remission (TNF-naïve):**<br>VDZ 12.6% difference from placebo (95% CI 3.7–21.4)<br>**Week 6 Clinical Remission (TNF-failure):**<br>VDZ 4.1% difference from placebo (95% CI −1.6–9.8)<br>**Week 52 Clinical Remission (TNF-naïve):**<br>VDZ 22.1% difference from placebo (95% CI 8.9–35.4)<br>**Week 52 Clinical Remission (TNF-failure):**<br>VDZ 14.9% difference from placebo (95% CI 4.7–25) |
| Sands et al. (13) | *Post-hoc* analysis of GEMINI 2 and 3 | **GEMINI 2 Week 6 Remission:**<br>VDZ+CS 19.0% vs. Placebo+CS 4.6% (14.4% difference; 95% CI −1.3–29.6)<br>VDZ 10.9% vs. Placebo 8.6% (without CS) (2.3% difference; 95% CI −6–10.6)<br>**GEMINI 3 Week 6 Remission:**<br>VDZ+CS 198% vs. Placebo+CS 10.2% (9.6% difference; 95% CI 0.3–19)<br>VDZ 18.6% vs. Placebo 14.4% (without CS) (4.1% difference; 95% CI −6.3–14.6) |

*CD, Crohn's Disease; UC, Ulcerative Colitis; RR, Relative Risk; CI, Confidence Interval; CS, Corticosteroid; IS, Immunosuppression; TNF, Tumor Necrosis Factor; SES-CD, Simple Endoscopic Score for Crohn's Disease; CRP, C-reactive Protein; CDAI, Crohn's Disease Activity Index.*

prospective cohort of 42 CD and 43 UC patients receiving VDZ and assessed microbial composition related to disease activity (25). Changes in microbiome diversity were associated with clinical remission in those with CD but not UC. Assessment of biochemical pathways revealed a significant increase in week 14 remission in patients with CD who had baseline enrichment of BCAA pathways, suggesting a functional component in addition to taxonomic differences as baseline predictors. Of note, the microbial changes of those who achieved remission at week 14 persisted at 1 year, suggesting an early marker rather than a baseline predictor of response. While this study suggests multiple microbial markers of baseline and early predictors of response to VDZ (ie taxonomic differences, diversity, and function) it lacks applicability as microbiome sequencing has not reached clinical point of care. It is also limited by its small, single-center cohort with limited follow-up and assessment of diet and would require further validation; but nonetheless an interesting pilot study to complement the data regarding TNF effect on microbiota and worth further investigation.

## PREDICTORS OF ENDOSCOPIC RESPONSE OR REMISSION

Endoscopic response is an important part of disease assessment and is becoming a larger part of the treatment target in IBD. The recent VERSIFY phase 3b clinical trial (26) assessed endoscopic response to VDZ in CD and found that endoscopic remission rates (SES-CD score $\leq$ 4) were greater in patients naïve to TNF antagonists, those with moderate compared to severe baseline endoscopic disease, and shorter disease duration (26). Endoscopic remission rates at week 26 and 52 were higher in TNF-antagonist naïve (9.6 and 25%) vs. TNF-antagonist exposure (5.5 and 8.3%), higher in moderate disease (SES-CD 7-15) (17 and 20.7%) vs. severe disease (SES-CD > 15) (6.7 and 14.8%), and higher in shorter disease duration (<1 year) (37.5 and 100%) vs. longer disease duration ($\geq$7 years) (7.1 and 11.5%).

*Post-hoc* analysis of the GEMINI 1 trial found that mucosal healing rates (Mayo endoscopic subscore of $\leq$1) were higher among VDZ treated patients with UC at 6 weeks (RR = 1.6; 95% CI 1.2–2.3) and 52 weeks (RR = 2.7; 95% CI 1.9–4) as compared to placebo (12).

The VICTORY cohort evaluated endoscopic response to VDZ in UC and found that 17% of patients achieved endoscopic remission (Mayo endoscopic sub-score 0) at 12 months. Prior TNF-antagonist was associated with reduced probability of achieving endoscopic response (HR 0.51, 95% CI 0.29–0.88) (18).

In a Canadian real-world cohort evaluating endoscopic and radiologic remission, VDZ patients with CD were less likely to obtain objective remission at 6 months (adjusted OR 0.30; 95% CI: 0.11–0.79, $p = 0.02$) and 12 months (adjusted OR 0.27; 95% CI: 0.09–0.78, $p = 0.02$) compared to UC (27). There were no differences in rates of remission due to disease severity, previous biologic failure, and pretreatment of CRP. Of note, this study did not separate endoscopic and radiographic remission.

**TABLE 2 |** Predictors of clinical response to VDZ from real-world cohorts.

| References | Cohort | Outcomes | Positive predictors of response | Negative predictors of response |
|---|---|---|---|---|
| Amiot et al. (4) | 272 patients (161 CD) with prior conventional or TNF antagonist therapy who completed induction. A multicenter French cohort | Steroid-free clinical remission at 54 weeks (HBI ≤4 or partial Mayo score <3 with a combined stool frequency and rectal bleeding subscore of ≤1) | CD: Week 6 response (OR = 7.41; 95% CI 2.85–19.23) UC: Week 6 response (OR = 7.51; CI: 95% 3.00–18.88) | CD: Corticosteroids at induction (OR = 0.37; 95% CI 0.16–0.88). HBI score > 10 at induction (OR = 0.15; 95% CI 0.06–0.37) UC: WBC > 9000 × 109/L (OR = 0.36; 95% CI 0.14–0.92). Mayo score > 9 at induction (OR = 0.37; 95% CI 0.15–0.92) |
| Amiot et al. (5) | 294 patients (173 CD) with prior conventional or TNF antagonist therapy. A multicenter French cohort | Steroid-free clinical remission at 14 weeks (HBI ≤4 or partial Mayo score <3 with a combined stool frequency and rectal bleeding subscore of ≤1) | CD: Week 6 response (OR = 11.2; 95% CI 4.3–28.8; $p$ = <0.001) UC: Week 6 response (OR = 5.3; 95% CI 2.2–13.1; $p$ = <0.001) | CD: Corticosteroid use at induction (OR = 0.35; 95% CI 0.16–0.77; $p$ = 0.009). HBI score > 10 at induction (OR = 0.11; 95% CI 0.05–0.27; $p$ = <0.001) UC: CRP > 20 mg/L at induction (OR = 0.30; 95% CI 0.11–0.80; $p$ = 0.02). Mayo score > 9 at induction (OR = 0.21; 95% CI 0.08–0.57; $p$ = 0.002) |
| Baumgart et al. (7) | 212 patients (97 CD) eligible for VDZ. Single site, prospective, German cohort | Clinical remission at 14 weeks (HBI ≤4 or partial Mayo score ≤1 plus a bleeding subscore of 0) | CD: Low HBI score ($p$ = 0.02). No hospitalization in prior year ($p$ = 0.01) UC: No predictors | |
| Chaparro et al. (8) | 521 patients (259 CD) with ≥1 induction VDZ dose. Multicenter Spanish cohort | Clinical remission at 14 weeks (partial Mayo score <2 or HBI score <5) | UC: Mild vs. severe disease (OR = 6.6; 95% CI 3–14.7) | CD: Higher baseline HBI (OR = 0.6; 95% CI 0.5–0.7) UC: Higher baseline CRP (OR = 0.8; 95% CI 0.8–0.9) |
| Dulai et al. (3) | 212 CD patients eligible for VDZ from a multicenter US cohort | Clinical remission (complete resolution of all CD-related symptoms) | | Prior TNF-antagonist exposure (HR = 0.40; 95% CI 0.20–0.81) Active or historical smoking (HR = 0.47; 95% CI 0.25–0.89) Active perianal disease (HR = 0.49; 95% CI 0.27–0.88) Severe disease activity (HR 0.54; 95% CI: 0.31–0.95) |
| Dulai et al. (18) | 180 UC patients eligible for VDZ from a multicenter US cohort | Clinical remission (complete resolution of all UC-related symptoms) and response (clinically significant response defined as >50% reduction in symptom activity by PGA) | | Achieve response with prior TNF-antagonist exposure (HR, 0.58; 95% CI, 0.39–0.86) Achieve remission with prior TNF-antagonist exposure (HR, 0.55; 95% CI, 0.35–0.88) |
| Kopylov et al. (6) | 204 patients (130 CD) treated with VDZ with at least 14 weeks of follow-up from a multicenter Israeli cohort | Clinical remission at 14 weeks (HBI <5 and a partial Mayo score <2 or SCCAI <4) | CD: Mild clinical activity at induction($p$ = 0.001) UC: no predictors | |
| Kopylov et al. (17) | 193 patients (133 CD) who completed 52 weeks of VDZ treatment with follow-up from a multicenter, retrospective, Israeli cohort | Clinical remission at 52 weeks (HBI ≤4, CDAI <150; SCCAI <2, partial Mayo score ≤2) | CD: Clinical response at 14 weeks (OR = 3.5; 95% CI 1.4–8.6) UC: Clinical response at 14 weeks (OR = 7.3; 95% CI 1.8–29.1) | |
| Lenti et al. (19) | 203 patients (135 CD) treated with VDZ from a multicenter UK retrospective cohort | Clinical response and remission at 14 and 52 weeks (partial vs. complete/significant symptom relief by PGA) | No predictors | |
| Shelton et al. (23) | 172 patients (107 CD) receiving ≥3 VDZ infusions at 2 US academic centers | Clinical response and remission at 14 weeks | Baseline CRP >8.0 mg/L (OR = 0.33; 95% CI 0.15–0.95. $p$ = 0.04) | |

*(Continued)*

**TABLE 2** | Continued

| References | Cohort | Outcomes | Positive predictors of response | Negative predictors of response |
|---|---|---|---|---|
| Stallmach et al. (16) | 127 patients (67 CD) eligible for VDZ from a single site, prospective German cohort | Clinical remission at 54 weeks (HBI ≤4 or a partial Mayo score ≤1 with a bleeding subscore of 0) | CD: Response or remission at week 14 ($p = < 0.001$). Lower CRP at week 14 as compared to baseline ($p = 0.01$) UC: Remission at week 14 ($p = <0.0001$). No prior TNF antagonist treatment (OR = 5.3; 95% CI 1.3–21.4). Less than 25% use of steroids within prior 6 months (OR = 5.4; 95% CI 1.3–22.1). Lower CRP at week 14 as compared to baseline ($p = 0.003$). Lower fecal calprotectin at week 14 ($p = 0.002$) | |

*CD, Crohn's Disease; UC, Ulcerative Colitis; OR, Odds Ratio; HR, Hazard Ratio; CI, Confidence Interval; HBI, Harvey-Bradshaw Index; SCCAI, Simple Clinical Colitis Activity Index; CRP, C-reactive Protein; CDAI, Crohn's Disease Activity Index; PGA, Physician's Global Assessment.*

## PREDICTORS OF ADVERSE EVENTS

Colombel et al. provided an integrated VDZ clinical trial analysis from the GEMINI trials and their follow-up long-term safety data (>4000 PYs) (28). They found VDZ to be well-tolerated with an acceptable safety profile. Overall, patients with UC and CD exposed to VDZ had less adverse events (AE) than placebo when adjusted for exposure (247.8/100 vs. 419.4/100 PYs). This included infectious AEs, with overall incidence in VDZ-exposed being lower than placebo (63.5/100 vs. 82.9/100 PYs). Due to the gut-selective mechanism of action of VDZ there may be concern that these patients are at higher risk for enteric infections. However, the rates of enteric infections were very low (≤0.8/100 PYs), excluding gastroenteritis. Predictors of serious infection in total cohort of UC and CD were younger age, opioid use, and corticosteroid use. When separated by type of IBD, prior TNF-antagonist failure was found to be a predictor of serious infection in the UC cohort but not younger age or concomitant steroid use.

In our analysis of real-world data from the VICTORY cohort, we also found VDZ to be well-tolerated with a similar safety profile to the GEMINI trials (20). Predictors of infection included active smoker status and number of concomitant immunosuppressive agents. VDZ monotherapy and VDZ plus immunomodulator had comparable rates of AEs (5.9/100 vs. 5.8/100 PYE), but the addition of corticosteroids to either resulted in increased risk of infection in an incremental fashion (VDZ+CS 9.5/100 PYE vs. VDZ+IM+CS 12/100 PYE). This is important to note and discuss with patients as the gut-selective mechanism of VDZ is thought to convey this favorable safety profile which cannot be relied on with the addition of other immunosuppressants.

## PREDICTION MODELING

There are many potential predictors of response that have been identified from clinical trial and real-world data (see **Tables 1**, **2** for summary), however, translating these findings into clinical practice can be challenging. The ability to cluster these data into a tool that can inform patients and clinicians about potential response early in treatment course, or ideally before starting, would allow for greater personalization within IBD therapy. Waljee et al. and Dulai et al. have both developed prediction models of response from *post-hoc* analyses of the GEMINI trials to address this need. Although both used a similar dataset for model derivation, differences exist between them which are important to highlight.

First, Waljee et al. utilized a machine-learning approach that incorporated baseline patient characteristics and labs in combination with changes in lab values during induction (29, 30). Our group in contrast used regression methodology with a primary focus on baseline patient characteristics and labs (31, 32). This distinction is important because the machine-learning model therefore requires a trial of induction therapy prior to determining if a patient is likely to respond to VDZ whereas baseline regression models can help classify patients before treatment initiation thereby avoiding the need to prove a lack of response or sub-optimal response after induction. Second, both groups used corticosteroid-free clinical remission and endoscopic remission as dependent outcomes for CD and UC, but our model also incorporated predictors of clinical remission and durable remission for CD into the assessment. Third, both groups transformed these models into clinical decision support tools (CDST) with Waljee et al. creating a simplified equation using variable importance plots and our group creating a point scoring system based CDST. Fourth, although both models demonstrated modest accuracy and performance within the GEMINI cohort, only the regression models underwent external validation in routine practice cohorts of patients treated with VDZ. Finally, the regression-based prediction models and CDSTs have now been shown to be able to predict not only clinical and endoscopic effectiveness, but also rapidity of treatment response, measured drug exposure, and biomarker response; thereby providing a more comprehensive prediction of key

**TABLE 3 |** Prediction models.

| | Regression Models (Dulai) | |
|---|---|---|
| | **CD** | **UC** |
| **Derivation-GEMINI Cohorts** | | |
| Performance | Week 26 CREM AUROC 0.69 Week 26 CSF-REM AUROC 0.69 Week 52 CREM AUROC 0.68 | Week 52 CSF-REM AUROC 0.69 |
| **Validation-VICTORY Cohorts** | | |
| Primary Outcome | Clinical and Endoscopic Remission at Week 26 | Sensitivity/Specificity (95% CI) of CDST at Week 26 |
| Performance | Week 26 CREM AUROC 0.67<br>Week 26 CSF-REM AUROC 0.66<br>Week 26 Mucosal Healing AUROC 0.72 Week 26 Deep remission AUROC 0.73<br>Week 26 CF-REM with MH AUROC 0.75 | **13 points:**<br>CREM Sensitivity: 92%<br>CREM Specificity: 25%<br>CSF-REM Sensitivity: 94%<br>CSF-REM Specificity: 30%<br>MH Sensitivity: 98%<br>MH Specificity: 30%<br>CSF-DR Sensitivity: 100%<br>CSF-DR Specificity: 31%<br>**19 points:**<br>CREM Sensitivity: 33%<br>CREM Specificity: 80%<br>CSF-REM Sensitivity: 37%<br>CSF-REM Specificity: 77%<br>MH Sensitivity: 40%<br>MH Specificity: 80%<br>CSF-DR Sensitivity: 46% (19–75%);<br>CSF-DR Specificity: 78% (69–85%) | Sensitivity/Specificity (95% CI) of CDST at Week 26<br>**26 points:**<br>CSF-REM Sensitivity: 93%<br>CSF-REM Specificity: 15% **32 points:**<br>CSF-REM Sensitivity: 51%<br>CSF-REM Specificity: 68% |
| POC Transformation | Absence of prior TNF antagonist exposure (+3 points)<br>Absence of prior bowel surgery (+2 points)<br>Absence of prior fistulizing disease (+2 points)<br>Baseline level of albumin (+0.4 points per g/L)<br>Baseline concentration of C-reactive protein (reduction of 0.5 points for values between 3.0 and 10.0 mg/L and 3.0 points for values >10.0 mg/L) | Absence of prior TNF antagonist exposure (+3 points)<br>Disease duration ≥2 years (+3 points)<br>Baseline endoscopic activity (moderate vs. severe) (+2 points)<br>Baseline albumin concentration (+0.65 points per g/L) |

| **Secondary Outcomes from Dulai Prediction Models** | | | | | | |
|---|---|---|---|---|---|---|
| | | | **Low probability** | **Intermediate probability** | **High probability** | ***p*-value** |
| Drug exposure | | | **Pre-Dose VDZ Concentrations (ug/mL) by Probability of Response** | | | |
| | Week 2 | UC | 22.9 | 27.4 | 32 | <0.001 |
| | | CD | 24.7 | 28.45 | 32.7 | <0.001 |
| | Week 6 | UC | 17.2 | 23.5 | 34.9 | <0.001 |
| | | CD | 15.3 | 23.5 | 33.4 | <0.001 |
| | Week 22 | UC | 18.0 | 23.8 | 32.5 | <0.001 |
| | | CD | 15.8 | 23.4 | 30.3 | <0.001 |
| | Week 46 | UC | 22.5 | 27.8 | 31.5 | 0.016 |
| | | CD | 18.7 | 25.8 | 32.6 | 0.0008 |
| Onset of action | | | **Change in Partial Mayo Score (UC) or Harvey-Bradshaw Index (CD) from Baseline by Probability of Response** | | | |
| | Week 6 | UC | −1.22 | −1.89 | −2.21 | <0.001 |
| | | CD | −1.69 | −2.61 | −4.22 | <0.001 |
| | Week 22 | UC | −2.68 | −3.2 | −3.75 | 0.003 |
| | | CD | −3.76 | −4.53 | −5.82 | <0.001 |
| | Week 38 | UC | −3.24 | −4.21 | −4.13 | 0.002 |
| | | CD | −4.62 | −5.57 | −6.76 | <0.001 |
| | Week 52 | UC | −3.64 | −4.42 | −4.33 | 0.029 |
| | | CD | −4.68 | −6.32 | −7.17 | <0.001 |

*AUROC, Area Under Receiver Operator Curse; CSFR, Corticosteroid-Free Remission; CSFER, Corticosteroid-Free Endoscopic Remission; CREM, Clinical Remission; CSF-REM, Corticosteroid-free Remission; MH, Mucosal Healing; DR, Deep Remission.*

*Dulai et al. CDST for CD Probability of response: Low (Intermediate ≤ 13), Intermediate (>13 to ≤19 points), High (>19 points).*

*Dulai et al. CDST for UC Probability of response: Low (≤26 points), Intermediate (>26 to ≤32 points), High (>32 points).*

components to patient outcomes and opportunities for treatment optimization (**Table 3**).

## FUTURE

Novel comparative head-to-head trials are forthcoming with the first such trial recently published. The VARSITY trial directly compared Vedolizumab vs. Adalimumab (ADA) as maintenance therapy in UC (33). Clinical remission rates at 52 weeks were 31.3% vs. 22.5% in VDZ vs. ADA (95% CI, 2.5–15.0; $p = 0.006$) and 52 week endoscopic improvement rates of 39.7% vs. 27.7% (95% CI, 5.3–18.5; $p < 0.001$). Rates of serious infections were low and similar between cohorts. This trial shows that VDZ is superior to ADA in achieving clinical remission and endoscopic improvement at 52 weeks maintenance therapy. Similar trials are sure to follow which will further inform on biologic positioning while adding more data to interpret predictors of response.

## CONCLUSION

VDZ is known to be safe, well tolerated, and effective. These are important points for personalization, but can we predict response to further guide therapy and shared decision-making? Subgroup analyses from the GEMINI trials were not powered for this question but they do provide evidence supplemented by real-world observational studies that increase generalizability for a heterogenous IBD population. Overall, it appears that patients with less severe disease (clinical, biomarkers) without prior biologic exposure and who demonstrate early response to VDZ have the highest rates of durable clinical and endoscopic response and remission. Prediction models and CDST confirmed these predictors and can be utilized to identify patients with higher probability of nonresponse so that either before initiation or after a short duration of treatment a decision to continue, discontinue, or even dose-escalation would be more informed. As biologics have become a mainstay of therapy, cost-analysis will help determine if prediction modeling can improve cost-effectiveness of VDZ by determining responders, nonresponders, and those with response latency needing dose escalation.

## AUTHOR CONTRIBUTIONS

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

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REVIEW
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# Personalizing Treatment in IBD: Hype or Reality in 2020? Can We Predict Response to Anti-TNF?

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The advent of anti-TNF agents as the first approved targeted therapy in the treatment of inflammatory bowel disease (IBD) patients has made a major impact on our existing therapeutic algorithms. They have not only been approved for induction and maintenance treatment in IBD patients, but have also enabled us to define and achieve novel therapeutic outcomes, such as combination of clinical symptom control and endoscopic remission, as well as mucosal healing. Nevertheless, approximately one third of treated patients do not respond to initiated anti-TNF therapy and these treatments are associated with sometimes severe systemic side-effects. There is therefore the currently unmet clinical need do establish predictive markers of response to identify the subgroup of IBD patients, that have a heightened probability of response. There have so far been approaches from different fields of IBD research, to descry markers that would empower us to apply TNF-inhibitors in a more rational manner. These markers encompass findings from disease-related and clinical factors, pharmacokinetics, biochemical markers, blood and stool derived parameters, pharmacogenomics, microbial species, metabolic compounds, and mucosal factors. Furthermore, changes in the intestinal immune cell composition in response to therapeutic pressure of anti-TNF treatment have recently been implicated in the process of molecular resistance to these drugs. Insights into factors that determine resistance to anti-TNF therapy give reasonable hope, that a more targeted approach can then be utilized in these non-responders. Here, IL-23 could be identified as one of the key factors determining resistance to TNF-inhibitors. Growing insights into the molecular mechanism of action of TNF-inhibitors might also enable us to derive critical molecular markers that not only mediate the clinical effects of anti-TNF therapy, but which level of expression might also correlate with its therapeutic efficacy. In this narrative review, we present an overview of currently identified possible predictive markers for successful anti-TNF therapy and discuss identified molecular pathways that drive resistance to these substances. We will also point out the necessity and difficulty of developing and validating a diagnostic marker concerning clinically relevant outcome parameters, before they can finally enter daily clinical practice and enable a more personalized therapeutic approach.

**Keywords: inflammatory bowel diseases, anti-TNF, response, prediction, personalized medicine**

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## INTRODUCTION

Inflammatory bowel diseases (IBD) encompasses chronic inflammatory disorders of the gastrointestinal tract whose phenotypic entities mainly comprises Crohn's disease (CD) and ulcerative colitis (UC) (1, 2). These chronic, relapsing, and remitting diseases are characterized by intestinal inflammation and epithelial injury, causing lifelong morbidity (3). Both IBD subtypes are progressive conditions that can lead to bowel damage and disability, having a major impact on an individual's quality of life. Furthermore, ongoing inflammatory activity is causative for occurrence of strictures, fistula, abscesses (1), as well as heightened incidence of colitis-associated neoplasia (4). Optimized anti-inflammatory therapy is therefore essential in the management of IBD patients.

Growing insights into underlying immunopathogenic mechanisms of IBD have led to the advent of targeted therapies, which selectively inhibit crucial mediators of the inflammatory process (5). The first class of biological therapies approved for the treatment of IBD patients were agents inhibiting the pro-inflammatory cytokine tumor necrosis factor (TNF). This substance class encompasses the chimeric monoclonal antibody infliximab, the monoclonal human antibody adalimumab, corresponding infliximab and adalimumab biosimilars, the fully human monoclonal antibody golimumab, and the PEGylated humanized Fab' fragment certolizumab pegol (6). These inhibitors of TNF are applied for induction and maintenance therapy and have made a major impact on our existing therapeutic algorithms. Their advent and the following introduction of targeted therapies (anti-alpha4beta7 integrin inhibitor vedolizumab, anti-IL-12/IL-23p40 antibody ustekinumab and JAK-inhibitor tofacitinib) have helped us to shift current therapeutic strategies toward achievement of deep and prolonged clinical and endoscopic remission, aiming for prevention of complications and halting the progressive course of disease, improving the quality of life of IBD patients (7).

However, depending on the duration of anti-TNF treatment and the outcome parameters chosen, approximately one third of treated patients do not demonstrate response to therapy (primary non-response). Available data indicate that primary non-response should not be assessed prior week 8–12 after initiated therapy (8). Furthermore, 30–50% of initial responders are prone to loose response to therapy in the course of anti-TNF treatment (secondary non-response). A review of studies evaluating loss of efficacy and requirement of infliximab dose intensification, estimated that the annual risk for loss of response to infliximab is ∼13% per patient-year of treatment (9).

There is therefore an urgent clinical need to establish predictive markers of response to identify the subgroup of IBD patients, which have a heightened probability of response to anti-TNF therapy. Such an approach would enable us to prevent a delay of initiating an effective treatment, create a substantial benefit for the patients via selection of the most appropriate agent for rapid response to therapy and improved quality of life (10–13). Treatment with a beneficial therapy also reduces the risk of being exposed to potential systemic side effects of an ineffective therapy. Although anti-TNF agents are generally well-tolerated in clinical practice, they have been shown to increase the susceptibility to serious infections (14), possibly melanoma skin cancer (15), and treatment-related complications, such as lupus-like syndromes or allergic reactions.

Recent cost analyses also identified anti-TNF antibodies as the main cost driver in IBD patients, necessitating the need for predicative biomarkers to enable health-economic sound use of these substances (16, 17). Reliable biomarkers predicting likelihood of therapeutic success to subsequent anti-TNF therapy, would allow utilization of a personalized medicine concept with optimized use of this substance class, providing a substantial benefit for the treated IBD patient (13).

In the following, findings from different fields of research to identify predictors to anti-TNF treatment are discussed. Therapeutic drug monitoring studies, which assessed the influence of trough levels and anti-drug antibody formation on therapeutic response were not considered in this review, as we only selected predictive markers which had to be measured before initiation of anti-TNF therapy.

Potential markers were derived from insights into disease-related and clinical factors, blood and fecal markers, molecular tissue expression, immunogenicity, previous therapies, pharmacogenomics, microbial, and metabolite markers, as well as blood and stool derived parameters.

Utilization of theses markers will hopefully lead to a more strategic approach of patient selection before initiating anti-TNF therapy in IBD. Furthermore, mechanisms underlying the failure to respond to anti-TNF therapy are not completely understood. An improved understanding of molecular resistance mechanisms would similarly be essential to optimize personalized medicine approaches in IBD (10).

## PATIENT AND DISEASE RELATED PREDICTORS TO ANTI-TNF THERAPY

Several patient and disease related factors have been described to be associated with treatment response to anti-TNF therapies.

### Age, Gender, Weight

On the one hand, younger age at initiation of therapy has been implied to predict better primary response to therapy in CD (18–20) and UC (21), but on the other hand several studies have not been able to demonstrate any relationship between age and therapeutic success (22–27). Similarly, contradicting data have also be described for gender, as single reports indicated better primary response in male CD (28) and female UC patients (25), but the majority of studies did not find any association (19, 22, 26, 27, 29, 30). Inconsistent results have also been obtained for correlation between weight of the anti-TNF treated patient and primary therapeutic response (13). Pooled analysis of individual participant data from clinical trials of infliximab in IBD did not demonstrate that obesity led to worse therapeutic response (31). Altogether, none of the stated patient related factors can be clearly associated with response to anti-TNF therapy.

## Smoking

From all environmental factors that have been described to affect the disease course in IBD patients, smoking has been identified as one of the most influential. Smokers with CD have a more complicated disease course and discontinuation led to better outcomes (32–34). Although, some studies have indicated worse outcomes of anti-TNF treated smoking CD patients in comparison to non-smokers (35, 36), two meta-analyses found no effect of smoking on primary effectiveness of infliximab in CD patients (37, 38). In UC, smokers have reduced colectomy rates, less primary sclerosing cholangitis and less back-wash ileitis than never smokers (39). In UC, few studies do (25) and most studies do not implicate influence of smoking on anti-TNF primary efficacy (21, 29, 30, 40).

## Disease Duration and Location

In patients with CD, shorter disease duration has been repeatedly described to predict higher responsiveness to anti-TNF drugs. In *post hoc* analyses of phase 3 clinical trials, patients with disease duration below 2 years had significantly better primary response rates to adalimumab (41) and certolizumab pegol (42) than those with long-standing disease. In UC, available data could not find a similar association (25, 40, 43).

Regarding disease location, differences between isolated ileal and colonic disease manifestation have been described. *Post-hoc* analysis of a placebo-controlled trial with certolizumab pegol showed higher probability of patients with colonic compared to isolated ileal disease to achieve clinical remission at week 6 of induction therapy (44). Several cohort studies also indicated better short-term and sustained clinical response to anti-TNF therapy in isolated colonic than in ileal CD (45, 46). Endoscopic and histologic healing were also more frequent in colon that the ileum after 1 year of adalimumab therapy in the EXTEND trial (47). For UC, there was no association between disease extend and probability of therapeutic induction and maintenance response to anti-TNF treatment (25, 27, 30).

## Disease Phenotype

Regarding the phenotypic manifestation, better short- and long-term response rates of anti-TNF therapy have been shown for non-stricturing and non-penetrating disease (Montreal Classification B1) in comparison to stenosing (B2) or fistulising disease (B3) (22, 48–51).

## Comorbidities

A recently published study showed that the presence of the comorbidities chronic obstructive pulmonary disease as well as extra-intestinal hepato-pancreato-biliary conditions were associated with primary non-response and myocardial infarction and skin disease were significantly associated with loss of response to anti-TNF treatment (52). Further studies will have to investigate these findings.

## Disease Severity

For disease severity, clearest data are available for UC. Here, anti-TNF therapy in severe disease showed diminished primary efficacy rates compared to treatment of less severe disease (25, 53–55). This might be due to the demonstrated fecal loss of anti-TNF through ulcerated intestinal mucosa into the stool of patients with high inflammatory burden (56). Another possible explanation might be that severe inflammation with high local TNF tissue concentrations could act as a sink for anti-TNF agents. This would explain why patients with high serum drug concentrations still fail to benefit from anti-TNF therapy, as insufficient tissue levels of anti-TNF are unable to neutralize heightened local TNF production (57).

## CRP, Fecal Calprotectin, Hemoglobin, Neutrophil-Lymphocyte Ratio, Albumin

A correlation between elevated C-reactive protein (CRP) levels and primary and sustained response to anti-TNF drugs has also been found in CD patients for all approved anti-TNF agents (41, 42, 49, 58–60). Analyses of the SONIC study have shown that elevated CRP levels were indicative of underlying inflammatory activity, thus predicting higher primary and long-term response rates than patients without inflammation (59). Nevertheless, not all CD patients with active disease exhibit elevated CRP-levels (61). In UC, higher anti-TNF induction and maintenance efficacy could be found in patients with low CRP-levels (21, 62).

Fecal calprotectin measurements have established themselves as surrogate measure for inflammatory activity in IBD (63). However, there have so far not been any conclusive results in relation to an association between fecal calprotectin levels and response to therapy (13).

Higher hemoglobin levels at baseline have only been shown to be associated with short- and long-term response to anti-TNF therapy in UC (53, 64, 65), but not CD (66).

One study reported that a high baseline neutrophil–to–lymphocyte ratio (cut-off value of 4.488) predicts secondary loss of response to infliximab treatment in UC patients (67).

Several studies have indicated that pre-treatment albumin levels correlate with primary response to anti-TNF therapy in UC, with lower levels showing worse response (29, 54, 64, 68). This might be due to diminished anti-TNF drug levels in hypoalbuminaemic patients (68).

## Previous Anti-TNF Exposure and Combination Therapy

There are several studies that have shown that previous anti-TNF therapy is associated with heightened probability of primary treatment failure and secondary loss of response of subsequent anti-TNF therapy (25, 43, 66, 69). A systematic review and meta-analysis reported that the efficacy of a second anti-TNF in CD patients was largely dependent on the cause for switching, as remission rates were higher in patients with previous anti-TNF intolerance (61%), compared with secondary (45%) or primary failure (30%) (70). Two randomized trial results underlined the primary benefit of concomitant immunomodulator therapy in infliximab treated IBD patients. In the SONIC trial, corticosteroid-free clinical remission at week 26 was seen in statistically significant more CD treated with azathioprine and infliximab, compared to those receiving infliximab or azathioprine alone (59). In the

randomized SUCCESS trial in UC patients, corticosteroid-free remission at week 16 was achieved by more patients under infliximab and azathioprine treatment, compared with those receiving infliximab or azathioprine alone (71).

### Previous Surgery

Previous surgery in CD patients has been described as a negative factor for primary therapeutic response to anti-TNF therapy (18, 19), but this finding was not confirmed by other studies (22, 26, 48).

### Serological Antibody Markers

Antinuclear antibody (ANA) seropositivity has been associated with anti-TNF secondary non-response (72). Anti-OmpC positivity was associated with a lack of response to anti-TNF therapy at 1 year and increased likelihood of therapy discontinuation in UC patients (73). Low baseline levels of IgG antibodies against the pattern recognition receptors IFI16 were associated with clinical response to infliximab induction treatment in UC (74). Several studies tested the capacity of the serological marker perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) to predict response to anti-TNF agents. A meta-analysis showed that pANCA negative patients had nearly a 2 fold higher response to anti-TNF therapy compared with patients who were pANCA positive. However, testing for pANCA positivity to predict non-response to infliximab therapy showed a sensitivity of only 25% and a specificity of 85%, leading to a positive predictive value of 41%, and a negative predictive value of 74%. These data indicate that pANCA testing are not applied in daily clinical practice for predicting response to therapy (75).

### Matrix Metalloproteinases

Loss of responsiveness might also be caused by heightened activity of matrix metalloproteinases in IBD non-responders, as they mediate proteolytic mucosal degradation of anti-TNF antibodies (76). Heightened clearance of TNF–anti-TNF antibody immune complexes through Fc receptor-mediated endocytosis and subsequent proteolytic degradation by the hyperactive reticuloendothelial system, might also contribute to non-response in UC patients (77).

## PHARMACOGENOMICS

Genome-wide association studies (GWAS) have been able to identify susceptibility loci in IBD (78), and analyses of germline genetic variants have repeatedly been investigated for their predictive capacity in anti-TNF treated patients.

### Crohn's Disease

NOD2 which has been identified as a susceptibility gene for CD, did not show an association with primary response to infliximab treatment (79, 80). Missing association for primary response was also described for polymorphisms in the genes encoding TNFR1 and TNFR2 (81, 82). In patients with luminal CD, the −843 CC/CT genotype of the apoptosis inducing protein Fas ligand was associated with higher primary clinical response rates (75 vs. 38%; $p = 0.002$) to infliximab than patients with the TT genotype. Same was seen for patients with fistulizing CD (85 vs. 40%; $p = 0.001$). In addition, patients with the caspase-9 93 TT ($n = 9$) genotype all responded, in contrast with 67% ($n = 147$) with the CC and CT genotype ($p = 0.04$) (83). Subsequently, the author group then proposed an apoptotic pharmacogenetic index based on their pharmacogenetic study of apoptosis genes (Fas ligand −843 C/T, Fas −670 G/A and caspase-9 93 C/T) and clinical predictors as a model for prediction of low, medium, and high primary responses to the first infusion of infliximab in patients with CD (84). Further associations between genetic loci and primary response to anti-TNF therapy have been described for the IBD5 locus in CD (85). Another study indicated that single-nucleotide polymorphisms (SNPs) associated with genetically determined high activity of TLR5 among primary CD responders (86). Polymorphisms at the FCGR3A locus, encoding IgG Fc receptor IIIa, have been shown to be associated with a CRP decrease in primary response to infliximab in CD (87). This finding was confirmed by subsequent studies in CD (88, 89). The FCGR3A V158F polymorphism seems to be associated with anti-drug antibody formation in anti-TNF treated CD patients, correlating with dose intensification in these patients. Moreover, anti-drug antibody formation has been shown to be significantly associated with the HLA-DQA1*05 allele in CD patient, leading to heightened probability of secondary loss of response to anti-TNF monotherapy, necessitating the need for immunosuppressive combination therapy (90). CD patients with FCGR3A polymorphisms or HLA-DQA501 might therefore need combination therapy with immunomodulators and anti-TNF drugs in the subgroup to inhibit anti-drug antibody formation and subsequent loss of response. The autophagy related gene ATG16L1 was indicative for primary response to anti-TNF therapy in one study (91), but data from a subsequent study could not confirm this finding (92). Recently, response of 427 CD patients to their first anti-TNF therapy was characterized. Here, 15 risk alleles were associated with primary non-response, as these patients had a significantly higher genetic risk score. A combined clinical-genetic model more accurately predicted primary non-response, when compared with a clinical only model (0.93 vs. 0.70; $p < 0.001$) (23). Furthermore, the combination of two–risk genotypes, involving both apoptosis and the TNF region, was associated with primary anti-TNF non-response (93).

### Ulcerative Colitis

There was an association of homozygous high-risk (rs1004819, rs2201841, rs10889677m rs11209032, rs1495965) compared to low-risk (rs7517847m rs10489629, rs11465804, rs1343151) IL-23 receptor polymorphisms with primary response to infliximab therapy in UC patients (94). Another study identified eight alleles associated with primary non-response in UC. Here, a combined clinical-genetic model significantly more accurately predicted primary non-response compared with a clinical-only model. Importantly, genetic risk scores for primary non-response were not associated with infliximab levels or antibody formation (95). Unlike in CD, no association between primary response to anti-TNF therapy and the IBD5 locus could be found in UC (85). Another study indicated SNPs associated with genetically

determined high activity of IL-12 and IL-18 levels among patients with UC were associated with primary non-response to anti-TNF treatment (86).

### Crohns's Disease and Ulcerative Colitis

Nuclear Factor kappa-light-chain-enhancer of activated B cells (NFκB) has been identified as a pivotal transcription factor in IBD pathogenesis (96) and polymorphisms in genes implicated in the NFκB-mediated primary response have been linked to anti-TNF treatment response in an IBD patient cohort study (97). Another study found that polymorphisms in genes involved in the regulation of the NFκB pathway (TLR2, TLR4, and NFKBIA), the TNF-α signaling pathway (TNFRSF1A), and other cytokine pathways (NLRP3, IL1RN, IL18, and JAK2) were associated with primary response to anti-TNF therapy in IBD patients (98).

In a recently published study, two successfully replicated genetic loci (rs116724455 in TNFSF4/18, rs2228416 in PLIN2) and four with suggestive evidence were found, that increased predictability of an exploratory risk model for primary non-response from initially 0.72 (clinical predictors) to 0.89 after adding the genetic predictors (99). A systematic review and meta-analysis of available studies with at least 100 BD patients included, indicated that apart from afore mentioned FCGR3A, polymorphisms in TLR4, TNFRSF1A, IFNG, IL6, and IL1B genes were also significantly associated with heightened primary response, whereas TLR2 and TLR9 variants with reduced response (100). Altogether, the mentioned studies indicate the potential of gene polymorphisms to predict response to anti-TNF therapy, but further large trials are needed to validate the mentioned findings.

## INTESTINAL MICROBIOME

Several studies have indicated that the gut microbiome and its interaction with the mucosal immune system is critically involved in driving the inflammatory reaction in IBD patients (101). Dysregulation of the microbiome has been reported in IBD patients with reduced diversity and temporal instability of the dominant taxa compared with healthy controls (102).

### Microbiota Changes

First studies investigated a possible relationship between specific changes in the microbiota and prediction of clinical response to anti-TNF therapy. In a prospective study in pediatric IBD patients, higher amounts form the groups of *Bifidobacterium ssp.*, *Eubacterium rectale*, *Clostridium colinum*, uncultured *Clostridiales*, and *Vibrio* and lower presence of *Streptococcus mitis* were found in primary responders than in non-responders (103). In another study, besides the antimicrobial peptides defensin 5 and eosinophilic cationic protein, lower dysbiosis indices and higher abundance of *Faecalibacterium prausnitzii* at baseline were also found in primary responders compared to non-responders to anti-TNF treatment (104).

### Metabolomic Predictors

As differences in the composition of the intestinal microbiota have been linked to changes in metabolite concentrations, recent studies also focussed on possible metabonomic predictors of primary response. Total metabolic exchange was significantly disrupted at baseline in fecal samples from IBD non-remitters. Butyrate and substrates involved in butyrate synthesis, such as ethanol or acetaldehyde, were less frequently exchanged among bacterial communities from patients who did not show primary therapeutic efficacy in response to anti-TNF therapy (105). Disturbances in an association network containing taxa of the *Lachnospiraceae* and *Ruminococcaceae* families, typically producing short chain fatty acids, were shown to characterize poor primary responses to treatment with anti-TNF-α therapeutic antibodies (106). A recently published prospective, longitudinal cohort study in CD patients identified metabolic profiles, which were predictive of primary anti-TNF non-response with alterations in bile acid, amino acid, and lipid pathways (107).

## IMMUNOLOGICAL MARKERS

### Proteomics

Large-scale detection, identification and characterization of proteins is a another domain of biomarker research in IBD (108). So far, only few studies have evaluated the capacity of proteomics for the prediction response to treatments. Serum proteomic profiling by surface enhanced laser desorption ionization time of flight-mass spectrometry (SELDI-TOF-MS) was applied in CD patients prior initiation of infliximab treatment. The author group found an association between platelet metabolism, in particular platelet aggregation factor four, and primary response to infliximab (109).

In another study, serum samples were subjected to two-dimensional gel electrophoresis, and after evaluation of densitometrical data, protein spots exhibiting differential expression among the groups, were further characterized by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). The proteins apolipoprotein A-I, apolipoprotein E, complement C4-B, plasminogen, serotransferrin, beta-2-glycoprotein 1, and clusterin were found to be up-regulated in the primary non-responder and responder groups, whereas their levels displayed no changes in the remitters group when compared to baseline samples. Additionally, leucine-rich alpha-2-glycoprotein (A2GL), vitamin D-binding protein (VTDB), alpha-1B-glycoprotein (A1BG), and complement C1r subcomponent (C1R) were significantly increased in the serum of primary remitters.

The label-free physiological intermolecular modulation spectroscopy (PIMS) was applied in peripheral blood mononuclear cells of IBD patients to identify responders to infliximab treatment. PIMS takes into account a combination readout based on changes in the resonance of water molecules and macromolecular conformation. PIMS data predicted primary response to anti-TNF therapy with an accuracy of 96% (110). All mentioned pioneering proteomic pilot study data require validation in larger cohort of patients.

# BLOOD MARKERS

## Cytokines

There are also several studies that primarily assessed the predictive value of blood parameters regarding prediction of response to anti-TNF therapy. High serum IL-1β concentrations were associated with lower primary clinical remission to infliximab in CD (111). IL-8 concentrations at baseline were higher in primary non-responders compared to responders in CD patients treated with infliximab. Multiple logistic regression identified TNF/CRP ratio at baseline as predictive for primary non-response to infliximab at week 14 (112).

Another study investigated the *in vitro* capacity of anti-TNF antibodies on cultured peripheral blood cells to suppress T cell surface receptor expression and cytokine release. The study found that anti-TNF suppressed the expression of CD25 on T cells and secretion of interleukin 5, to a higher degree in UC primary responders than in non-responders. A created prediction model was subsequently tested in a validation cohort. Correct classification of future therapy response was here achieved in 91% of the cases (113). In UC patients, primary anti-TNF non-responders had significantly increased TNF, IFNγ, IL-1β, and IL-10 levels compared to responders. Non-responders also demonstrated significantly lower TNF and IL-1β production by cultured peripheral blood mononuclear cells to various Toll-like receptor stimulation compared to responders, as well as reduced TLR9-induced IL-6 and TLR-3,−4,−8, and−9-induced IL-10 (114).

A recently published study investigated TNF production by cultured and lipopolysaccharide stimulated peripheral blood mononuclear cells from IBD patients prior to infliximab therapy initiation. Primary responders demonstrated significantly higher TNF and IL-6 production than non-responders. In CD patients, a certain threshold of TNF levels identified responders with 100% sensitivity and 82% specificity. This finding was confirmed in multivariate analysis. The percentage of TNF-positive cells was higher in CD14+ monocytes compared to lymphocytes after stimulation (115).

## Vitamin D

Recent studies investigated a possible correlation between vitamin D levels and clinical response to infliximab therapy. Here, low baseline vitamin D concentration was associated with heightened probability of primary clinical remission at week 14 in CD patients (116). Another study in IBD patients, found a significant link between deficiency of vitamin D and the presence of ANA, which were found to be associated with failure to anti-TNF therapy and also reported as significant risk factors for anti-TNF induced adverse events associated with anti-TNF therapy (72).

# TISSUE MARKERS

The analyses of gene expression via RNA sequencing in inflamed tissue or intestinal immune cells of patients have enlarged our insights into the immunopathogenesis of IBD.

## Different Gene Signature Profiles

A study in patients with colonic CD, identified a gene signature profile composed of TNFAIP6, S100A8, IL11, G0S2, and S100A9, which predicted primary infliximab response with 100% accuracy (117). A subsequent study performed by another group in their cohort of CD patients supported the role of the reported expression signature as predictive for primary anti-TNF outcome (118). High baseline IL13RA2 levels were associated with lack of mucosal healing in anti-TNF treated CD patients. The authors also showed TNF-driven pathways were significantly enriched in primary non-responders to infliximab and linked to increased mucosal IL13RA2 expression (119). GATA3 expressing lamina propria CD4+ T lymphocytes were increased in anti-TNF endoscopic primary non-responders compared to responders in CD patients (120).

One of the first studies to investigate the predictive capacity of gene expression profiles in UC patient samples and primary response to subsequent anti-TNF therapy was undertaken in 2009. Here, colonic tissue transcriptomics in biopsy samples that were taken prior to initiation of infliximab therapy in two cohorts of UC patients led to the identification of a five-gene signature consisting of osteoprotegerin, stanniocalcin-1, prostaglandin-endoperoxide synthase 2, IL-13 receptor alpha 2 (IL13RA2), and IL-11, that are all involved in the adaptive immune response. This panel of genes separated responders from non-responders with 95% sensitivity and 85% specificity (121).

Other studies investigated cytokine transcript changes in pre-treatment mucosal biopsies. One study in UC patients reported higher expression of genes encoding IFN-γ and IL-17 in the mucosa of anti-TNF therapy primary responders compared to non-responders (122). On the other hand, another study showed that UC week 14 responders had lower mucosal mRNA expression of interleukin IL-1β, IL-17A, IL-6, and IFN-γ than primary non-responders. In a study with CD patients, high expression of IL-17 and IL23 was found in infliximab responders in comparison to primary non-responders (123).

In a study with UC patients, mucosal healing upon initiated anti-TNF therapy was associated with lower pre-treatment mucosal expression of transcription factor Th1-Tbet and higher expression of Th17-Rorc (124) in primary responders. Furthermore, GATA3 expressing lamina propria CD4+ T lymphocytes were increased in anti-TNF endoscopic primary non-responders compared to responders in CD patients (120). In a recently published study, the authors used a colonic 13-gene transcript panel that had previously shown an association with efficacy of anti-TNF therapy, to predict therapeutic response to golimumab in UC patients. The baseline gene expression signature predicted mucosal healing with a sensitivity of 87%, but with a specificity of only 34%, indicative of a high false positive rate. The gene expression signature was not able to identify patients who would achieve primary clinical response or clinical remission (125).

## TREM-1

Another study found increased baseline presence of mucosal plasma cells and inflammatory macrophages in colonic biopsy

samples from IBD patients who did not primarily respond to anti-TNF therapy. Abundance of inflammatory macrophages were associated with increased expression of the triggering receptor expressed on myeloid cells (TREM-1), chemokine receptor type 2 (CCR2), and chemokine ligand 7 (CCL7). Blood gene expression analysis of an independent cohort, identified TREM-1 downregulation in primary non-responders at baseline, which was predictive of clinical response with an AUC of 94%. This was also one of the few studies, where results were validated in independent cohorts (126). Strikingly, another study described downregulated TREM1 expression in the blood of IBD patients with endoscopic remission upon anti-TNF therapy (127). These contrary findings regarding TREM-1 expression in primary responder and non-responders to anti-TNF therapy, although regarding differing endpoints consisting of, respectively, clinical and endoscopic parameters, demonstrate the need for further studies.

### TNF

Several studies have shown that TNF levels are markedly increased in the serum and intestinal tissue of IBD patients (128), centrally regulating the intestinal inflammatory process in multiple ways. Here, studies have shown that the transmembrane precursor protein mTNF expressed on immune cells rather than soluble TNF (sTNF) is the pivotal factor in perpetuating the inflammatory reaction in IBD, thereby also representing the decisive target for effective anti-TNF therapy (129, 130). Induction of mucosal T cell apoptosis has been described as the main mechanism of action of efficacious anti-TNF treatment in IBD, as intestinal T cell resistance to apoptosis is important for sustaining chronic intestinal inflammation (131, 132). Application of anti-TNF drugs to disrupt the costimulatory interaction between mTNF on CD14+ macrophages and tumor necrosis factor receptor 2 (TNFR2) on T cells from the mucosa of patients with IBD has been shown to induce T cell apoptosis (133). Thus, a correlation between the level of mucosal TNF expression and the efficiency of the TNF antibody directed against it was subsequently analyzed.

One study harnessed the diagnostic method of molecular endoscopy (134–136), to prospectively analyse a correlation between mucosal mTNF expression and effectiveness of anti-TNF therapy in CD patients. Mucosal mTNF expressing cells were visualized *in vivo* by topical application of a fluorescent anti-TNF antibody in conjunction with confocal laser endomicroscopy (CLE) during a conventional colonoscopy procedure. Patients with high numbers of intestinal mTNF+ cells showed statistically significantly higher primary clinical response rates at week 12 than patients with low numbers mTNF+ cells. Patients with high mTNF expression rates also reached endoscopic remission more often over a follow-up period of 1 year (137).

One study in UC patients found an inverse and independent association between pre-treatment mucosal TNF expression levels and primary clinical and endoscopic remission of infliximab treatment (138).

## MECHANISMS OF RESISTANCE TO ANTI-TNF THERAPY

Recently, the concept that changes in the composition of immune cell infiltrates in response to therapeutic pressure lead to molecular resistance to the applied drug has been introduced to the IBD filed (10). An improved understanding of molecular resistance is essential to optimize personalized treatment in IBD. First studies have indicated mechanisms that drive primary resistance to biological therapy in IBD.

### IL-23 and IL23R+TNFR2+ T Cells

A recent study indicated that excessive IL-23 production by CD14+ gut macrophages is one of the main drivers of evasion of apoptosis upon anti-TNF antibody therapy in CD non-responders. This results in the expansion of apoptosis-resistant IL23R+TNFR2+ T cells that mediate resistance to anti-TNF therapy (139).

### OSM

One of the best validated studies indicating activation of a TNF-independent signaling pathway in anti-TNF resistant patients (10), was based on analyzing mRNA expression levels in mucosal biopsies taken prior anti-TNF therapy. The study associated oncostatin M (OSM) with primary failure to anti-TNF therapy in IBD patients. These data were found by analysis of over 200 patients with IBD, including two well-described cohorts from phase three clinical trials of infliximab and golimumab. Fittingly, in an animal model of anti-TNF-resistant intestinal inflammation, genetic deletion, or pharmacological blockade of OSM significantly diminished colitis activity (140). Further studies also associated elevated plasma OSM and nCD64 expression in pediatric CD patients with poor biochemical outcomes (<50% reduction in FC from baseline at week 12) to infliximab treatment (141). Another recent study demonstrated that serum OSM levels were significantly lower in CD patients with mucosal healing at week 54 upon infliximab treatment than in patients not achieving this endpoint (142).

### IL7R Depending Signaling Pathway

Another study elucidated heightened expression of the IL7R and the IL-7 dependent signaling pathway in the inflamed colon of IBD patients non-responsive to anti-TNF therapy. The IL-7R signaling specifically regulates effector but not regulatory T cell homing to the gut by controlling alpha4 and beta7 integrin expression, thereby implicating blockade of the IL-7R as a novel therapeutic option in IBD (143).

### IL-22BP

A recent study delineated the pathogenic role of the IL-22 binding protein (IL-22BP) in IBD. Data of the study suggested that efficacious anti-TNF treatment may block IL-22BP expression by intestinal T cells, enabling IL-22 induced mucosal healing. Correspondingly, T cell derived IL-22BP was not downregulated in anti-TNF primary non-responders, thereby suggesting that direct

targeting of IL-22BP might represent an effective treatment option (144).

## GIMATS Module

Recently, single-cell analysis of inflamed intestinal tissue from CD patients depicted that cellular heterogeneity contributes to anti-TNF treatment resistance. A unique cellular composition that consisted of IgG plasma cells, inflammatory mononuclear phagocytes, activated T cells, and stromal cells, which was classified as the GIMATS module, in active lesions was associated with failure to achieve durable remission upon anti-TNF therapy. Results of the study suggest that combining anti-TNF antibodies with drug targets that block key nodes in the GIMATS response may represent an opportunity to overcome anti-TNF resistance in patient with high GIMATS expression. Here, inflammatory macrophage-derived stimulatory mediators such as IL-1ß or OSM were implicated to trigger stromal activation in GIMATS$^{high}$ lesions (145).

## CONCLUSION

Although significant amount of scientific data has been collected to identify a reliable biomarker for prediction of therapeutic response to anti-TNF treated IBD patients, none of them have entered daily clinical practice as a decisive tool to enable an individualized therapeutic approach. Even 20 years after introduction of this substance class to our therapeutic armamentarium, there is still the unmet need for a reliable marker that would allow a more rational application of anti-TNF treatment in IBD. The currently applied clinical practice of randomly commencing a biological treatment and assessing response to therapy several weeks after initiation is coupled with progression of tissue damage in non-responders, risk of systemic side-effects, and substantial health-care costs of an inefficient therapy. Prediction of therapeutic response would allow optimization of the risk/benefit ratio of anti-TNF inhibition in IBD.

The potential of molecular stratification of patients to enable a personalized treatment approach (146) is best visible in pediatric patients with early onset IBD, which is driven by high penetrance alleles or by the dysfunction of a single gene (147, 148). Here, identification of monogenic IBD forms led to initiation of specific targeted therapies that were able to ameliorate intestinal inflammation (149). However, personalized treatment of polygenic IBD has so far not been able to be based on genetic information alone.

Current data demonstrate that response to anti-TNF therapy may be influenced by many factors that consist of disease-related and clinical characteristics, biochemical markers, blood and stool derived parameters, pharmacogenomics, microbial, and metabolic factors, as well as local mucosal factors. These studies are important contributions toward identification of a clinically applicable biomarker.

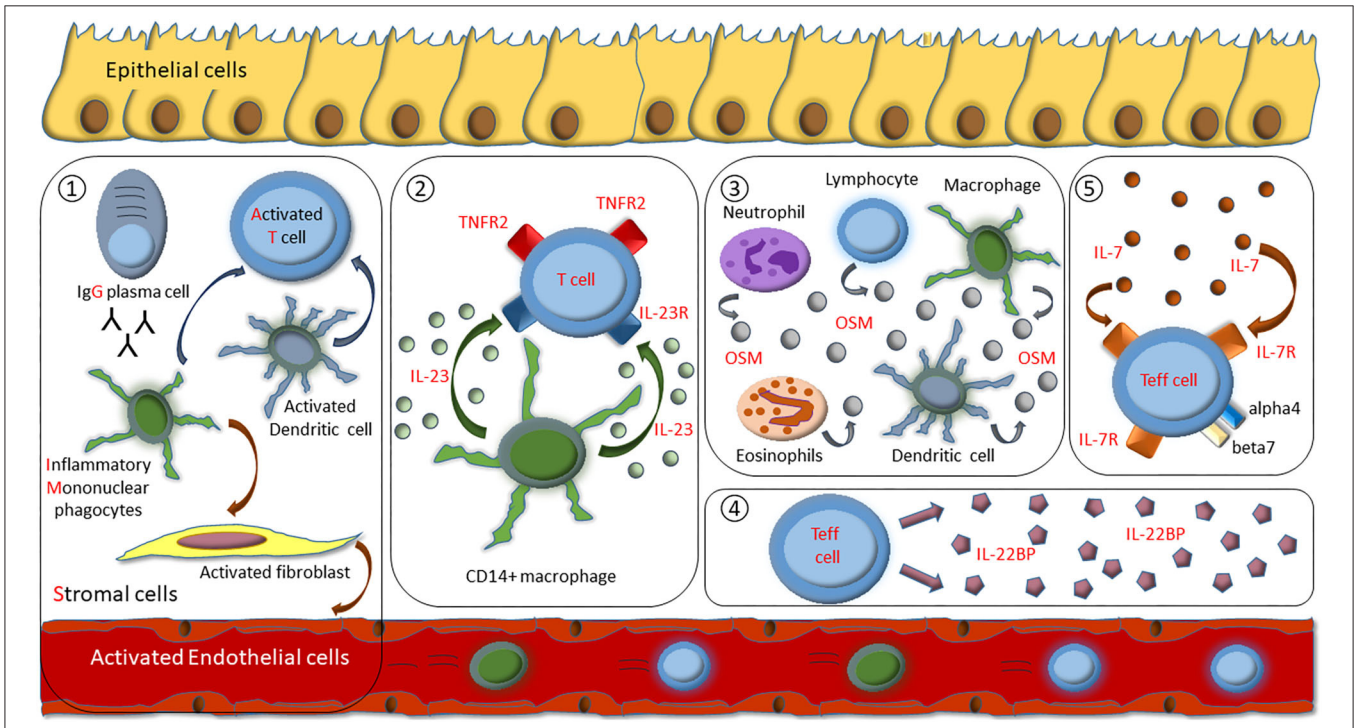


**FIGURE 1 |** Overview of identified mechanisms of molecular resistance to anti-TNF therapy in IBD patients. (1) Cellular composition of IgG plasma cells, inflammatory mononuclear phagocytes, activated T cells, and stromal cells (GIMATS module). (2) Excessive IL-23 production by CD14+ gut macrophages drive expansion of apoptosis-resistant IL23R+TNFR2+ T cells. (3) Overexpression of intestinal oncostatin M (OSM). (4) Overexpression of T cell derived IL-22BP. (5) Heightened expression of the IL-7R dependent signaling pathway that specifically regulates effector T cell homing to the gut by controlling alpha4 and beta7 integrin expression.

A suitable biomarker should ideally be non-invasively assessed, validated, rapidly quantifiable, inexpensive to measure, easily reproducible, and importantly not influenced by various confounders. Future trials that aim to validate a predictive biomarker of response must therefore also take into account other factors that have been shown to influence the efficacy of biological therapies, reflecting the complexity of such an approach. Nevertheless, interpretation of these findings must also take into account possible decisive influence of pharmacological factors, as a recently published prospective cohort study in CD patients (PANTS study), demonstrated that the only factor independently associated with primary anti-TNF non-response was low drug concentration at week 14 (24). Future studies should therefore also implement measurement of anti-TNF trough levels in the trial design to ideally identify predictive factors independent of serum drug levels. There is sufficient evidence that implies that pharmacokinetic factors alone are rather insufficient to reflect non-response, as even patients with sufficient drug levels fails to benefit from anti-TNF therapy, strongly implying mechanistic reasons for failure (10, 150). Trials should be performed separately in each IBD entity with clear definition of the studied end-point that defines response to therapy, which ideally should include endoscopic outcomes (151). Potential biomarkers need prospective validation in multi-center studies with large cohorts of patients and should incorporate short-term and long-term observations. Endoscopic, clinical, and laboratory baseline characteristics should ideally be evenly distributed when comparing responders and non-responders to therapy, to exclude influence of confounding factors. As reasons for non-response are possibly multifactorial, studies should also not restrict themselves to only analyzing one factor, but rather incorporate many markers and investigate in how far they might even influence each other, especially for molecular markers. This is best visible in the area of transcriptomic studies, which have helped us to understand disease-associated changes, but one must be aware that the functional relevance of these findings are unclear, as they do not take into account potential post-translational modifications. These studies should therefore ideally be backed up by corresponding protein quantification.

It is reasonable to expect that exposure to anti-TNF inhibitors induces emergence of TNF-independent inflammatory pathways that mediate resistance to anti-TNF therapy. Recent insights into mechanisms that drive resistance to anti-TNF therapy provide a comprehensive cellular and molecular basis to overcome this process with novel therapeutic approaches, like inhibitory agents targeting IL-23, OSM, IL-7R, IL-22BP, or IL-1ß (**Figure 1**). These insights might help us to not only understand mechanistic reasons for anti-TNF failure, but could also lead the way to tailor subsequent treatment options for the benefit of the patient.

In summary, currently no single marker fulfills all criteria for being an appropriate prognostic indicator for response to any anti-TNF treatment in IBD, and therefore the suggested biomarkers appear of limited clinical utility. Upcoming research should aim to develop a predictive model that incorporates all relevant factors derived from ongoing research, as indicated in our narrative review, to establish a reliable and validated tool that allows us to open new avenues for personalized medicine. The development of predictors of anti-TNF response is of central clinical importance and might be essential to their future use in the therapeutic algorithm of treating IBD patients.

## AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, drafting the article, revising it for important intellectual content, and final approval of the version to be submitted.

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# Gut Microbiota and Metabolic Specificity in Ulcerative Colitis and Crohn's Disease

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**Background:** Inflammatory bowel disease (IBD) represents multifactorial chronic inflammatory conditions in the gastrointestinal tract and includes Crohn's disease (CD) and ulcerative colitis (UC). Despite similarities in pathobiology and disease symptoms, UC and CD represent distinct diseases and exhibit diverse therapeutic responses. While studies have now confirmed that IBD is associated with dramatic changes in the gut microbiota, specific changes in the gut microbiome and associated metabolic effects on the host due to CD and UC are less well-understood.

**Methods:** To address this knowledge gap, we performed an extensive unbiased meta-analysis of the gut microbiome data from five different IBD patient cohorts from five different countries using QIIME2, DIAMOND, and STAMP bioinformatics platforms. *In-silico* profiling of the metabolic pathways and community metabolic modeling were carried out to identify disease-specific association of the metabolic fluxes and signaling pathways.

**Results:** Our results demonstrated a highly conserved gut microbiota community between healthy individuals and IBD patients at higher phylogenetic levels. However, at or below the order level in the taxonomic rank, we found significant disease-specific alterations. Similarly, we identified differential enrichment of the metabolic pathways in CD and UC, which included enriched pathways related to amino acid and glycan biosynthesis and metabolism, in addition to other metabolic pathways.

**Conclusions:** In conclusion, this study highlights the prospects of harnessing the gut microbiota to improve understanding of the etiology of CD and UC and to develop novel prognostic, and therapeutic approaches.

**Keywords: gut microbiome, metabolism, ulcerative colitis, Crohn's disease, prognosis**

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## INTRODUCTION

Inflammatory Bowel Diseases (IBDs) consist of a series of autoimmune chronic inflammatory conditions of the gut and include Crohn's Disease (CD) and Ulcerative Colitis (UC) (1). The hallmark of both IBDs is inflammation. Also, CD and UC share disease symptoms, including diarrhea, abdominal pain, and weight loss. However, despite the

symptomological similarities, CD and UC have quite distinct pathobiology regarding the spatial distribution and penetrance of inflammation along the intestine and therapeutic responses (2). In the United States, CD and UC affect ∼1 person in every 200 people (3) and a 5–10 and 2–10 fold increase has been noted in the prevalence of CD and UC, respectively, in developed countries over the past decade (4).

While, the etiology of IBD is not well-understood, environmental factors and the host genetics play important roles in regulating the disease's pathology and prognosis (1, 5). Here, one of the most recognized theories is that abnormal immunological responses to the gut microbiota play a central role in IBD susceptibility and progression. In this regard, recent studies have demonstrated that the gut microbiota acts as a metabolic organ and contributes to human health by active participation in various physiological functions of the host (6). Accordingly, composition of the gut microbial communities is critically different between healthy individuals and IBD patients (7). Such compositional changes of the gut microbiota, commonly referred to as "gut dysbiosis," are now being comprehended for developing promising strategies for prognosis and treatment of the disease (8). However, it remains unclear whether gut dysbiosis associated with the CD and UC is disease-specific, as it may help develop accurate disease predictive and management models. Moreover, an improved understanding of such differences and associated metabolic changes may help in devising novel therapeutic intervention strategies.

The current study was aimed at addressing the above described knowledge gaps. We examined fecal metagenomics sequencing data derived from CD and UC patients from five developed countries with known prevalence of IBD. The fecal metagenomics data and associated disease metadata were analyzed to identify microbial associations with CD, UC and healthy controls. Outcomes from these analyses were then subjected to "*in silico*" community modeling and metabolic pathway construction. Overall, despite the known diversity of the gut microbial communities, we found consistent differences between the gut microbiota of CD and UC patients. The gut microbial metabolic modeling further suggested disease specificity in the microbial metabolic fluxes/pathways for CD vs. UC. We believe these findings aid in the current understanding of microbial dysbiosis in CD and UC patients and toward development of effective diagnostic and therapeutic strategies.

# MATERIALS AND METHODS

## Data Collection

Fecal metagenomics sequencing data from IBD patients (CD and UC) and corresponding healthy controls (HC) were retrieved from the National Center for Biotechnology Information (NCBI). We used five different datasets belonging to the IBD patients from developed countries including USA, Canada, and three European countries (UK, Spain, and Netherlands). Among these, four datasets were generated using 16S rRNA gene amplicon sequencing while the fifth dataset was generated using the whole metagenome sequencing [NCBI SRA accession: SRP129027] (9). The NCBI SRA accession numbers for the four 16S rRNA datasets are: SRP183770 (10), SRP128892 (11), SRP115494 (12), and ERP008725 (13). The criterion in the selection of these datasets was that each dataset must contain data from at least 20 subjects each from the CD, UC and healthy cohorts. Details of samples used for the analysis from these five datasets are provided in **Supplementary Figure 1**.

## Metagenomic Data Analysis

Raw sequencing reads (fastq files) from publicly available datasets were analyzed using QIIME2 (Quantitative Insights Into Microbial Ecology version 2) software, a next-generation microbiome bioinformatics platform to determine the taxonomic diversity profiles of the microbiota in healthy and IBD samples (14). The QIIME2 plugin, DADA2 algorithm was used for quality-score based filtering of the input sequences and construction of feature table, which also contains the count of each unique sequence of each sample. To assign the taxonomy of the Feature Data (unique sequences), the pre-trained Naive Bayes and q2-feature classifiers were used. The sequences were clustered into Operational Taxonomic Units (OTUs) using a closed-reference OTU picking workflow against the Greengenes (15) 13_8 reference set from V4 region, based on an average percent identity of 99%. To avoid the problem of spurious OTUs, the singletons and doubletons were removed, and the ultimate counts/sample were generated. The whole metagenome dataset SRP129027 was aligned using DIAMOND (16) against the full NCBI NR database, which uses the "seed and extend" method to find all matches between a query sample and the reference database. The aligned sample data was saved in a compressed format called DAA (DIAMOND alignment archive). DAA files were then imported into the MEGAN6 (17) for functional classification using InterPro2GO, eggNOG, KEGG, and SEED classification schemes.

## Comparison of the Five Different Datasets

The alpha diversity (Shannon diversity) and beta diversity (Bray-Curtis distance) of all the IBD datasets were calculated and plotted using VEGAN R package (18) based on relative frequency of taxonomic profiles. The diversity of statistically significant species between HC, UC, and CD was assessed using Wilcoxon rank-sum tests and corrected for multiple testing hypothesis (Benjamini-Hochberg method) with the $p$-value $<0.05$ considered as statistically significant. The differential microbial features for HC vs. IBD, HC vs. CD, HC vs. UC and CD vs. UC in all the five datasets were identified using Statistical Analysis of Metagenomic Profiles (STAMP; v2.1.3) (19) software. The differential taxa (at order level) identified from all the datasets were plotted using UpSetR (20) to show the microbial taxa shared among the datasets. For metabolic modeling of HC, CD, and UC microbial communities, we selected the differential microbial species that were present in at least three of the five datasets to avoid the biasness based on the dataset.

## Pan-Genome Analysis and Metabolic Model Construction

A total of 12 significant microbial species were identified in our meta-analysis as differential taxa among the HC, CD, and

UC comparisons. To identify the metabolic fluxes of these differentiating taxa in HC, CD, and UC gut, we performed *in silico* metabolic modeling. For this, we retrieved the complete genome or draft genome sequences of 12 differentiating taxa from NCBI. For the draft genome, the strain that has the lower number of contigs with the highest fold coverage in a particular species was taken and used for the further analysis. Thereafter, we predicted the similarity between the bacterial genomes using Gegenees (21), which uses a fragmented alignment approach to facilitate the comparative analysis of microbial genomes. As proposed by Tettelin et al., a pan-genome can be defined as being the entire gene content of all strains in the study group (22). Thus, the Pan-genome consisted of the core genome, accessory or dispensable genome as well as unique or novel genome. Genes present in all microbial strains were considered as the core genome, and those missing in at least one strain of a microbial species were called the accessory genome, while genes present only in a single strain were considered unique. *KBase* (23) is a collaborative, open environment platform for studying the systems biology of plants, microbes, and their communities. It also has several analysis tools and data for systems biology. The *Compute Pan-genome* (v.0.07) and *Compare Genomes from pan-genome* (v.0.07) tools from KBase were used for the pan-genome construction. For disease-specific microbes, metabolic models were built using the *Build Metabolic Model* (v.1.7.6) tool from the KBase. In the metabolic modeling, bacterial growth rates were determined using *in silico* methods; we used the biological media as complete media or default media in *KBase* to construct the gap-fill model. The constructed 12 metabolic models were then compared using the *Compare Model* (v.1.7.6) app from *KBase*, which helps identify pan-genes, pan-reactions, pan-metabolites involved in disease-related microbes.

## Integrating the Metabolic Model Into the Community Model

Metabolic models were constructed for all three groups (CD, UC and HC), where each group contained four group-specific microbes. We then used the KBase tool *Merge Metabolic Model into Community Model v.1.7.6* to construct three community models, where similar reactions among the four microbes within each group were merged by a mixed-bag model. After building three community models, we performed the flux balance analysis in KBase using *Run Flux Balance Analysis v.1.7.6*, with the default media and Biomass reaction to predict metabolic fluxes in a metabolic model. Then, we identified the reactions with flux values that are involved in pathways.

## Statistical Analyses

OTU tables were used for downstream analysis to identify the functional and taxonomic profiles. Data were further analyzed using the following statistical methods: STAMP; v2.1.3 (19) software package was used to estimate the diversity of microbial communities between: (i) HC and IBD samples; (ii) CD and UC samples; and (iii) HC, CD and UC samples. For comparison between the two specific groups, for example: HC vs. IBD and CD vs. UC, Welch's *t*-test was applied. To predict the effect size and confidence intervals, the differences in mean proportion effect size measure along with Welch's confidence intervals were used. ANOVA was done for statistical comparison of the data from multiple groups, i.e., CD vs. HC vs. UC. Statistically significant features were examined using *post-hoc* tests (e.g., Tukey–Kramer) to determine how CD vs. HC vs. UC profiles differ from each other. Eta-squared effect size measure was used to predict the effect size (<0.80) and confidence intervals. To determine the false discovery rate (FDR), the multiple test correction method, Benjamini-Hochberg was used in all the comparisons. A statistical difference of at least $P < 0.05$ was used to select the significant features within a group of profiles.

## Datasets Used for Validation

For validation purposes two different whole metagenomic datasets consisting of CD, UC, and HC samples that were generated from subjects in USA were used. These datasets were retrieved from NCBI SRA SRP108708 (24) and SRP115812 (25), which consists of 157 and 300 samples, respectively. These datasets were processed using DIAMOND, MEGAN and STAMP packages using the same parameters as described above.

# RESULTS

This study was undertaken in view of the established fact that gut dysbiosis promotes susceptibility to IBD and disease severity. However, significance of this causal association for disease specificity for the CD and UC and molecular modalities of the host-microbe interaction remain poorly understood. Overall, we attempted to address the following critical questions: (i) how conserved are the gut microbial communities among IBD patients; (ii) whether gut dysbiosis precipitates in a disease-specific manner in UC and CD; and (iii) whether gut dysbiosis has disease-specific effects on the host metabolism. We focused on the meta-analysis of published raw sequenced data on gut microbiome from matched cohorts of healthy and IBD-patients from developed countries including the USA, Canada, Spain, UK, and Netherlands (**Supplementary Figure 1**). All these datasets were retrieved from NCBI to our local server for the meta-analysis. Each dataset was individually analyzed and compared in four pair-wise combinations (i.e., IBD vs. HC, CD vs. UC, CD vs. UC vs. HC), to predict the specific microbes associated with healthy control and/or IBD, based on the statistical FDR *p*-value (<0.05). To reduce false positives, we followed stringent criteria and focused only on those microbial species that were conserved in at least three of the five datasets analyzed. The alpha diversity, as measured by the Shannon diversity index, was determined using the number and types of observed OTUs within each dataset (**Figure 1A**). The Shannon index increases as both the richness and evenness of the community increases. In most cases the HC group showed higher Shannon diversity over both the CD and UC groups, and UC recorded higher diversity over CD. In contrast, the diversity index was relatively uniform across all three groups in the SRP115494 dataset. We also calculated the beta diversity between the groups using Bray-Curtis distance measure for HC vs. CD, HC vs. UC and CD vs. UC groups to understand the level of species overlap between the groups. Beta diversity was smaller when there was more overlap

of species between groups, and vice-versa. In all five datasets, beta diversity between HC vs. UC was lower compared to HC and CD, indicating that there are more overlapping species in UC with HC than in CD with HC (**Figure 1B**). On the other hand, CD vs. UC had consistently showed higher beta diversity indicating very low overlap of species between these two groups.

## Gut Microbial Composition in IBD Significantly Differ From That of Controls

We first performed an unbiased analysis of the five datasets by comparing the gut microbiota of healthy controls against all IBD patients (including all CD and UC patients). We analyzed the order-level OTUs and identified 25 orders across five datasets that were significantly different (FDR corrected *p*-value <0.05) between the healthy controls and IBD patients (**Supplementary Datasheet 2**). Out of these, members of two orders, Bacteroidales and Clostridiales were conserved in all five datasets while members of Lactobacillales and Erysipelotrichales were conserved in at least three datasets (**Figure 2A**). Of note, we classified all the significant OTUs from the kingdom to the species level in these datasets (**Supplementary Table 1**), but only order-level differences were used to compare between the IBD vs. the HC groups (**Figure 2A**). Further analysis revealed more significant differences between HC and IBD at the species-level with number of significant species ranging from 11 to 63 across all five datasets analyzed (**Supplementary Table 2** and **Supplementary Datasheet 3**). A combined total of 146 unique species were identified to be significantly different between the HC and IBD group; however, only seven of them were conserved in at least three of the five datasets. The mean relative frequencies of these seven species were then compared between the HC and IBD groups (**Figure 2B**). Microbial species such as *Gemmiger formicilis* (*p*-value = $1.51^{e-8}$) and those from the order *Clostridiales* were highly enriched in the HC group compared to the IBD groups. Similarly, microbial species from family *Ruminococcaceae,* in specific, from genus *Ruminococcus* showed significantly high abundance in HC compared to the IBD (*p*-value = $8.66^{e-4}$). In contrast, *Blautia producta* (*p*-value = $6.75^{e-4}$) and *Clostridium ramosum* (*p*-value = $8.86^{e-5}$) were highly enriched in IBD compared to the HC group (**Supplementary Datasheet 3**). Overall, above analyses confirmed the existence of major differences in the diversity and abundance of the gut microbial communities between healthy individuals and IBD patients.

## Microbial Species Specificity for CD and UC Patients Compared to the Healthy Individuals

In the light of above findings, we wondered if disease-specificity of the gut microbiota in UC and CD patients will persist even when compared with the gut microbial composition in the HC group. To this end, IBD patients from all five datasets were divided into the CD or UC cohorts using the corresponding tags in the metadata. A multi-group analysis was done while keeping the parameters for inclusion/exclusion of specific microbes the same as above. In this comparison, we identified 28 OTUs at the order-level taxa (**Supplementary Datasheet 2**). However, members of only one order, Clostridiales, were found to be conserved in all five datasets. The members of the Bacteroidales and Coriobacteriales were found to be conserved in four datasets while those belonging to the Bifidobacteriales, Erysipelotrichales and RF39 were identified in at least three datasets (**Figure 3A**). Similarly, below the order level we found higher divergence. These OTU distributions from the kingdom to species level are provided in the **Supplementary Table 1**. Overall, this comparison predicted 10 to 109 significant OTUs across the five datasets at the species-level (**Supplementary Table 2**) with a total of 168 unique OTUs (**Supplementary Datasheet 4**). Out of these, 12 OTUs were identified as conserved (present in at least three datasets) (**Figure 3B**). In particular, the species *G. formicilis* and *Coprococcus catus* were highly enriched in HC when compared to the IBD patients (**Figure 3B** and **Supplementary Datasheet 4**). The species *C. ramosum* (*p*-value = $2.64^{e-19}$) however showed a significant enrichment in the CD patients (**Supplementary Datasheet 4**). The *Caprococcus eutatus*, *Ruminococus bromii* and *G. formicilis* were all highly enriched in CD patients compared with the HC samples (**Supplementary Datasheet 4**). Notably, these organisms play a significant role in distinguishing healthy patients from IBD patients.

Overall, we identified 12 unique microbial species in our multi-group analysis, which included four differentiating species for each: the CD, UC, and HC cohorts, as listed in the **Supplementary Table 3**. The species that showed significant association with the HC included *C. catus, C. eutatus*, *R. bromii*, and *G. formicilis*. The CD-specific organisms included the *C. ramosum*, *Ruminococcus lactaris*, and *Clostridium clostridioforme* and *Clostridium bolteae, two* species that belonged to the genus *Clostridium* and family Lachnospiraceae. Similarly, the four differentiating microbial species that showed significant association with UC included the *Ruminococcus albus*, *Ruminococcus callidus*, *Faecalibacterium prausnitzii,* and *Clostridium celatum.*

## Disease-Specific Microbial Association in CD vs. UC

We further investigated how microbial communities differ between CD and UC patients. At the order-level, a total of 30 OTUs were identified as significantly different in the CD cohort vs. the UC cohort (corrected *p*-value ≤0.05) (**Supplementary Datasheet 2**). Similar to the IBD vs. HC comparison, both *Bacteroidales* and *Clostridiales* were conserved in all five datasets. Likewise, Bifidobacteriales were conserved in four datasets while Coriobacteriales, Erysipelotrichales, and Fusobacteriales were present in at least three datasets (**Figure 4A**). However, this analysis showed higher levels of divergence from kingdom to the species level comparison (**Supplementary Table 1**). Further analysis revealed a cluster of 21-88 OTUs to be significantly different in CD vs. UC at the species level (**Supplementary Table 2** and **Supplementary Datasheet 5**). From the five datasets combined, a total of 195 OTUs were predicted to be significantly different

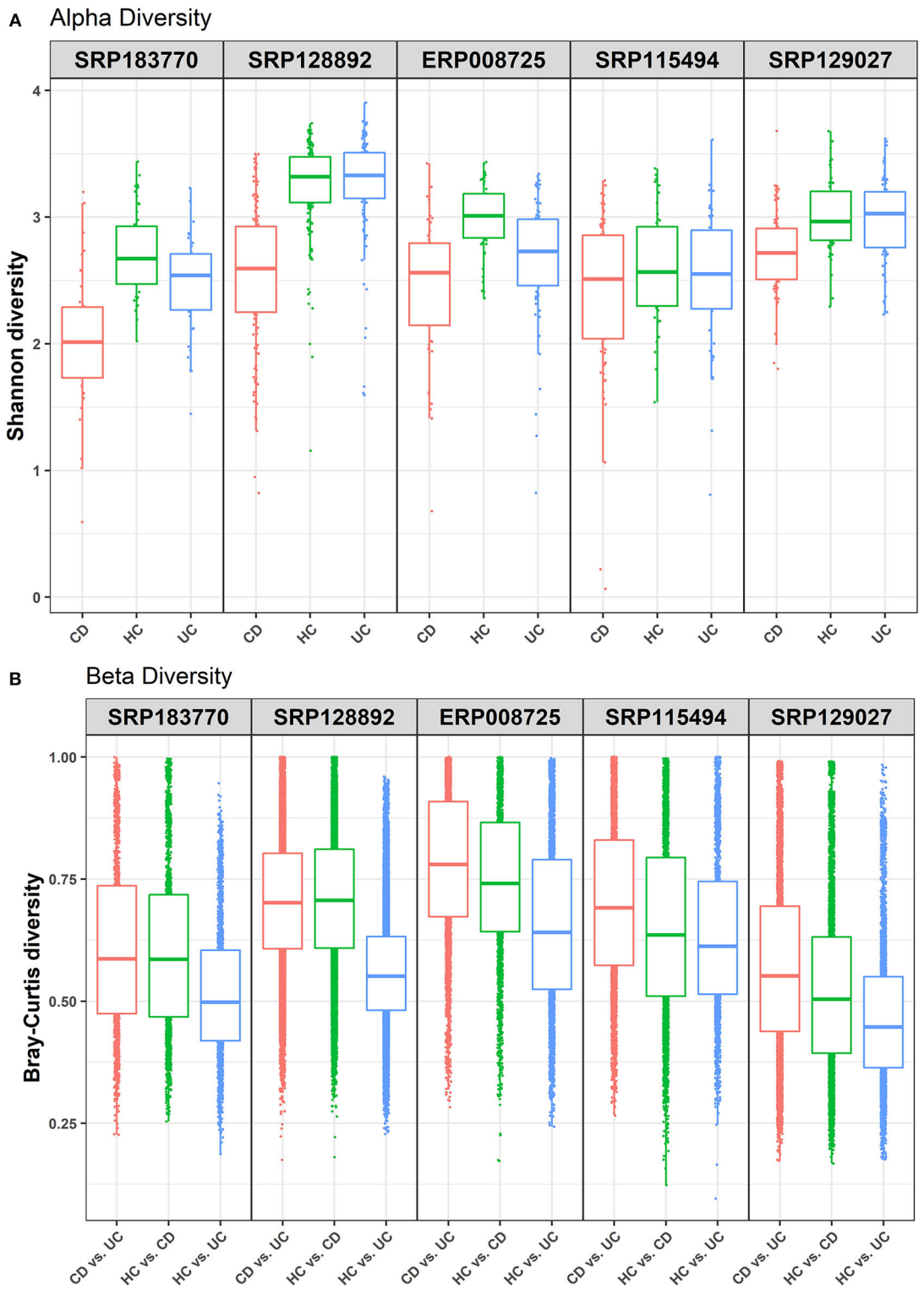


**FIGURE 1 |** Alpha and beta diversity comparisons among HC, CD and UC cohorts. Analyses were performed on species-level taxa. **(A)** Boxplot showing Shannon diversity of each group. Each dot represents a sample and the lines in the boxes correspond to the median of samples; **(B)** Bray–Curtis distances between the comparison pair. Dots represent the distance between the samples in each comparison group and the lines in the boxes correspond to the median.

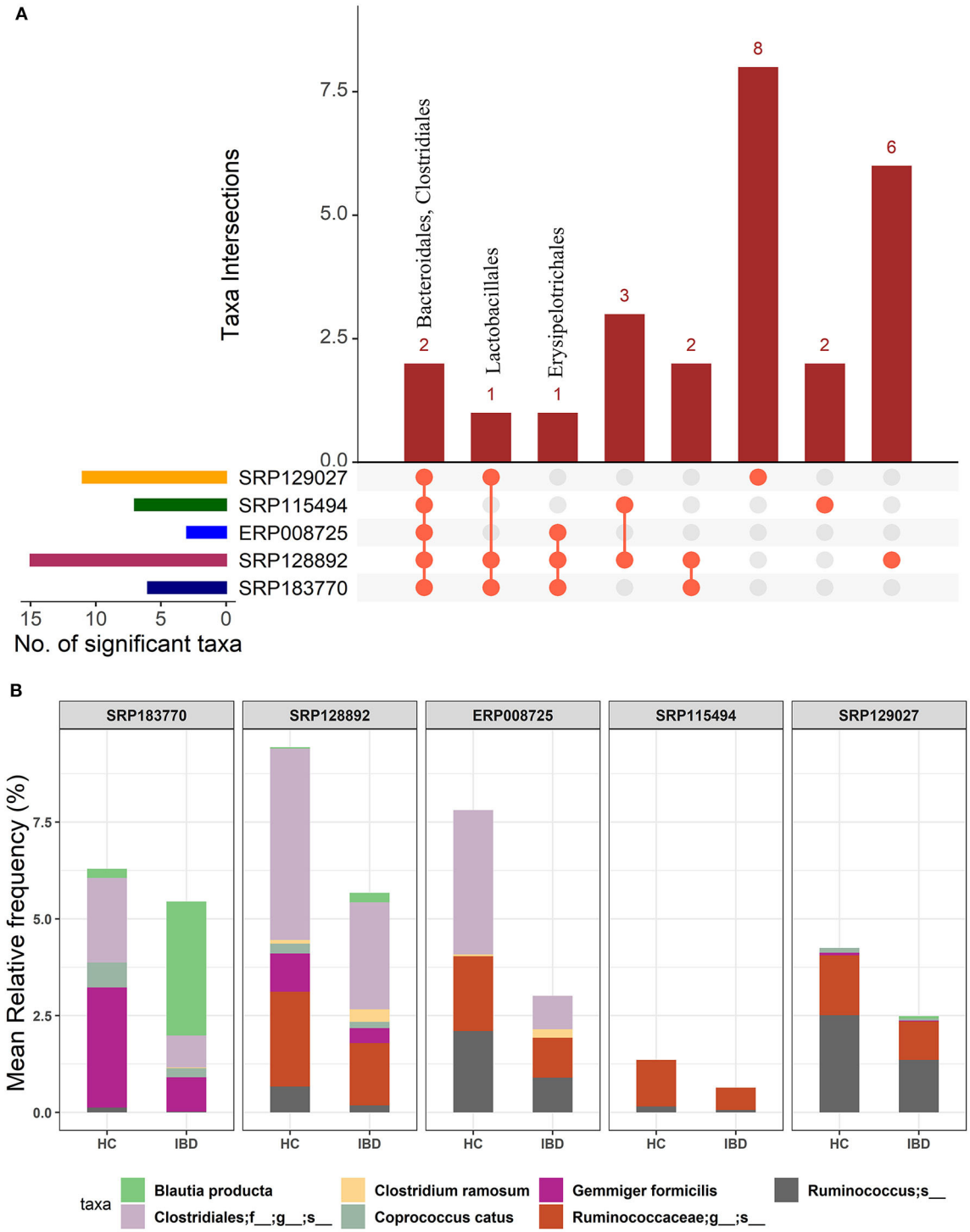


**FIGURE 2 |** Comparison of microbial communities between IBD and HC cohorts across five different datasets. **(A)** An upset plot showing taxonomic intersections across the five datasets at the Order-level. Each bar represents the number of orders in that category and the orange dot below the bar indicates their conservation across the datasets. For instance, members of Bacteriodales and Clostridiales are conserved in all five datasets; **(B)** Stacked bar plots show the relative mean frequencies of significant species-level communities in IBD or HC that are present in at least in three out of five datasets. Corresponding values are provided in the 146 OTUs sheet in **Supplementary Datasheet 3**, where the columns contain data for five different datasets.

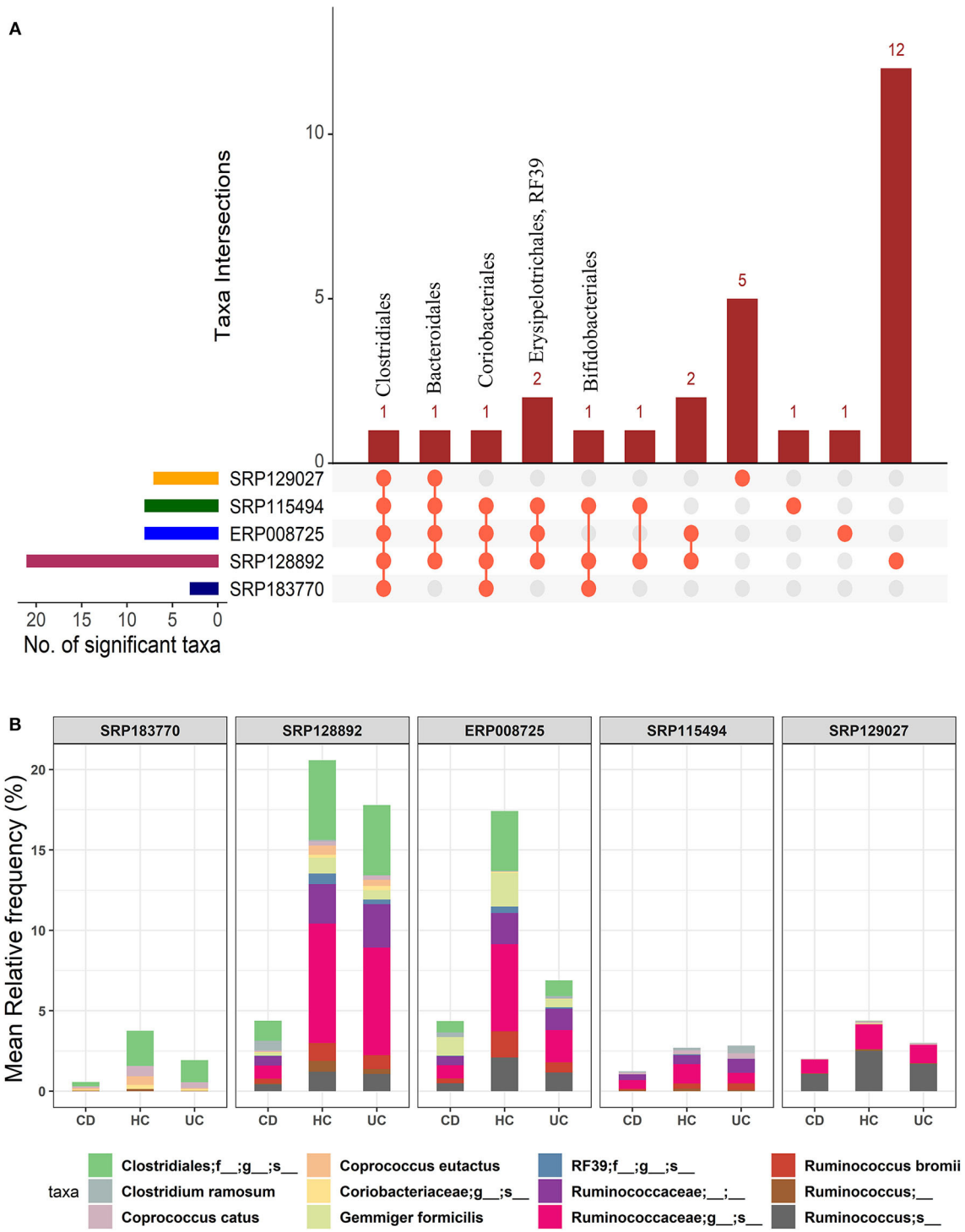


**FIGURE 3 |** Comparison of microbial communities among CD, UC, and HC cohorts across five datasets. **(A)** An upset plot showing taxonomic intersections across the five datasets at the Order-level. Each bar represents the number of orders in that category and the orange dot below the bar indicates their conservation across the datasets. For instance, members of Clostridiales are conserved in all five datasets; **(B)** Stacked bar plots show the relative mean frequencies of significant species-level communities in CD, HC or UC that are present in at least in three out of five datasets. Corresponding values are provided in the 168 OTUs sheet in **Supplementary Datasheet 4**, where the columns contain data for five different datasets.

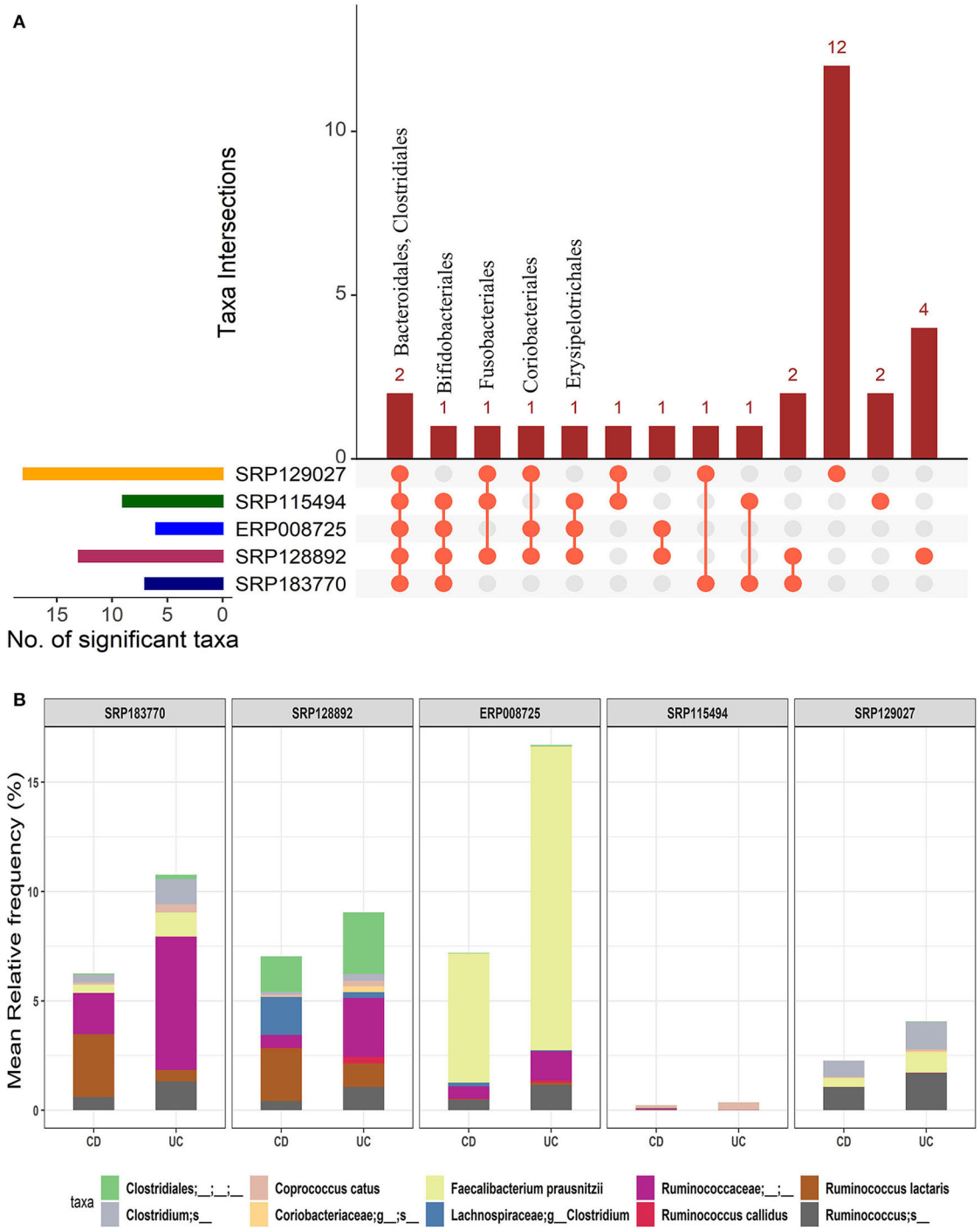


**FIGURE 4 |** Comparison of microbial communities between CD and UC cohorts across five datasets. **(A)** An upset plot showing taxonomic intersections across the five datasets at the Order-level. Each bar represents the number of orders in that category and the orange dot below the bar indicates their conservation across the datasets. For instance, members of Bacteroidales and Clostridiales are conserved in all five datasets; **(B)** Stacked bar plots show the relative mean frequencies of significant species-level communities in CD or UC that are present in at least three out of five datasets. Corresponding values are provided in the 195 OTUs sheet in **Supplementary Datasheet 5**, where the columns contain data for five different datasets.

between the CD and UC cohorts. Among these, ten OTUs were identified as conserved, based on the criteria that an OTU must be present in at least three of the five datasets examined (**Figure 4B** and **Supplementary Table 2**). Importantly, we found that the members of genus *Clostridium* belonging to two different families, Lachnospiraceae and Clostridiaceae, were

rather specific for CD or UC, respectively. The genome sizes of the members of the genus *Clostridium* also varied, depending on the family they belong to (**Table 1**). Similarly, members of the genus *Ruminococcus* also belonged to multiple families; their disease-specific association was distinguishable by their family, Lachnospiraceae and Ruminococcaceae in CD and UC, respectively (**Supplementary Datasheet 5**). At the species level, *R. lactaris* (from family, 2.8% higher relative frequency ($p$-value $= 0.016$) in CD compared to UC (**Supplementary Datasheet 5**). In addition, *C. catus*, *R. callidus*, and *F. prausnitzii* were also able to differentiate the UC patients from CD patients at a statistically significant threshold level (**Supplementary Datasheet 5**). Similar trends were seen for the *Lachnospiracae* and *Ruminococcaceae* families as they were decreased in the CD patients in comparison with the UC patients, while *Ruminococcus gnavus* was increased vice versa (**Supplementary Datasheet 5**). Overall, these studies helped designate typical changes in the composition of gut microbial composition in UC vs. CD patients.

Taken together, our analysis supported the initial postulation that the gut dysbiosis presents itself in a disease-specific manner and can be harnessed for diagnostic and/or prognostic purposes. Therefore, we further investigated to determine if the metabolic profiles of the above-identified microbial species also confer specificity for CD, UC, and HC to help distinguish between the IBD disorders and with healthy controls.

## Validation of Disease-Specific Species Using Distinct Datasets

For the validation purpose, we have used the two whole metagenomics datasets (**Supplementary Figure 4A**). The alpha diversity (Shannon diversity) and beta diversity (Bray-Curtis distance) were analyzed, which showed similar results with our previous comparisons. HC group showed higher Shannon diversity over both the CD and UC groups (**Supplementary Figure 4B**). Beta diversity was smaller when there was more overlap of species between CD and UC groups (**Supplementary Figure 4C**). We analyzed the order- and species-level comparisons for CD vs. HC, UC vs. HC, and CD vs. UC (**Supplementary Datasheet 8**). In the prior comparison, members of order *Bacteroidales* and *Clostridiales* were enriched in all the three comparisons and a similar trend was observed in these datasets too (**Supplementary Figures 5A–C**). Similarly, at the species-level, in comparison to the previously identified significant OTUs, seven out of seven in CD vs. HC (**Supplementary Figure 6A**), 11 out of 12 in UC vs. HC (**Supplementary Figure 6B**) and ten out of ten in CD vs. UC (**Supplementary Figure 6C**) were also identified in these two datasets (**Supplementary Datasheet 8**). These results using distinct datasets validate our prior results using five datasets and demonstrate that the disease-specific species identified in this study can be reliably advanced to metabolic modeling studies.

## Metabolic Modeling Using the Pan-Genomic and Pan-Metabolomic Data

The 12 disease-specific microbial species that we identified in CD, UC, and HC cohorts showed a large variation in their

**TABLE 1 |** Metabolic characterization of disease-specific species involved in CD, HC, and UC.

| Diseases | Species | Accession no. | Strain type | Size | Total genes | Reactions | Metabolites | Genes in Model | FBA# | Reactions* | Metabolites | FBA# |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CD | *Clostridium bolteae* ATCC BAA-613 | ABCC00000000 | Gram-positive | ~6.6 | 6,074 | 614 | 758 | 312 | 1.12636 | 809 (331) | 864 | 17.9152 |
| | *Erysipelatoclostridium ramosum* DSM 1402 | ABFX00000000 | Gram-positive | ~3.2 | 3,041 | 535 | 658 | 221 | 1.17318 | | | |
| | *Ruminococcus lactaris* CC59-002D | AZJE00000000 | Gram-positive | ~2.7 | 2,855 | 587 | 706 | 166 | 1.18649 | | | |
| | *Clostridium clostridioforme* CM201 | AGYS00000000 | Gram-positive | ~5.6 | 6,074 | 583 | 707 | 273 | 0.974817 | | | |
| HC | *Coprococcus catus* GD/7 | NC_021009 | Gram-positive | ~3.5 | 2,972 | 551 | 706 | 197 | 36.1197 | 899 (380) | 930 | 7.41658 |
| | *Ruminococcus bromii* AM32-13AC | QSIY01000000 | Gram-positive | ~2.5 | 2,007 | 459 | 598 | 134 | 27.6451 | | | |
| | *Coprococcus eutactus* 2789STDY5834963 | CYXU00000000 | Gram-positive | ~3.1 | 2,665 | 585 | 722 | 179 | 27.9975 | | | |
| | *Gemmiger formicilis* ATCC 27749 | FUYF00000000 | Gram-negative | ~3.2 | 2,882 | 524 | 650 | 178 | 0.616684 | | | |
| UC | *Ruminococcus callidus* ATCC 27760 | AWVF01000000 | Gram-positive | ~3.1 | 2,791 | 493 | 621 | 156 | 1.18834 | 871 (368) | 915 | 3.23853 |
| | *Faecalibacterium prausnitzii* A2165 | NZ_CP022479 | Gram-negative | ~3.1 | 2,956 | 528 | 655 | 177 | 20.9628 | | | |
| | *Clostridium celatum* DSM 1785 | AMEZ01000000 | Gram-positive | ~3.5 | 3,211 | 580 | 698 | 228 | 22.7295 | | | |
| | *Ruminococcus albus 7* DSM_20455 | NC_014833 | Gram-positive | ~4.4 | 3,983 | 555 | 680 | 197 | 1.15961 | | | |

**The number given in the brackets are number of reactions with fluxes. #FBA- Flux Balance Analysis.*

genome size, indicating a diverse metabolic footprint across the organisms. *R. bromii* and *C. bolteae* contained the smallest and largest genomes (at ∼2.5 and ∼ 6.6 Mb), respectively (**Table 1**). First, we looked at the genome-level similarities among these 12 species using the Gegenees similarity analysis tool, which showed the similarity range between 18 and 78% at the nucleotide level (**Supplementary Figure 2**). Then, species-level metabolic models were reconstructed for all 12 organisms by choosing appropriate templates from the Gram-positive or Gram-negative species. These predicted models are provided in the SBML (.xml) and excel (.xls) formats in the **Supplementary Folder**: Model.zip.

For each of the 12 reconstructed metabolic models, we identified all possible biological reactions and chemicals/metabolites involved in the complete reaction. These reactions included forward, reverse as well as bi-directional biological reactions. The total number of genes, reactions, and metabolites that are potentially involved in these metabolic models, for all the 12 microbial genomes, are listed in **Table 1**. The combined set of genes, reactions and metabolites from each group were then used for CD vs. HC, UC vs. HC and CD vs. UC comparisons, to identify the pan, core, accessory and unique sets of genes, and corresponding reactions and metabolites (**Supplementary Table 4**). To identify the reactions that are specific to CD, UC, and HC cohorts, we excluded all the core reactions that are present in all 12 genomes and separated the unique and accessory reactions that are exclusive to each cohort. Likewise, we identified disease-specific or control-specific genes and metabolites. From these metabolic models, we obtained the number of specific reactions, metabolites and genes in each diseased condition (CD and UC) and healthy control (HC). However, only a limited number of the specific reactions were present within the communities of CD, UC, and HC when compared with each other (**Supplementary Table 5**). For example, in comparison of the CD vs. HC, only 141 reactions were identified as CD specific. Likewise, in UC vs. HC, 153 reactions were identified as UC specific. While comparing disease associated reactions, CD vs. UC 124 and 186 reactions were identified as specific to CD and UC, respectively. Since the identified disease-specific microbes belonged to a different genus, there are many reactions that were identified as single specific reactions in each metabolic model, even though they were not shared with their community. Similarly, we compared the metabolites and genes involved in the metabolic models and the total numbers of identified items have been listed in **Supplementary Table 5**. The entire list of the reactions, compound and genes in the metabolic model and their specific reactions, compound and genes, which differentiate CD vs. HC, UC vs. HC and CD vs. UC, are provided in **Supplementary Datasheet 6**.

## Community Metabolic Modeling Using Disease-Specific Microbes

In this analysis, we combined the metabolic models of all organisms in each cohort to build a community model for each of the CD, UC and HC cohorts. For example, metabolic models of *C. bolteae*, *C. ramosum*, *R. lactaris*, and *C. clostridioforme* were combined to generate a single community metabolic model for CD. These models are provided in SBML (.xml) and excel (.xls) formats in the **Supplementary Folder**: Model.zip. Notably, from the CD, HC, and UC comparisons, the total identified reactions from the community model were 809, 899, and 871, respectively. To further determine the reaction fluxes, flux balance analysis was performed for each community model with a goal to determine the maximum reaction biomass for each model. The growth rate of the biomass yield for CD, HC, and UC showed the objective values as 17.91, 7.41, and 3.2, respectively (**Table 1** and **Supplementary Datasheet 7**). Here, the identified metabolites in CD were highly enriched in pathways including metabolism of the cofactors and vitamins, amino acid metabolism, metabolism of other amino acids, and metabolism of terpenoids and polyketides. However, the UC metabolites were enriched more in the glycan biosynthesis and metabolism, biosynthesis of the other secondary metabolites, and polyketide sugar unit biosynthesis pathways (**Figure 5**). On the other hand, metabolic pathways such as lipid metabolism and xenobiotic biodegradation and metabolism were rather high in the HC, while pathways relating to the carbohydrate metabolism, nucleotide metabolism, and energy metabolism were equally distributed in all three groups. We also identified that there were 331, 380 and 368 enhanced flux reactions involved in 44, 55 and 47 sub-pathways of CD, HC, and UC, respectively (**Supplementary Figure 3**). Based on the flux values and their reactions, we then compared the HC, UC, and CD to detect cohort specific reactions (**Supplementary Datasheet 6**). Interestingly, these comparisons led to the identification of specific metabolic reactions that differentiate for CD, UC, and HC (**Table 2**).

## The Disease-Specific Gut Microbiome Affects Specific Host Metabolic Pathways

We found disease-specific enrichment of the gut microbial communities in IBD compared to HC. Therefore, we further examined specific metabolic pathways that can be altered based on the microbial communities specific to UC and CD cohorts (**Table 2**). Also, to understand the potential impact on the host metabolism due to disease-specific enrichment of microbial communities, we explored the metabolic footprints of these communities. As expected, our meta-analysis showed that microbial species unique to HC are involved primarily in the breakdown of non-digestible carbohydrates and resistant starch alongside generation of lactate, acetate, propionate, and butyrate. However, the microbial communities differentially enriched in CD patients (vs. UC) potentially impact the higher carbohydrate utilization as reflected by the enrichment of pathways involved in the metabolism of simple carbons such as fructose, mannose, and galactose (**Supplementary Figure 3** and **Table 2**). Also, glyoxylate and dicarboxylate metabolic pathways involved in carbohydrate biosynthesis from the fatty acids were increased in association with differential enrichment of the CD microbiota vs. UC (**Supplementary Figure 3** and **Table 2**). Benzoate degradation, a metabolic process associated with the induction of inflammation, was also upregulated

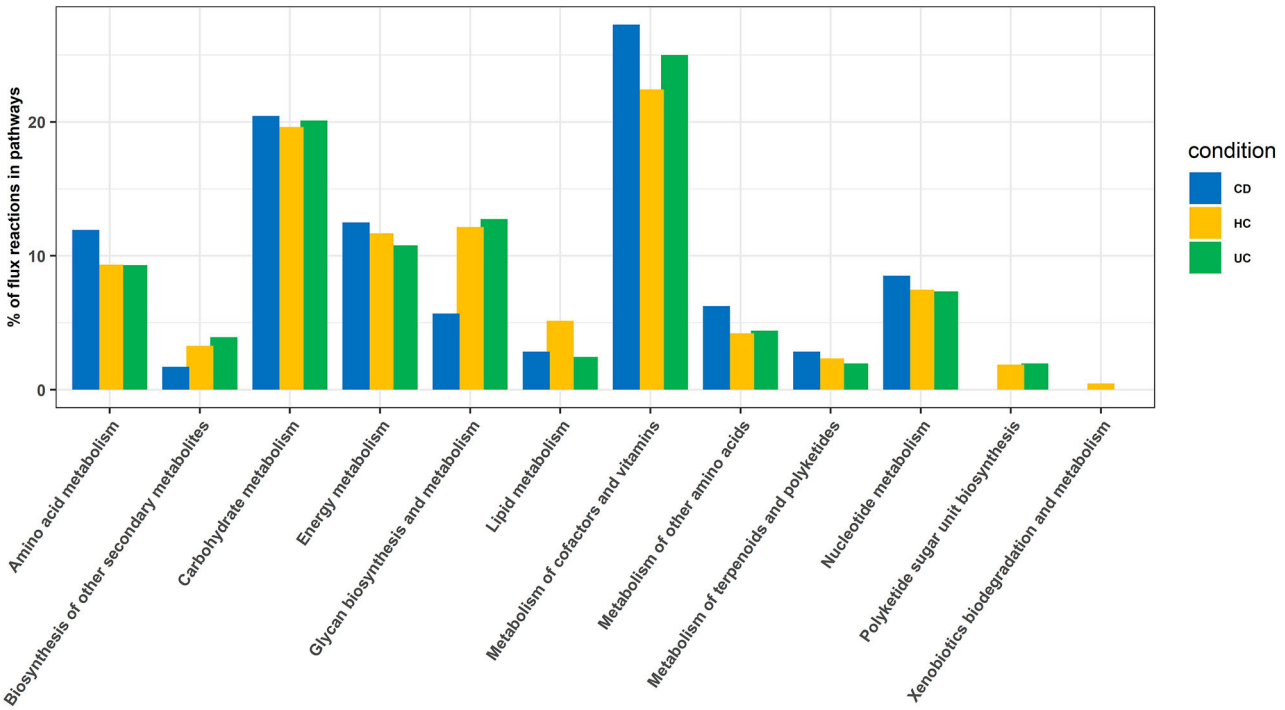


**FIGURE 5 |** Enriched pathways identified based on enriched metabolic reactions in disease-specific organisms for HC, CD, and UC cohorts.

specifically in the CD. Interestingly, the microbiota enriched in the CD also exhibited increased antioxidant defense molecule processing, including ascorbate and glutathione metabolism (**Supplementary Figure 3** and **Table 2**). On the other hand, UC enriched microbiota were associated with an increase in the metabolic pathways related to glycolytic and gluconeogenic metabolic pathways that are involved in maintaining the normal energy hemostasis. We also found that the pyruvate metabolic pathway was increased in the UC enriched microbiota compared to the CD enriched microbiota (**Supplementary Figure 3** and **Table 2**). Overall, our data suggested that disease-specific enrichment of microbial communities affect the host metabolic pathways in disease-specific manners.

## DISCUSSION

Our study represents one of the first efforts to discover the IBD-associated microbes and cohort-specific reactions from 16S rRNA and whole metagenome datasets using computational methods. Microbiota diversity has been known to play a key role in IBD (26). Earlier studies have shown an association between salmonella and campylobacter infections with an increased risk of IBD (27). However, another report did not show any consistent association between *Mycobacterium avium* subspecies *paratuberculosis* with CD (28). Some viruses, including the measles virus, were initially thought to be a risk factor for IBD (29). Later, *Clostridioides difficile*, cytomegalovirus infection, and other causes of sepsis have been noted to cause exacerbation of IBD, but no causal link has been detected (30). As mentioned before, UC and CD are sufficiently different in their pathobiology despite the similarities in disease symptoms and pathologies (31). Multiple studies have observed significant differences in the gut intestinal microbiomes of IBD patients when compared to the healthy individuals (2, 32, 33). These studies have led to the general perception that dysregulation of gut microbial diversity is potentially similar in CD and UC patients, and is characterized by a lower proportion of the *Firmicutes* and an increase in Gamma proteobacteria (34).

Due to the high prevalence of IBD in the developed countries, we performed data analysis on IBD samples (with at least 20 patient samples in each of the CD, UC, and HC cohorts) only from the developed countries. First, we looked at the alpha and beta diversity of the samples and cohorts using the Shannon index and Bray-Curtis distance measure, respectively. As expected, the alpha diversity trended higher in most of the health control datasets compared to the two IBD groups (CD and UC) (**Figure 1A**). Likewise, beta diversity as measured by the Bray-Curtis distance measure between the cohorts showed notable differences (**Figure 1B**) with the highest beta diversity recorded in CD vs. UC comparison and the lowest in HC vs. UC. These results indicate that there is only a small overlap of microbial species between CD and UC, which supports our notion that gut dysbiosis precipitates in a disease-specific manner. On the other hand, there's relatively a higher overlap of microbial species (less beta diversity) between UC and HC samples indicating that the UC microbiome is relatively closer to healthy controls compared to that of CD.

**TABLE 2 |** Differential microbiome patterns, metabolites, and metabolic function changes in CD vs. UC.

| Disease | Differential microbiota change | Key enzymes involved | Functions |
|---|---|---|---|
| UC | *Ruminococcus albus* | Pyruvate synthase | Digestion of plant fibers |
| | *Ruminococcus callidus* | (S)-Malate:NADP+ oxidoreductase(oxaloacetate-decarboxylating) | Cellulose metabolism |
| | *Clostridium celatum* | CoA-transferases | Starch degradation |
| | *Faecalibacterium prausnitzii* | glycoside hydrolases | Glycan degradation |
| | | D-glucose 1-epimerase | Decreases pro inflammatory cytokines |
| | | Cellulases | Methane production |
| | | Galactosidase | Reduces nitrate to nitrite |
| | | | Hydrolyse Hippurate and starch |
| | | | Involved in glucose and mucin production |
| | | | Anti-inflammatory effect |
| | | | T-reg cells regulation |
| CD | *Clostridium bolteae* | sn-Glycero-3-phosphocholine | Lower carbohydrate oxidation |
| | *Clostridium ramosum* | glycerophosphohydrolase | Increased fat oxidation (Reduced fat accumulation) |
| | *Ruminococcus lactaris* | D-psicose 3-epimerase | Involved in tryptophan metabolism |
| | *Ruminococcus callidus* | isocitrate lyase | Involved in polyamines metabolism |
| | | malate synthase | Increases production of enterolignans, enterodiol and |
| | | D-glyceraldehyde-3-phosphate aldose-ketose-isomerase | enterolactone from plant lignin |
| | | 4-Carboxymuconolactone carboxy-lyase | Involved in lactose (important enzyme: Galactosidase) |
| | | L-Rhamnose ketol-isomerase | and fructose metabolism |
| | | NAD-dependent threonine 4-phosphate dehydrogenase | Increased production of butyrate (acetyl Co A, glutrate, |
| | | Pyridoxamine-5′-phosphate:oxygen oxidoreductase | lysine and amino butyrate pathways) |
| | | D-Galactonate hydrolyase | Negatively regulate leucine and bile acids |
| | | (R)-Glycerate:NAD+ oxidoreductase | Increased fat transporter |
| | | | Involved in wound healing, neutrophil recruitment and |
| | | | intestinal motility |
| | | | Stimulate the production of pro inflammatory cytokines |

Then, we looked at the detailed profiles of bacterial species at different hierarchical taxonomic levels (kingdom to species) between the disease and healthy cohorts. Because the differences are minimal at the higher taxonomic levels, we focused on the profiles at the order level and below. Specific differences in microbes were noted by comparing the healthy and disease cohorts in three different ways, i.e., HC vs. IBD (**Figure 2**); HC vs. CD vs. UC (**Figure 3**); and CD vs. UC (**Figure 4**). Using a strict criteria that a species must be present in at least three out of the five datasets analyzed, we identified a combined 12 different species, four for each cohort that can be used as unique microbial markers (**Supplementary Table 3**). The genus *Clostridium* and *Ruminococcus* were highly prevalent in CD and UC, respectively. In HC, *Coprococcus* and *Gemmiger* played a vital role in differentiating healthy individuals from disease cohorts. Taken together, our results validated a similar outcome from other studies that the diversity of microbial communities is altered in IBD patients (9, 11). Similarly, He et al. compared 74 mucosal biopsies from 15 participants, including nine CD patients and six healthy individuals. They reported that 65 genera were identified as differentially abundant between active and quiescent CD, with a loss of *Fusobacterium* and a gain of potentially beneficial bacteria, *Lactobacillus*, *Akkermansia*, *Roseburia*, *Ruminococcus*, and *Lachnospira* after the induction of remission (35). These taxa also showed a positive correlation with clinical disease severity and a negative correlation with species richness. Our analysis also reported the *Clostridium* from two different families Lachnospiraceae and Clostridiaceae. It is noteworthy to point out that the UC-specific *C. celatum* is a member of the family Clostridiaceae while the two CD-specific Clostridium species are members of the family Lachnospiraceae (36). Similarly, *Ruminococcus* was also reported in two different families, Ruminococcaceae in UC and Lachnospiraceae in CD.

Our study noted that there are significant changes in *F. prausnitzii,* which differentiate the UC patients from CD patients. Of interest, *F. prausnitzii,* the most abundant bacterium in the healthy human gut is the major member of the Firmicutes phylum (37). Importantly, *F. prausnitzii* has immune-suppressive effects. It produces a protein that inhibits the NF-κB pathway, stimulates production of anti-inflammatory cytokine IL-10, and inhibits ulcerative colitis in BALB/c mice (37). *F. prausnitzii* is depleted in several intestinal disorders; however, more consistently in CD patients (38). Our analysis confirmed similar depletion of this microbial species in the CD patients. However, it revealed a contrasting enrichment in the UC patients. Notably, *F. prausnitzii* also produces the short-chain fatty acid, butyrate, an essential nutrient for the intestinal epithelial cells and its increase in UC patients may represent an adaptive enrichment. Furthermore, the proportions of the Clostridia were altered in CD patients: the Roseburia and *Faecalibacterium* genera of the *Lachnospiracae* and *Ruminococcaceae* families were decreased while *R. gnavus* was increased (32).

Comparison of the genome size and sequence similarities among the twelve species (**Supplementary Figure 2** and **Table 1**) revealed vast variations. The sequence similarity between some species was as low as 40% indicating that the diversity of these genomes also contributes to a diverse metabolic footprint

that affects the host metabolism in a disease-specific manner. Remarkably, several recent studies suggest that microbial diversity affects disease conditions by impacting the host-microbe interaction in regulating the host metabolism (39). To understand these interactions, we further analyzed the metabolic profiles of disease-specific species that we identified above using metabolic modeling and flux balance analysis. We identified significant pathways in CD and UC, which included enriched pathways related with amino acid and Glycan biosynthesis and metabolism.

Studies have shown that gut microbiota impact the host potentially by influencing the metabolism by producing specific enzymes and/or metabolites (40, 41). Interestingly in our findings, species unique to the HC are involved primarily in the breakdown of non-digestible carbohydrates and resistant starch, and the generation of short-chain fatty acids. Of interest, butyrate plays a crucial physiological role in maintaining the health and integrity of the colonic mucosa (42). CD enriched microbial species were mostly involved in fructose, mannose, and galactose metabolism. In this regard, *C. bolteae* and *R. callidus* enriched in CD are known to use above sugars and metabolize them into glyceraldehyde-3 phosphate, a key metabolite of the glycolytic pathway, the principal energy-generating mechanism in human body (43). Additionally, the glutathione and ascorbate pathways, involved in the maintenance of normal homeostasis during oxidative stress, were enriched in CD.

In comparison, the UC enriched microbiota are associated with an increase in the glycolytic, gluconeogenic, and pyruvate metabolic pathways. Notably, pyruvate can be catabolized into succinate, lactate, or acetyl-CoA and can be metabolized into acetate, propionate, and butyrate (43). We speculate these changes will help promote adaptive responses against inflammatory insults to heal the mucosa. *F. prausnitzii*, a "health-promoting" microbiota, was also explicitly increased in the UC patients. Studies have reported anti-inflammatory properties of this microbiota by promoting IL-10 production while and inhibiting NF-kB activity in the host cells. Also, *F. prausnitzii* is linked with butyrate production (37). Taken together, our data suggested that the enzymes involved in specific host metabolic pathways can be impacted differentially by the gut microbiota in CD vs. UC, though a systematic experimental investigation is warranted to uncover further details. This study supports the identification of disease-specific microbial communities and their effects on the host metabolism, which helps researchers differentiate between IBD (CD and UC) diseases in the initial stages.

## CONCLUSIONS

In conclusion, this article represents an unbiased determination of the relative status of the gut microbial communities in IBD patients compared with healthy controls, using meta-analysis of five different IBD datasets available in the public domain representing populations from five different developed countries. While this analysis confirmed the generally recognized association of the gut microbial dysbiosis with IBD, it also revealed that this dysbiosis bears disease specificity, as we found significant changes in microbiota enrichment in UC vs. CD at different taxonomic levels down to the genus and species. The metabolic modeling further demonstrated the significance of dynamic host-microbe interactions in affecting host metabolism, which potentially is mediated by the release of specific microbial enzymes and metabolites. We believe that such information will not only help development of potential biomarkers for disease validity in non-invasive manner but also therapy response. Obviously, further detailed analysis is needed to satisfy such needs and is part of our ongoing studies.

## DATA AVAILABILITY STATEMENT

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

## AUTHOR CONTRIBUTIONS

AS and CG supervised the study. JS, AS, and CG designed experiments. JS, RA, and NA generated and analyzed data. JS, NA, and CG made the figure panels. JS and RA wrote the original draft. NA, AS, and CG reviewed and edited the manuscript. All authors have 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.2020.606298/full#supplementary-material

**Supplementary Folder |** Model.zip. This folder contains the predicted model for 12 organisms and the community model of CD, HC, and UC in SBML (.xml) and Excel (.xls) format. Available online at: https://doi.org/10.6084/m9.figshare.13208204.v1.

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# Global Studies of Using Fecal Biomarkers in Predicting Relapse in Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract mainly comprising two forms including Crohn's disease (CD) and ulcerative colitis (UC). IBD is a lifelong relapsing remitting disease and relapses occur at random patterns which are unpredictable. Fecal biomarkers have been increasingly used to assess disease activity in IBD due to their positive correlations with intestinal inflammation. Recent studies have also assessed the use of fecal biomarkers in predicting relapse and post-operative recurrence. This review provides information from global studies of using fecal calprotectin, lactoferrin and S100A12 to predict relapse in IBD. Strategies for further studies and the use of these fecal biomarkers for personalized management in IBD are also discussed.

**Keywords: inflammatory bowel disease, Crohn's disease, ulcerative colitis, fecal biomarkers, prediction, calprotectin, lactoferrin, S100A12**

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## INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract comprising of two major subsets, Crohn's disease (CD) and ulcerative colitis (UC) (1). Inflammatory bowel disease is a lifelong disease and patients often experience multiple episodes of relapse and remission. Relapses in IBD occur at a random pattern, which are unpredictable. Endoscopy is not used routinely for disease monitoring due to its invasiveness and cost. Current monitoring of disease relapses in patients with IBD is symptom based (1). In order to improve patient management, various studies have assessed the use of fecal biomarkers in predicting disease relapse (2).

Fecal biomarkers have attracted a great attention owning to their non-invasiveness and cost effectiveness. Fecal biomarkers used in IBD are bioproducts resulted from inflammatory responses in the intestinal mucosa. Calprotectin is the most studied fecal biomarker. Lactoferrin, S100A12 and other fecal biomarkers have also been examined in recent years. Most of the studies have reported that these biomarkers correlate well with the endoscopic score and histological inflammation in patients with IBD (3–12).

Recent studies have also assessed the use of fecal biomarkers in predicting relapse and post-operative recurrence. In this review article, we provide comprehensive and updated information from global studies on the use of fecal calprotectin, lactoferrin and S100A12 to predict relapse in IBD. We have also discussed strategies for further studies and the use of these fecal biomarkers for personalized management in IBD.

## BIOLOGY OF CALPROTECTIN, LACTOFERRIN, AND S100A12

Fecal biomarkers used in IBD are either actively secreted by or released from necrotic immune cells during inflammatory responses at the intestinal mucosa. They have a wide variety of biological functions including antimicrobial activity, proinflammatory activity, degradation of extracellular matrix and intracellular pathogens, as well as cellular and metabolic activities.

### Calprotectin

Calprotectin is a cytoplasmic protein prominently found in neutrophils that accounts for more than 40% of the cytosolic proteins in neutrophils, and to a lesser extent in monocytes and macrophages. Calprotectin is released to extracellular environment during inflammatory responses upon neutrophil activation or necrosis and induces neutrophil chemotaxis and adhesion. Calprotectin is stable for up to 1 year when stored at −20°C, and stable for 7 days when stored at 4°C and room temperature (13–15).

The physiologically active conformation of calprotectin is a heterodimer complex consisting of S100A8 and S100A9 and both proteins belong to the S100 family. The S100A8 and S100A9 subunits consist of 93 and 113 amino acids with molecular weight of 10.8 and 13.2 kDa, respectively (16, 17). Each subunit is able to bind two calcium ions. In addition to the calcium binding site, each heterodimer displays two transition metal binding sites at the interface of S100A8/S100A9, the first site binds manganese and zinc, while the second site binds zinc only (18–21).

As a metal chelating agent, calprotectin binds transition metals with high affinity and efficiently sequester them away from invading microbial pathogens, thereby starves invading pathogens, limiting their growth and resulting in a process called "nutritional immunity" (22–25). At the site of infection, calprotectin is not only abundantly released by neutrophils, but also epithelial cells and other immune cells, thereby playing a critical role in host defense against various bacterial species such as *Listeria monocytogenes*, *Salmonella* Typhimurium, *Borrelia burgdorferi*, *Helicobacter pylori*, *Staphylococcus aureus*, as well as fungal pathogens including *Candida albicans* (26–33). Interestingly, some bacterial pathogens harbor mechanisms allowing them to evade the harmful environment created by calprotectin. For examples, *H. pylori* is able to alter its outer membrane *via* lipid A modification, thus evading the antimicrobial activity of calprotectin. The growth of *S.* Typhimurium was actually elevated over competing commensal microbes in the presence of calprotectin due to the presence of ZnuABC zinc transporter, which enables the bacterium to acquire zinc under zinc-limiting conditions (34, 35).

### Lactoferrin

Lactoferrin is present in most exocrine secretions such as milk, saliva, tears, mucosal secretions, and plasma (36). Secretory epithelia and neutrophils are the main sources of lactoferrin. Lactoferrin is stable for up to 7 days when stored at 4°C or room temperature (37–39).

Human lactoferrin is an 80 kDa glycoprotein containing ∼700 amino acids. The single polypeptide chain forms two homologous globular domains, namely N-terminal lobe and C-terminal lobe, respectively, depending on their localization, and each terminal lobe contains two domains (N1, N2, C1, and C2), resulting in a deep cleft conformation for iron-binding (40).

Lactoferrin has antimicrobial activity. Lactoferrin binds free iron, which inhibits the growth of iron-dependent bacterial species and reduces bacterial biofilm formation (41). Lactoferrin can also bind to receptors on bacterial surface, which induces death of Gram-negative bacteria due to a disruption in the cell wall and inhibits the formation of bacterial biofilms. Under inflammatory conditions, the levels of lactoferrin are increased.

### S100A12

S100A12 is also a protein of the S100 family that is predominately expressed and secreted by neutrophils. Human S100A12 contains 91 amino acids with a molecular weight of 10.4 kDa and the protein is stable for 7–10 days when stored at room temperature (42–44). Similar to calprotectin, S100A12 is able to bind calcium, iron and zinc. As a metal chelating agent, S100A12 also has antimicrobial activity (45–47). Furthermore, S100A12 has chemotactic characteristic that recruits mast cells and monocytes to the site of inflammation (48–50). S100A12 is able to bind a number of cellular receptors. Recent evidence suggest that S100A12 stimulate proinflammatory responses in monocytes *via* Toll-like receptor 4, leading to upregulated monocyte expression of proinflammatory cytokines including interleukin (IL)-1β, IL-6, and IL-8 (51). S100A12 is overexpressed in inflammatory conditions.

## CALPROTECTIN, LACTOFERRIN, AND S100A12 IN PREDICTING RELAPSE IN IBD

The gold standard of defining clinical remission or relapse relies on endoscopic mucosal healing and histological scoring of inflammation. Majority of the quiescent IBD patients have residual inflammation in the colonic mucosa, and when the degree of inflammation reaches a critical level, symptomatic relapse occurs (52). Various research groups have examined the use of fecal biomarkers as predictive markers for relapse and they are summarized in **Table 1**. Most of these studies assessed calprotectin and few examined lactoferrin and S100A12. Of the 31 studies listed in **Table 1**, 29 studies examined calprotectin, three studies examined lactoferrin and one study examined S100A12. Some of these studies have examined multiple fecal biomarkers.

The reported sensitivities, specificities and the cut-off values in different studies assessing fecal calprotectin as a biomarker in predicting relapse varied greatly. Of the 29 studies of calprotectin listed in **Table 1**, the sensitivities for predicting CD, UC, and IBD ranged from 28 to 100%, 31 to 100%, and 38 to 100%, respectively. The specificities for predicting CD, UC, and IBD ranged from 43 to 52%, 63 to 100%, and 69 to 100%, respectively. The cut-off values for CD, UC, and IBD varied from 106.5 to 462 μg/g, 120

**TABLE 1 |** Summary of studies investigating fecal biomarkers for the prediction of relapses in inflammatory bowel disease.

| References | Location | Age median or mean* (range) | Disease | N | Time interval | Optimal cut-off | Median/mean* | | *P*-value | Sensitivity/ specificity % | PPV/NPV % | Method |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Relapse | Non-relapse | | | | |
| **Calprotectin** | | | | | | | | | | | | |
| Buisson et al. (53) | US | 25.9* | CD | 112 | 1 yr | 100 μg/g | - | - | - | 76/86 | 77/85 | ELISA (Genova diagnostics) |
| | | | UC | 48 | | | | | | | | |
| Ferreiro-Iglesias et al. (54) | Spain | 44 (18–78) | CD | 71 | 4 mons | >300 μg/g | 477 μg/g | 65 μg/g | <0.005 | 100/80 | 78.3/100 | Lateral flow assay (Buhlmann) |
| | | | UC | 24 | | | | | | | | |
| Kittanakom et al. (55) | Canada | CD: 14.6 *(11–17)* UC: 14.1 *(11–17)* | IBD | 40 | - | 400 μg/g | - | - | - | 100/75.9 | 58.8/100 | ELISA (PhiCal) |
| | | | | | - | 800 μg/g | - | - | - | 100/72.4 | 55.6/100 | Fluorescence enzyme immunoassay (Phadia) |
| | | | | | - | 500 μg/g | - | - | - | 100/72.4 | 55.6/100 | ELISA (Buhlmann) |
| Diederen et al. (56) | Netherlands | *14.9 (all <18)* | IBD | 114 | 6 mons | 350 μg/g | 370 μg/g | 122 μg/g | 0.003 | 82/79 | 41/96 | - |
| Roblin et al. (57) | France | 35 | CD | 119 | 6 mons | >250 μg/g and TLI < 2 μg/mL | - | - | - | 94/84 | 73/97 | Lateral flow assay (Buhlmann) |
| Theede et al. (58) | Denmark | 39* | UC | 70 | 6 and 12 mons | 321 mg/kg | - | - | - | 46.7/85.5 | 46.7/85.5 | ELISA (Buhlmann) |
| Ferreiro-Iglesias et al. (59) | Spain | 46 (18–68) | IBD | 53 | 2 mons | 160 μg/g | 332 μg/g* | 110 μg/g* | <0.005 | 91.7/82.9 | 68.7/96.1 | Lateral flow assay (Buhlmann) |
| | | 41 (18–43) | CD | 33 | | 160 μg/g | 287 μg/g* | 94 μg/g* | <0.005 | 87.5/84.0 | 66.9/94.8 | |
| | | 51 (19–68) | UC | 20 | | 198 μg/g | 420 μg/g* | 136 μg/g* | <0.005 | 100/81.3 | 48.5/100 | |
| Ferreiro-Iglesias et al. (60) | Spain | 38 (24–64) | CD | 30 | 4 mons | 204 μg/g | 625 μg/g | 45 μg/g | <0.005 | 100/85.7 | 74.1/100 | Lateral flow assay (Buhlmann) |
| Delefortrie et al. (61) | Belgium | 43 | CD | 29 | 6 mons | 183.5 μg/g | 667 μg/g | 109 μg/g | <0.05 | 100/76.2 | 61/100 | Lateral flow assay (Buhlmann) |
| | | | | | | 124.5 μg/g | 339.5 μg/g | 71.4 μg/g | <0.05 | 87.5/66.66 | 50/93.5 | Chemiluminescent immunoassay (Liaison). Samples extracted with Liaison extraction device |
| | | | | | | 106.5 μg/g | 261.5 μg/g | 37.6 μg/g | <0.05 | 87.5/95.2 | 87.5/95 | Chemiluminescent immunoassay (Liaison). Samples extracted with weighing protocol |
| Mooiweer et al. (62) | Netherlands | 50 (19–71) | CD | 20 | 12 mons | 56 μg/g[&] | 284 μg/g | 37 μg/g | <0.01 | 64/100 | 20/100 | ELISA (Ridascreen) |
| | | | UC/IBD-U | 52 | | | | | | | | |
| Yamamoto et al. (63) | Japan | 35 (18–74) | UC | 80 | 40 wks | Elevated level ≥55 μg/g | 76.5 μg/g | 15.5 μg/g | <0.0001 | 88/80 | 66/94 | ELISA (Cell sciences) |
| Scaioli et al. (64) | Italy | 40 (16–89) | UC | 74 | 1 yr | 193 μg/g | 218 μg/g | 48 μg/g | <0.01 | 65/98 | 92/88 | ELISA (Calprest) |
| Yamamoto et al. (65) | Japan | 35.1* (20–75) | UC | 80 | 12 mons | 170 μg/g | 173.7 μg/g* | 135.5 μg/g* | 0.02 | 76/76 | -/- | ELISA (Cell sciences) |
| Jauregui-Amezaga et al. (66) | Spain | 46* | UC | 64 | 1 yr | 250 μg/g | 200 μg/g | 75 μg/g | 0.75 | 41/85 | -/80 | ELISA (Cerba internacional) |
| Naismith et al. (67) | UK | 47* (>18) | CD | 92 | 12 mons | 240 μg/g | 414 μg/g | 96 μg/g | 0.005 | 80.8/74.4 | 28/97 | ELISA (Buhlmann) |
| Vos et al. (68) | Belgium and Norway | 48* (19–79) | UC | 87 | 52 wks | 300 μg/g | 125 μg/g* | 27 μg/g* | <0.001 | 58.3/93.3 | -/- | ELISA (PhiCal) |
| | | | | | | Two consecutive measurements of >300 μg/g within 1 mon | | | | 61.5/100 | -/- | |
| Lasson et al. (69) | Sweden | 33 (18–74) | UC | 69 | 1 yr | 169 μg/g | 263 μg/g | 102 μg/g | 0.009 | 64.4/70.8 | 80.6/51.5 | ELISA (Buhlmann) |
| | | | | 67 | 2 yrs | 262 μg/g | 263 μg/g | 124 μg/g | <0.05 | 51.1/81.8 | 85.2/45.0 | |

*(Continued)*

**TABLE 1 |** Continued

| References | Location | Age median or mean* (range) | Disease | N | Time interval | Optimal cut-off | Median/mean* | | *P*-value | Sensitivity/ specificity % | PPV/NPV % | Method |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Relapse | Non-relapse | | | | |
| | | | | 67 | 3 yrs | 262 μg/g | 280 μg/g | 118 μg/g | 0.01 | 52.2/85.7 | 88.9/45.0 | |
| Meuwis et al. (70) | France and Belgium | 32 | CD | 79 | 28 mons | 250 μg/g | - | - | - | -/- | -/- | ELISA (PhiCal) |
| van Rheenen et al. (71) | Netherlands | *14.1* (<18)* | CD | 31 | 3 mons | 500 μg/g | - | - | - | 67/81 | -/- | ELISA (Calpro) |
| | | *13* (<18)* | UC | 31 | | | | | | | | |
| Louis et al. (72) | France and Belgium | 32 (>17) | CD | 115 | 1 yr | 300 μg/g | - | - | - | -/- | -/- | ELISA (PhiCal) |
| Laharie et al. (73) | France | 30.4 (15–69) | CD | 65 | 14 wks | 130 μg/g | 200 μg/g | 150 μg/g | Ns | 61/48 | -/- | ELISA (Buhlmann) |
| | | | | | | 250 μg/g | | | | 43/57 | -/- | |
| García-Sánchez et al. (74) | Spain | 36.9* | CD | 66 | 1 yr | 200 μg/g | 524 μg/g | 123 μg/g | <0.01 | 80/65 | 46/88 | ELISA (Calprest) |
| | | 40.4* | UC | 69 | | 120 μg/g | 298 μg/g | 105 μg/g | <0.01 | 81/63 | 49/88 | |
| Kallel et al. (75) | Tunisia | 33 (15–66) | CD | 53 | 12 mons | 340 μg/g | 380.5 μg/g | 155 μg/g | <0.001 | 80/90.7 | -/- | ELISA (Calprest) |
| Sipponen et al. (76) | Finland | *12.9 (2–17)* | IBD | 72 | 12 mons | 108.5 μg/g | 409 μg/g | 282 μg/g | 0.44 | 38/72 | -/- | ELISA (PhiCal) |
| | | | | | | 100 μg/g | | | | -/- | 39.6/75 | |
| Gisbert et al. (77) | Spain | 43* | IBD | 163 | 12 mons | 150 μg/g | 239 μg/g | 136 μg/g | <0.001 | 69/69 | 30/92 | ELISA (PhiCal) |
| | | | CD | 89 | | 150 μg/g | 266 μg/g | 145 μg/g | 0.002 | 28/93 | -/- | |
| | | | UC | 74 | | 150 μg/g | 213 μg/g | 126 μg/g | 0.03 | 31/91 | -/- | |
| D'incà et al. (78) | Italy | - | IBD | 162 | 1 yr | 130 μg/g | - | - | - | 68/67 | 52/79 | ELISA (Calprest) |
| | | 43 (18–77) | CD | 65 | | 130 μg/g | 207 μg/g | 88 μg/g | 0.055 | 65/62 | 44/80 | |
| | | 46 (15–80) | UC | 97 | | 130 μg/g | 190 μg/g | 49 μg/g | 0.02 | 70/70 | 60/79 | |
| Diamanti et al. (79) | Italy | - | IBD | 73 | 3 yrs | 275 μg/g | - | - | - | 97/85 | 85/97 | ELISA (Calprest) |
| | | 16 (1.5–18) | CD | 32 | | 462 μg/g | - | - | - | 100/71 | 78/100 | |
| | | 12 (6–18) | UC | 41 | | 275 μg/g | - | - | - | 94/95 | 94/95 | |
| Costa et al. (80) | Italy | 35.7* | CD | 38 | 12 mons | 150 μg/g | 220.1 μg/g | 220.5 μg/g | 0.395 | 87/43 | 50/83 | ELISA (Calprest) |
| | | 41.2* | UC | 41 | | 150 μg/g | 220.6 μg/g | 67 μg/g | <0.0001 | 89/82 | 81/90 | |
| Tibble et al. (81) | UK | 33 | CD | 43 | 12 mons | 100 μg/g | 244 μg/g | 84 μg/g | <0.0001 | 90/83 | -/- | ELISA (In-house) |
| | | 49 | UC | 37 | | | 246 μg/g | 58 μg/g | <0.0001 | | | |
| **Lactoferrin** | | | | | | | | | | | | |
| Yamamoto et al. (65) | Japan | 35.1* (20–75) | UC | 80 | 12 mons | 140 μg/g | 161.5 μg/g* | 130.7 μg/g* | 0.03 | 67/68 | -/- | Colloidal gold agglutination assay (Alfresa Pharma Corp.) |
| Gisbert et al. (77) | Spain | 43* | IBD | 163 | 12 mons | - | 62%^ | 35%^ | <0.05 | 62/65 | 25/90 | ELISA (TechLab) |
| Walker et al. (82) | US | 13.4* (2–21) | IBD | 55 | 2 mons | - | 845 μg/g* | 190 μg/g* | 0.003 | -/- | -/- | ELISA (TechLab) |
| **S100A12** | | | | | | | | | | | | |
| Däbritz et al. (83) | Germany | 37.4 (3.5–74.6) | IBD | 181 | Predicting relapse 8–12 wks earlier | 0.43 μg/g | - | - | - | 70/83 | -/- | ELISA (In-house) |
| | | | CD | 61 | | | | | | | | |
| | | | UC | 120 | | | | | | | | |

*Time interval: cut-off values for predicting relapse within a specified period. Concentrations of fecal markers in relapsers and non-relapsers are expressed as mean (*) or median. Age of patients are presented as mean (*) or median. Studies on pediatric patients are in italic. &Cut-off value for prediction of absence of relapse. ^Positive lactoferrin test was more frequent in relapsing than in non-relapsing patients. TLI, trough level of infliximab; IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; IBD-U, inflammatory bowel disease-unclassified; PPV, positive predictive value; NPV, negative predictive value; Ns, Not statistically significant; Wk, week. Mon, month; Yr, year. -, information not available.*

to 321 μg/g, and 100 to 800 μg/g, respectively (**Table 1**). Twenty-one studies compared the levels of calprotectin of relapsed and non-relapsed patients, of which 18 studies (85.7%) found that the levels of fecal calprotectin in relapsed patients were significantly higher, indicating that the levels of fecal calprotectin reflect the levels of inflammation in the intestinal mucosal tissues. A meta-analysis by Mao et al. analyzed combined data from six studies in **Table 1**, comprising a total of 672 adult IBD patients (318 UC and 354 CD) (84). They reported that the pooled sensitivity and specificity of fecal calprotectin in predicting relapse in quiescent IBD to be 78 and 73%, respectively (84). However, this meta-analysis did not state the cut-off values of the pooled data, the cut-off values in the six original studies varied from 100 to 340 μg (74, 75, 77, 78, 80, 81).

The time intervals observed in studies examining fecal calprotectin in **Table 1** were from 2 months to 3 years. More than 50% of these studies observed patients for a time interval of 1 year or above. The remaining studies observed patients for shorter terms such as 2, 4, or 6 months. There were no specific traits associated with observation term intervals in respect of cut-off values, sensitivities and specificities.

Most of the studies on fecal calprotectin in predicting IBD relapse were from Europe. Of the 29 studies examining calprotectin in **Table 1**, 23 were from Europe, two from North America, two from UK, one from Africa, and there were only two studies from Asian populations, both of which were from the same research group in Japan (63, 65).

Enzyme-linked immunosorbent assay (ELISA) was used in quantifying the levels of calprotectin in stools in 23 out of the 29 studies in **Table 1**. The remaining studies used other methods such as Lateral Flow Assay, chemiluminescent immunoassay, colloidal gold agglutination assay, and fluorescence enzyme immunoassay. The ELISA kits used by these studies were from eight different manufacturers and one study used in-house ELISA. The studies by Kittanakom et al. and Delefortrie et al. have compared different methods in quantifying fecal calprotectin for predicting relapse of IBD and CD, respectively (55, 61). Kittanakom et al. (55) reported the cut-off values of 400 and 500 μg/g when using ELISA kits supplied by two different manufacturers, however the cut-off was of a much higher value (800 μg/g) when fluorescence enzyme immunoassay was used. Delefortrie et al. showed cut-off values of 124.5 and 106.5 μg/g when the same chemiluminescent immunoassay was performed with different sample extraction methods, but the cut-off was much higher (183.5 μg/g) when Lateral Flow Assay was used (61). These results showed that variations can be introduced due to different detection methods used in various studies.

To date, only three studies have investigated the use of fecal lactoferrin in predicting relapse in IBD, of which only the study from Japan was able to identify an optimal cut-off value (65). However, this study did not find a statistically significant difference of fecal lactoferrin levels between relapsed and non-relapsed patients. The remaining two studies from Spain and US, although have found a significant difference of fecal lactoferrin levels between relapsed and non-relapsed patients, but they did not report optimal cut-off values for prediction of relapse (77, 82). Only one study had examined the use of S100A12 for predicting relapse in IBD. By using an in-house ELISA, Däbritz et al. showed that a cut-off value of 0.43 μg/g was able to predict relapse 8–12 weeks earlier with sensitivity and specificity being 70 and 83% respectively.

## CALPROTECTIN, LACTOFERRIN, AND S100A12 IN PREDICTING POST-OPERATIVE RECURRENCE IN CD

A non-invasive biomarker with predictive potential to identify patients without recurrence would be desirable to avoid post-operative endoscopies. In recent years, the use of fecal calprotectin in predicting post-operative recurrence in CD has been evaluated by various studies. Limited studies have also examined lactoferrin and S100A12. These studies are listed in **Table 2**.

These studies again reported varied sensitivities, specificities and cut-off values. Studies examining calprotectin reported sensitivities between 46 and 95% and specificities between 45.9 and 97%. The cut-off values also ranged from 60 to 274 μg/g. In the study by Lasson et al. (95) three different cut-off values (100, 200, and 250 μg/g) were assessed, and the corresponding sensitivities were 85, 54, and 46%, respectively. Nevertheless, this study did not detect a significantly different levels of fecal calprotectin in patients with and without post-operative recurrence while the other studies did (**Table 2**). A meta-analysis performed by Tham et al. on examining the use of fecal calprotectin for detection of post-operative endoscopic recurrence in CD showed that a significant threshold effect was observed for fecal calprotectin values of 50, 100, 150, and 200 μg/g; while the optimal diagnostic accuracy was obtained for fecal calprotectin value of 150 μg/g, with a pooled sensitivity and specificity being 70 and 69%, respectively (100).

Four studies have examined lactoferrin, which all showed significantly different fecal lactoferrin levels in patients with and without post-operative recurrence. However, the cut-off values ranged from 3.4 to 140 μg/g (**Table 2**). Only one study has examined S100A12 in pediatric patients using an in-house ELISA, which reported a sensitivity of 90% and specificity of 12%, and no significant difference in fecal S100A12 levels was observed in patients with and without post-operative recurrence (**Table 2**).

## DISCUSSION AND SUGGESTIONS

Studies from diverse geographical regions of the world, mainly from Europe, have examined the use of fecal biomarkers in predicting disease relapse and post-operative recurrence in patients with IBD. Calprotectin is the most studied marker, and several studies also examined lactoferrin and few have investigated S100A12. The consistent information from these studies is that the level of calprotectin increases along with the intestinal mucosal inflammation, which is consistent with the biological functions of this protein. However, whether it can be used to predict disease relapse and post-operative recurrence is inconclusive from the current studies.

**TABLE 2 |** Summary of studies investigating fecal biomarkers for the prediction of post-operative recurrence in patients with Crohn's disease.

| References | Location | Age median or mean* (range) | N | Time interval | Optimal cut-off | Median/mean* | | *P*-value | Sensitivity/ specificity % | PPV/NPV % | Method |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | With POR | Without POR | | | | |
| **Calprotectin** | | | | | | | | | | | |
| Cerrillo et al. (85) | Spain | 40.7* (18–74) | 61 | 24 mons | 160 μg/g | - | - | - | 85/70 | 26/98 | ELISA (Calprest) |
| Baillet et al. (86) | France | 34.9* | 30 | 1 yr | 100 μg/g | 354.8 μg/g* | 114 μg/g* | 0.0075 | 67/93 | 89/77 | Lateral Flow Assay (Buhlmann) |
| Verdejo et al. (87) | Spain | 46.2 | 86 | < 1 mon | 62 μg/g | 172.5 μg/g | 75 μg/g | 0.003 | 85.7/45.9 | 67.7/70.8 | Lateral flow assay (Buhlmann) |
| Garcia-Planella et al. (88) | Spain | 40 | 119 | ∼24 mons | 100 μg/g and 5 mg/L of CRP | 205 μg/g* | 94 μg/g* | < 0.0001 | 82/53 | 54/81 | ELISA (Calprest) |
| Wright et al. (89) | Australia and New Zealand | 36 | 135 | 18 mons | 135 μg/g | 275 μg/g | 72 μg/g | <0.001 | 87/66 | 56/91 | ELISA (Buhlmann) |
| Lopes et al. (90) | Portugal | 45* | 99 | 25 mons[#] | 100 μg/g | 196.5 μg/g | 42.1 μg/g | <0.001 | 74/75 | 61/91 | Fluorescence enzyme immunoassay (Thermo Fisher Scientifi) |
| Hukkinen et al. (91) | Finland | *13.6 (≤18)* | 22 | 5.7 yrs[#] | 139 μg/g | - | - | - | 73/64 | 68/70 | ELISA (PhiCal) |
| | | | | | Increase of 79 μg/g | - | - | - | 73/71 | 73/71 | |
| Herranz Bachiller et al. (92) | Spain | 48.6* | 97 | - | 60 μg/g | 192.45 μg/g | 94.39 μg/g | 0.0001 | 88/58 | 51.73/83.9 | ELISA (Calprest) |
| Yamamoto et al. (93) | Japan | 32 (21–48) | 30 | 24 mons | 140 μg/g | 199 μg/g | 82.5 μg/g | 0.002 | 75/91 | 75/91 | Colloidal gold agglutination assay (Alfresa Pharma Corp.) |
| Boschetti et al. (94) | France | 39.3* (18–70) | 86 | 18 mons | 100 μg/g | 473 μg/g* | 115 μg/g* | <0.0001 | 95/54 | 69/93 | ELISA (Buhlmann) |
| Lasson et al. (95) | Sweden | 36 (17–63) | 30 | 1 yr | 100 μg/g | 227 μg/g | 189 μg/g | 0.25 | 85/35 | 50/75 | ELISA (Buhlmann) |
| | | | | | 200 μg/g | | | | 54/53 | 47/60 | |
| | | | | | 250 μg/g | | | | 46/53 | 43/56 | |
| ^Yamamoto et al. (96) | Japan | 32* | 20 | 12 mons | 140 μg/g | 229.5 μg/g* | 102.3 μg/g* | 0.005 | 70/70 | 70/70 | ELISA (Cell sciences) |
| Lobatón et al. (97) | Spain | 40 | 115 | - | 272 μg/g | 788.5 μg/g* | 100 μg/g* | <0.001 | 79/97 | 98/76 | Lateral flow assay (Buhlmann) |
| | | | | | 274 μg/g | 1211.9 μg/g* | 101.8 μg/g* | <0.001 | 77/97 | 98/75 | ELISA (Buhlmann) |
| Yamamoto et al. (98) | Japan | - | 20 | 12 mons | 170 μg/g | - | - | - | 83/93 | -/- | ELISA (Manufacturer not specified) |
| Orlando et al. (99) | Italy | 38 | 50 | 3 mons | 200 mg/L | - | - | - | 63/75 | 70/68 | ELISA (Calprest) |
| **Lactoferrin** | | | | | | | | | | | |
| Wright et al. (89) | Australia and New Zealand | 36 | 135 | 18 mons | 3.4 μg/g | 5.7 μg/g | 1.6 μg/g | 0.007 | 70/68 | 53/81 | ELISA (TechLab) |
| Lopes et al. (90) | Portugal | 45* | 99 | 25 mons[#] | 7.25 μg/g | 23.27 μg/g | 2 μg/g | <0.001 | 74/68 | 61/91 | ELISA (TechLab) |
| ^Yamamoto et al. (96) | Japan | 32* | 20 | 12 mons | 125 μg/g | 161.4 μg/g* | 83.7 μg/g* | 0.02 | 70/60 | 64/67 | Colloidal gold agglutination assay (Alfresa Pharma Corp.) |
| Yamamoto et al. (98) | Japan | - | 20 | 12 mons | 140 μg/g | - | - | - | 67/71 | -/- | Colloidal gold agglutination assay (Manufacturer not specified) |
| **S100A12** | | | | | | | | | | | |
| Wright et al. (89) | Australia and New Zealand | 36 | 135 | 18 mons | 10.5 μg/g | 2.0 μg/g | 0.8 μg/g | 0.188 | 91/12 | 35/71 | ELISA (In-house) |

*Majority of the studies have examined the use of fecal biomarkers for prediction of endoscopic recurrence, except the study performed by Yamamoto et al. (96) (^) which was on clinical recurrence. Time-interval: median (#) or maximum follow up period. Concentrations of fecal markers in patients with and without POR are expressed as mean (*) or median. Age of patients are presented as mean (*) or median. Studies on pediatric patients are in italic. IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; POR, post-operative recurrence; CRP, C-reactive protein; PPV, positive predictive value; NPV, negative predictive value; -, information not available.*

Several factors from these studies have contributed to the uncertainty of using fecal biomarkers in predicting disease relapse and post-operative recurrence. Firstly, the cut-off values used in these studies varied remarkably, making it difficult to draw reliable conclusion. Secondly, different detection methods were used, which may produce inconsistent results. Thirdly, the time intervals observed in different studies were random, which again makes it difficult to compare the results between studies. Further studies therefore are warranted to determine whether these fecal biomarkers are reliable predicative markers in the management of IBD. We suggest the following strategies.

## Use Fecal Biomarkers as Markers for Personalized Management in IBD

The degree of mucosal inflammation, the level of inflammation that can cause clinical symptoms and the response to different therapeutic agents in individual patients with IBD vary greatly. Given this, fecal biomarkers are perhaps best used in personalized management. Fecal samples can be collected at different stages of IBD in individual patients and the levels of fecal biomarkers can then be measured. Changes in levels of fecal biomarkers can be used to monitor and predict disease progress in individual patients, which may lead to an enhanced patient management.

## Coordinated Multi-Center Analysis

Coordinated multi-center studies from different geographic regions are needed in order to determine whether fecal biomarkers can be used as reliable predictive markers for patients with IBD globally. Samples in different centers should be collected at multiple but consistently defined timepoints. Given that ELISA was the most commonly used quantification method in previous studies, perhaps this method should still be used. However, ELISA kits provided by different manufacturers should be compared. Consistently defined cut-off values should be used for data analysis. This approach is more likely to produce conclusive data regarding whether fecal biomarkers can be used as cohort markers to predict disease relapse in patients with IBD.

## AUTHOR CONTRIBUTIONS

FL played a major role in writing the manuscript. LZhu and LZha conceived the project. LZhu, LZha, SL, and SR provided critical feedback and helped in editing the manuscript. All authors have approved the final version of the manuscript.

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REVIEW
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# How to Optimize Treatment With Ustekinumab in Inflammatory Bowel Disease: Lessons Learned From Clinical Trials and Real-World Data

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Ustekinumab is a fully human IgG1 monoclonal antibody that has been approved for the treatment of moderate to severe Crohn's disease, and more recently moderate to severe ulcerative colitis. It binds with high affinity to the p40 subunit of human interleukin-12 and 23. This mechanism of action prevents the bioactivity of both interleukins, thus precluding their interaction with the cell surface receptor protein. The pivotal clinical trials (UNITI-1, UNITI-2 and IM-UNITI) demonstrated its clinical efficacy and safety, in naïve patients and also in those previously exposed to immunosuppressants and/or biologics. There is now an extensive experience with its use worldwide, corroborating its favorable profile even in patients with refractory disease. However, the number of medical treatment options available in inflammatory bowel disease are still limited. Hence, we should prioritize the treatments that have a greater probability of response in an individual patient. Our aim was to review and summarize all the available literature regarding the potential predictors of response to ustekinumab that can increase the success rate with this therapy in clinical practice.

**Keywords: Crohn's disease, predictive factors, ulcerative colitis, ustekinumab, biological therapy**

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## INTRODUCTION: DO WE NEED PREDICTIVE FACTORS IN CROHN'S DISEASE?

Inflammatory bowel diseases (IBD)—a term including both ulcerative colitis (UC) and Crohn's disease (CD)— are two chronic, disabling conditions causing an uncontrolled inflammatory process in the gastrointestinal tract, with a relapsing and remitting course (1, 2). It is considered that IBD appears in genetically predisposed subjects after the interaction with diverse environmental factors, therefore it is described as a complex disease where there is an interaction between multiple factors that has not been fully elucidated so far. The interaction between luminal antigens and the mucosal immune system seems to be crucial and mediated through an increased intestinal permeability, at least during the early stages of the disease (3). This interaction may trigger an abnormal and uncontrolled inflammatory response in susceptible individuals, leading to progressive bowel damage and symptomatic disease (4). Due to our increased knowledge of the immunological disturbances observed in these patients, new treatment options have been developed in recent years (5). Over the past 20 years, tumor necrosis factor (TNF)-antagonists have

transformed the medical management of IBD due to their ability to induce a complete control of symptoms, induce mucosal healing in a significant proportion of patients, and reduce the long-term requirements of surgery and hospitalization (6–8). Despite their impact in the paradigm of disease control, many challenges remain: around two-thirds of IBD patients demonstrate short-term clinical response to anti-TNF therapy and ∼40% of patients who initially improve subsequently lose response (9, 10). These data about the efficacy should be added to the potential adverse events associated to anti-TNF therapy, that underlines the urgent need of alternative therapeutic options targeting new disease pathways for refractory patients. In recent years, the experience with new biologics blocking leukocyte migration mediated through integrins—vedolizumab—or the immune pathways regulated by interleukin (IL)-12/23—ustekinumab (UST)—have increased the chance to obtain better disease control and improve quality of life. Hence, these new therapeutic options imply a greater probability of inducing disease remission in difficult-to-treat patients. Despite this important progress, the selection of first-line biologic therapy seems to be crucial, as it has consistently been shown that there is a stepwise reduced response rate with each subsequent biologic therapy (11, 12). Contrary to the aforementioned steps toward disease control, many regulatory authorities have approved UST only after anti-TNF failure, which significantly reduces the overall efficacy of the drug.

Taking into consideration that many new drugs involving other mechanisms of action are still to come, personalized medicine will gain importance in the near future (13, 14). This is a new concept in our field, but many new findings associated to the ability to predict response, relapse and even adverse events by using clinical data and biomarkers will allow us to choose the best drug, for an individual patient at the right time (15, 16). Several factors have been linked to the response to TNF blockade in IBD, including clinical factors, pharmacokinetics, biochemical markers, pharmacogenomics, microbiome signatures, metabolic compounds and mucosal markers (17, 18). While there are significant advances allowing a better identification of patients more likely to respond to anti-TNFs, including also a more profound understanding of its pharmacokinetics, few studies have investigated predictive factors of therapeutic efficacy to UST that may improve the probability of response and long-term benefit. This review will discuss all the possible factors and biomarkers associated to the initial and long-term response to UST in CD.

# THE UNMET NEEDS WITH USTEKINUMAB: EFFICACY IN RANDOMIZED CLINICAL TRIALS AND OBSERVATIONAL COHORTS

## Clinical Trials

UST is a fully human immunoglobulin G1 monoclonal antibody that blocks the p40 subunit of IL-12 and IL-23, precluding cytokine-mediated cellular activation. IL-23 promotes the differentiation of naïve T cells into Th17 phenotype, whereas IL-12 regulates the Th1 polarization. The downstream effect of the IL-12/23 blockade is the neutralization of human IL-12 and IL-23-mediated cell signaling, cell activation, and cytokine production involved in the pathogenesis of CD (19). UST has demonstrated its efficacy inducing response and remission in CD patients in randomized clinical trials and also in real-life studies. The UNITI study, a phase III multicenter, double-blind, placebo-controlled randomized clinical trial included an induction (UNITI-1 and 2) and a maintenance phase (IM-UNITI) (20). Patients started UST after primary non-response, loss of response or intolerance to anti-TNF agents (UNITI-1), but also failure or severe adverse events during conventional therapy with immunosuppressants or steroids (UNITI-2). The primary aim—defined as a reduction in the Crohn's Disease Activity Index [CDAI] $\leq$100 or CDAI<150 at week 6—was achieved by 22, 34, and 34% in the placebo, UST 130 mg and UST 6 mg/kg groups, respectively, in the UNITI-1 trial (714 patients) and 29, 52, and 56% in the UNITI-2 (628 patients). In the IM-UNITI study, including 397 responders during the induction, the primary endpoint—clinical remission (CDAI < 150) at week 44—was achieved by 36, 49, and 53% in the placebo, UST 90 mg q12w and 90 mg q8w arms, respectively. Long-term data from the IM-UNITI study show that 62 and 70% of patients in the q12w and q8w arms were in clinical remission at week 152, respectively (21). A treat-to-target approach based on endoscopic findings at week 16 has been evaluated with UST in the STARDUST trial (NCT03107793). This is the first randomized trial evaluating the efficacy of UST under a dose adjustment strategy based on biomarkers (fecal calprotectin and C-reactive protein) and symptoms (CDAI), compared with a standard, clinically-driven approach. Preliminary results have been presented at United European Gastroenterology Week 2020, were the treat-to-target strategy showed a numerically higher endoscopic response, but there were no clear differences between both treatment arms.

## Real-World Data

Additionally, several open-label observational cohort studies have also assessed and confirmed the efficacy and safety of UST for CD in clinical practice (22–41). Although these real-world studies have some obvious advantages over randomized clinical trials (as they reflect real clinical practice and in clinical scenarios where patients will not fulfill the rigorous inclusion criteria of clinical trials), their limitations need to be taken into account. Real-life studies are limited usually by smaller sample size, more limited follow-up, and are prone to bias due to their outcomes and frequent retrospective design. Nonetheless, real-world studies are an important source of information in addition to the results of clinical trials. Based on these assumptions, a recent multicenter retrospective Spanish study including 407 patients observed that 57 and 64% of patients with active disease starting UST achieved clinical remission at weeks 26 and 52, respectively (30, 31). Fecal calprotectin normalization was observed in 44 and 54% of patients at weeks 26 and 52, while C-reactive protein returned to normal in 36 and 37% of patients at the same time points, respectively. Biemans et al. recently reported results from the nationwide prospective observational Dutch cohort (40). This study included 221 CD patients, where corticosteroid-free clinical remission rates at weeks 24 and 52

were 38 and 37%, respectively. In conclusion, clinical trials and real-world studies have demonstrated that UST is safe and effective for the induction and maintenance of response and remission of refractory CD patients, but a significant proportion still fails to obtain the strict endpoints that should be regarded as our goals as steroid-free clinical remission and mucosal healing. Therefore, we will discuss the clinical factors and biomarkers that have been associated with a higher probability of clinical benefit with UST. As the IBD drug pipeline is still limited, the identification of predictive factors should be carefully considered as it may help us to enhance the probability of achieving disease remission.

## PREDICTIVE FACTORS ASSOCIATED TO USTEKINUMAB RESPONSE

### Clinical Factors

There are many aspects that influence the response to UST, but there have been no definite characteristics associated to a certain patient profile that may show a better response so far. However, some patient or disease-related aspects may help us to decide that anti-interleukin therapy is the best option in an individual patient (**Table 1**). Age is one of the most important factors, and it may be expected to plays an important role in the prognosis and treatment outcomes of CD. Nevertheless, some age subgroups of patients are usually underrepresented in most of the clinical trials and observational cohorts. Previous descriptions of the management of elderly IBD patients show that they usually receive steroids, immunosuppressants or anti-TNF agents less frequently (45). Though, regarding anti-TNF therapy there is no clear evidence about the influence of age on the response to these drugs (18). Data with UST is still limited, but in the 6 mg/kg and 130 mg treatment arm in the UNITI-1 trial, younger patients showed increased rates of clinical response at week 6 compared to placebo [odds ratio (OR), 2.4; confidence interval (CI) 95%, 1.3–4.3 and OR, 2.7; CI 95%, 1.5–4.9, respectively] (20). Only one additional retrospective case series has demonstrated that age influences treatment outcomes with UST (37). Here, Casas Deza et al. observed that older age was associated with reduced clinical response rates after 16 weeks of therapy. However, there are no consistent data across the remaining studies suggesting a different efficacy across different age groups (18). Hence, older patients may show a reduced clinical response to UST at least in the short-term, but data about treatment persistence and more robust outcomes are needed to confirm this relationship.

Important sociodemographic aspects, including gender and ethnicity are also attractive patient-related characteristics to consider. Again, subjects randomized to receive 6 mg/kg and 130 mg in the UNITI trials showed an improved clinical response rates between females and white patients compared to other races grouped together (20). Two observational cohorts support this finding, with reduced rates of combined response and remission rates after 24 weeks (35) and 48 weeks (39) in male patients, whilst the remaining short and long-term studies have not replicated this observation.

Low body weight has been associated with an improved response to anti-TNF therapy (46, 47), although controversial results have also been reported (48), and it is expected to be secondary to complex disease-related mechanisms that can influence pharmacokinetics. The currently approved loading dose of UST consists on a weight-based infusion of 260, 390, or 520 mg in patients 55, 56–85, or >85 kg, respectively. During the induction period with the initially approved dosing strategy, patients <60 kg of body weight receiving 90 mg subcutaneous UST showed a similar trend toward improved clinical response rates at week 8 (49). Consistent results had been observed in the UNITI program, were subjects in both treatment arms-−6 mg/kg and 130 mg iv—with low body weight showed improved clinical response rates in the short-term (20). Recent results from the Dutch IBD cohort have confirmed this trend, as body mass index was inversely correlated with the corticosteroid-free clinical remission at week 52 (40). Patient populations across different countries can have important differences, but body weight is a readily available information that could be easily implemented in clinical practice.

### Disease-Related Factors

Some factors associated with the characteristic of the disease should be also considered when starting UST therapy. Disease extension is one of the main items included in the Montreal classification and it defines one of the most important characteristics of the disease (50). Thus, it is of great importance to evaluate if it is associated with the response to certain immunosuppressive or biologic agents. The presence of lesions in the ileum and colon was shown to be associated with improved clinical response rates in those patients receiving 130 mg or 6 mg/kg UST in the UNITI trial, compared to placebo (OR, 2.8; CI 95%, 1.7–4.7, and OR, 5.0; CI 95%, 2.8–8.9, respectively) (20). In a Canadian retrospective cohort, Ma et al. described improved steroid-free clinical response and remission rates in ileocolonic CD (OR, 2.41; 95% CI, 1.01–5.79) (25). Further analysis from the same group were in line with their previous findings, as ileocolonic disease was associated with lower rates of loss of response during follow-up (OR, 0.26; 95% CI, 0.1–0.68) (26). Favorable results have been observed also when the disease is limited to the colon (26, 32). In contrast, the recent experience reported including 407 patients from Spain showed opposite results, as ileocolonic and colonic disease extension were associated with lower clinical response rates at week 26 (OR, 0.56 95% CI, 0.32–0.96, and OR, 0.34 95% CI, 0.16–0.69, respectively) (31).

Another important aspect that can significantly influence the response to biologics is the presence of penetrating or stricturing complications. It would be expected that patients who have shown a progression of the disease to a B2/B3 phenotype will have established and irreversible bowel damage that will be more difficult to control with medical therapy (51). In the UNITI-1 and UNITI-2 cohorts, 9–12% and 8–12% of patients had bowel strictures at baseline, while 18–20% and 15–16% had active fistulas (but there is no data available about type or location of the fistulas), respectively (20). No *post-hoc* analysis are available from these subgroups, hence data can be obtained only from observational studies. Both analysis performed in the Canadian cohort demonstrated that UST was less effective when stricturing complications have

**TABLE 1 |** Predictive factors of response in observational studies in patients with Crohn's disease.

| References | Study design | UST dosing | Clinical scenario | No patients | Endpoint | Predictive factors | |
|---|---|---|---|---|---|---|---|
| | | | | | | Positive association | Inverse association |
| Kopylov et al. (22) | Retrospective | Sc | CD refractory to at least one anti-TNF | 38 | Clinical response | - | - |
| Wils et al. (24) | Retrospective | Sc | CD refractory to immunosuppressants and anti-TNF | 122 | Clinical benefit at 3 months | Concomitant IM (OR 5.43; 95% CI 1.14–25.77) | - |
| Khorrami et al. (23) | Retrospective | Sc | CD refractory or intolerant to at least one anti-TNF | 116 | Clinical benefit | Previous intestinal resection (OR 2.09; 95% CI 1.16–3.79) | Initial response (OR 0.16; 95% CI 0.09–0.31) Previous use ≥2 IM (OR 0.5; 95% CI 0.28-0.88) |
| Harris et al. (28) | Retrospective | Sc | Complicated CD refractory to anti-TNF | 45 | Clinical response | - | - |
| Ma et al. (25) | Retrospective | Sc (89%) Iv (11%) | CD failing anti-TNF therapy | 167 | Steroid-free clinical response and remission | **Clinical response at 6 months** Ileocolonic disease (OR 2.41; 95% CI 1.01–5.79) | **Clinical response at 6 months** Harvey-Bradshaw index ≥ 7 (OR 0.26; 95% 0.11–0.61) Stricturing disease (OR 0.29; 95% 0.12–0.72) Immunomodulators at induction (OR 0.37; 95% CI 0.15–0.89) |
| Ma et al. (26) | Retrospective | Sc (88%) Iv (12%) | Primary clinical steroid-free response to UST | 104 | Loss of response among primary responders to UST | Harvey-Bradshaw index ≥ 7 (OR 4.63; 95% 1.64–13.11) Stricturing phenotype (OR 2.77; 95% 1.1–7.01) | Concomitant IM (OR 0.41; 95% 0.17–0.97) Colonic disease (OR 0.33; 95% 0.11–0.98) Ileocolonic (OR 0.26;95% 0.1–0.68) |
| Greenup et al. (27) | Retrospective | Sc | Real-world experience | 73 | Symptomatic response at 3, 3–12 and >12 months | Type of anti-TNF non-response: Primary non-response vs. secondary loss of response or intolerance (OR 17.33; 95% CI 2.34–128.47, and OR 26.56; 95% CI 3.46–203.62, respectively) | - |
| Wils et al. (38) | Retrospective | Sc | Real-world experience | 88 | Failure-free persistence in initial responders | - | - |
| Iborra et al. (30) | Retrospective | Iv induction | Luminal CD refractory to conventional therapy | 305 | Clinical remission at week 14 | - | No of previous anti-TNF (OR 0.67; 95% CI 0.44–0.95) Endoscopic severity (OR 0.08; 95% CI 0.01–0.37) Intolerance to last anti-TNF vs. primary or secondary failure (OR 0.66; 95% CI 1.13–6.30) |
| Iborra et al. (31) | Retrospective | Iv induction | Moderate-severe CD and no response or insufficient response to conventional therapy | 407 | Remission and clinical remission at week 26 and 52 | **Clinical remission at week 26** Response at week 14 (OR 9.90 95% CI 4.91–20.86) **Clinical remission at week 52** Response at week 14 (OR 8.45; 95% CI 3.97–18.8) | **Clinical remission at week 26** No of previous anti-TNF (OR 0.53; 95% CI 0.37–0.75) Colonic (OR 0.34; 95% CI 0.16–0.69) Ileocolonic (OR 0.56; 95% CI 0.32–0.96) **Clinical remission at week 52** No of previous anti-TNF (OR 0.52; 95% CI 0.35–0.78) Severe endoscopic activity (OR 0.35; 95% CI 0.16–0.71) |

*(Continued)*

**TABLE 1 |** Continued

| References | Study design | UST dosing | Clinical scenario | No patients | Endpoint | Predictive factors | |
|---|---|---|---|---|---|---|---|
| | | | | | | **Positive association** | **Inverse association** |
| Harris et al. (29) | Retrospective | Iv induction | Clinically active CD | 84 | Clinical response and drug persistence | - | - |
| Murate et al. (33) | Prospective | Iv induction | Moderate-severe CD | 22 | Clinical response at 24 weeks | Higher TNF-$\alpha$ concentration (cut-off 19.58 pg/ml; AUROC 0.819) | Lower SES-CD at baseline (cut-off <13; AUROC = 0.757) |
| Liefferinckx et al. (32) | Prospective | Iv induction | CD refractory to anti-TNF therapy | 152 | Clinical response | **Clinical response** Colonic (OR 3.5; 95% CI 1.34–9.41) | **Clinical remission** Body mass index <18 (OR 0.28; 95% CI 0.09–0.87) |
| Bar-Gil et al. (34) | Prospective | Iv induction | Active CD | 106 | Clinical response at week 24 | - | - |
| Bennet et al. (42) | Prospective | Sc (95%) Iv (5%) | Moderate-severe CD refractory to anti-TNF and/or vedolizumab | 96 | C-reactive protein, clinical activity and endoscopy | - | - |
| Saldaña Duenas et al. (36) | Prospective | Iv induction | Real-world experience in refractory CD | 61 | Clinical response and remission at week 16, 24, and 52 | - | - |
| Casas Deza et al. (37) | Retrospective | Iv (83%) Sc (17%) | Real-world experience in refractory CD | 69 | Clinical disease activity at week 16 | **Clinical response at week 16** Reason to stop prior biologic, adverse events (OR 96; CI 97.5% 10.15–1,273) or secondary loss of response (OR 7.07; 97.5% CI 1.22–48.02) | **Clinical response at week 16** Age (OR 0.95; 97.5% CI 0.90–0.99) Smoking habits (OR 0.19; 97.5% CI 0.04–0.78) |
| Hoffmann et al. (35) | Retrospective | Iv | Real-world experience in refractory CD | 68 | Steroid-free clinical remission or response at week 24 | **-** | **Steroid free-clinical response** Male (OR 0.11; 95% CI 0.02–0.61) Steroids at baseline (OR 0.071; 95% CI 0.011–0.464) Extraintestinal manifestations (OR 0.119; 95% CI 0.022–0.636) |
| Kubesch et al. (39) | Retrospective | Iv induction | Real-world experience | 106 | Clinical and biochemical remission at week 48 | Remission at week 8 (OR 4.75; 95% CI 1.21–18.58) Response at week 16 (OR 10.52; 95% CI 2.27–48.75) | Male gender (OR 0.26; 95% CI 0.08–0.88) Penetrating behavior (OR 0.25; 95% CI 0.07–0.89) |
| Biemans et al. (40) | Prospective | Iv induction | Real-world experience | 221 | Corticosteroid-free clinical remission at week 52 | - | Body mass index (OR 0.91; 95% CI 0.83–1.00) |
| Li et al. (43) | Clinical trial (UNITI-1, UNITI-2 and IM-UNITI) | Iv induction | Moderate-severe CD | 251 | Overall Global Histology Activity Score at week 8 | - | Baseline total SES-CD (OR 0.18; 95% 0.042–0.321) Baseline Overall Global Histology Activity Score (OR 0.374; 95% CI 0.213–0.535) |
| Waljee et al. (44) | Clinical trial (UNITI-1, UNITI-2 and IM-UNITI) | Iv induction | Moderate-severe active CD enrolled in pivotal RCT | 401 | Clinical and biochemical remission beyond week 42 | **Predictors during induction (week 8)** CRP at baseline, week 3, 6, and 8 | Baseline CRP cut-off 14.65 mg/L (AUROC 0.67; 95% CI 0.61–0.74) |

*(Continued)*

**TABLE 1** | Continued

| References | Study design | UST dosing | Clinical scenario | No patients | Endpoint | Predictive factors | |
|---|---|---|---|---|---|---|---|
| | | | | | | Positive association | Inverse association |
| | | | | | | Serum UST to CRP ratio at week 3 and 6<br>Albumin at week 8<br>**Pragmatic modeling**<br>Week-6 albumin to CRP ratio >4.92 (AUROC 0.76; 95% CI 0.71–0.82)<br>CRP at week 6 (AUROC 0.75; 95% CI 0.70–0.81) and 8 (AUROC 0.76; 95% CI 0.71–0.82) | |
| Kassouri et al. (41) | Retrospective | N/A | CD refractory or intolerant to at least one anti-TNF therapy | 29 UST and 71 vedolizumab | Effectiveness of third line biologic therapy and surgery-free survival | **Surgery-free survival (UST and vedolizumab combined)**<br>Ileal (OR 9.0; 95% CI 1.0–81.9)<br>Ileocolonic (OR 5.3; 95% CI 0.7–39.4)<br>Prior adalimumab and infliximab exposure (OR 2.2; 95% CI 0.9–5.1) | - |

*CD, Crohn's disease; CI, confidence interval; HBI, Harvey-Bradshaw index; IM, immunomodulator; OR, odds ratio; N/A, Not available; SES-CD, Simple Endoscopic Score-Crohn's Disease; TNF tumor necrosis factor.*

already developed, but most patients included in both cohorts received a subcutaneous induction regimen (25, 26). Similarly, a retrospective analysis of 106 CD patients receiving intravenous induction showed that penetrating complications were associated with lower rates of clinical and biochemical remission at week 48 (OR, 0.25; 95% CI, 0.07–0.89) (39). The remaining observational cohorts describing the experience across different countries with the intravenous induction did not show statistically significant differences according to disease phenotype (30–32). Additional data can be obtained from two recent analysis comparing the efficacy of UST and vedolizumab in CD (52, 53). Patients from five French university hospitals receiving either vedolizumab or UST for CD refractory or intolerant to TNF antagonists were analyzed (52). At week 48, UST was associated with higher clinical remission in patients with penetrating disease (OR, 6.58; 95% CI, 1.91–22.68). In a similar approach by the Dutch Initiative on Crohn and Colitis including 69 patients with UST and 69 with vedolizumab, there were no differences regarding the presence of intraabdominal complications at study entry (53). Therefore, accumulating evidence suggests that UST could be preferred in patients with inflammatory-predominant lesions and in those with penetrating behavior, at least after anti-TNF failure. Nevertheless, more quality data comparing the use of different biologic therapies would improve our management of patients with complicated disease.

Whereas, data about the efficacy of combination therapy with TNF antagonists has consistently shown an improvement in clinical and endoscopic outcomes (54, 55), evidence with UST or vedolizumab shows controversial results. Up to now, most of the evidence suggests no benefit of combination therapy with immunomodulators (56, 57). A recent meta-analysis including 15 studies found no improvement in clinical or endoscopic outcomes between patients receiving monotherapy or a combination of both drugs (OR, 1.1; 95% CI, 0.87–1.38; and OR, 0.58; 95% CI, 0.21–1.16, respectively) (57). Therefore, current evidence do not support a clear benefit of these strategy, but as UST is frequently used in refractory patients this decision should be carefully balanced in an individual basis.

Perianal fistulas and abscesses are severe complications that can lead to significant morbidity and reduced quality of life (58, 59). Up to 25% of patients develop perianal fistulas in the long-term, with a cumulative risk of 21% after 10 years and 26% after 20 years (60). Despite of its substantial impact on quality of life, there is a lack of randomized controlled trials about the best treatment options for this disabling complication. Immunomodulators and biologic anti-TNF agents, even alone or in combination, have been the most widely used treatments for perianal fistulas (61). However, no randomized controlled trial has evaluated the efficacy of UST in perianal fistula healing (62). Data from a *post-hoc* analysis of the CERTIFI, UNITI-1, UNITI-2 studies has reported its efficacy in active perianal fistulas—observed in 11 to 16% of patients at baseline -, although the results did not describe simple and complex fistula separately (63) (**Table 2**). Complete fistula healing was achieved in 24% of patients receiving 130 mg/kg and in 28% with the 6 mg/kg dosing, compared to 14% in the placebo arm. Although these results

**TABLE 2 |** Summary of studies evaluating the efficacy of ustekinumab for perianal complications of Crohn's disease.

| References | Study design | No perianal fistula | Type of fistula | Endpoint | Predictors of response |
|---|---|---|---|---|---|
| Khorrami et al. (23) | Retrospective | 18 | N/A | Clinical efficacy by physician assessment | None observed |
| Sands et al. (63) | RCT | 69 (1 mg/kg or 130 mg)<br>70 (6 mg/kg) | N/A | Fistula response and complete fistula resolution | None observed |
| Wils et al. (38) | Retrospective | 9 | N/A | Clinical efficacy by physician assessment | None observed |
| Chapuis-Biron et al. (64) | Retrospective | 207 (71% active) | N/A | Clinical success at 6 months | ≥3 prior anti-TNF agents (OR 0.4; 95% CI 0.15–1.08; $p = 0.056$) |
| Attauabi et al. (65) | Retrospective | 18 | 56% complex | Fistula response and remission at week 8, 24, and 52 | None observed |

*N/A, not available; RCT, randomized controlled trial.*

suggest a beneficial effect over placebo, a systematic review and meta-analysis did not show statistically significant differences for the induction of remission [relative risk (RR) 1.77; 95% CI 0.93–3.37] (66). However, this analysis included data only up to December 2016, so information from more recent cohorts may include additional and has the potential to obtain different conclusions. Data from uncontrolled real-world studies have reported heterogeneous results on fistula response and closure rates (23, 24, 64, 65). In 148 patients with active perianal disease included in a observational cohort from the GETAID, 39% achieved treatment success with UST (64). In this cohort, no predictive factors were associated with the main outcomes, and only the number of prior anti-TNF agents (≥3 drugs) showed a trend toward a reduced response rate. No additional predictive factors have been associated with fistula response or healing in real-world experience reported so far (23, 24, 38, 64, 65).

## Endoscopic and Histologic Factors

Increasing evidence supports the impact of mucosal and histologic healing in UC, as it has been extensively demonstrated that the resolution of the mucosal lesions improves the long-term clinical outcomes (67). Nevertheless, data supporting the influence of healing endoscopic lesions in CD is favorable, but the evidence is still more limited (68, 69). A recent systematic review with meta-analysis has shown increased clinical remission rates, but not influence on surgery risk (68). The current definition of mucosal healing suggested by the 2015 STRIDE recommendations is the resolution of ulcers at ileocolonoscopy or cross-sectional imaging (70), as it has been previously defined in the SONIC (54), ACCENT (7) and EXTEND (71) trials. The most frequently used scores are the Crohn's Disease Endoscopic Index of Severity (CDEIS) and the Simple Endoscopic Score for Crohn's Disease (SES-CD) (72, 73). However, no definite endpoints or endoscopic scores were included in the STRIDE statements based on these score (70), but the IOIBD has proposed the use of a SES-CD ≤2 (74).

No clear data can be obtained from the initial reports from the developing program of UST about potential endoscopic predictors of response (20, 49). However, a recent *post-hoc* analysis of the UNITI-1, UNITI-2, and IM-UNITI trials evaluating histologic disease activity also included some endoscopic outcomes (43). Here, Li et al. observed that baseline SES-CD inversely correlated with the histologic disease activity at week 8 (OR, 0.18; 95% CI, 0.042–0.321). The relationship between response rates and endoscopic disease severity has been also observed in real-world studies (30, 31, 33). Iborra et al. evaluated the short and long-term clinical and endoscopic response among patients included in the ENEIDA registry (30, 31, 75). Conversely, after 14 weeks of treatment endoscopic severity at baseline was negatively associated with clinical remission rates (OR, 0.08; 95% CI, 0.01–0.37) (30), and this observation was further confirmed in their follow-up at 52 weeks (OR, 0.35; 95% CI, 0.16–0.71) (31). There is only one prospective observational study that has reported real-life experience about this outcome (33). In this study, Murate et al. found that clinical response at week 24 was more frequently observed in subjects with lower SES-CD at baseline (cut-off <13) (33).

In the future, it is expected that routine assessment of endoscopic disease activity or even surrogate markers of mucosal colonic lesions will help us in the decision making process. Meanwhile, the evaluation of endoscopic severity in CD remains as an important unmet need for the stratification and follow-up assessments during medical therapy.

## Biomarkers

Even though some studies have suggested a more favorable response to UST in patients with more severe disease (25, 26), no clear conclusion can be obtained from disease scores or biomarkers associated with a specific immune pathway. No association has been observed between C-reactive protein and clinical outcomes in most of the observational cohorts (18).

Potential cut-off values of C-reactive protein and additional biomarkers have been analyzed with data from UNITI-1, UNITI-2, and IM-UNITI (44). Using machine learning algorithms, some biomarkers were able to identify non-responders to UST after 42 weeks of therapy. Interestingly, lower CRP levels at week 3, 6, and 8, higher serum UST through levels to CRP ratio and increased albumin levels were associated with increased treatment success rates. A prospective observational cohort from Japan recently found that responder to UST at week 8 showed higher TNF-$\alpha$ concentrations at baseline (33). Moreover, serum TNF-$\alpha$ levels in responders were significantly decreased during anti-IL12/23 therapy. No additional serum biomarkers associated with UST response have been identified.

Results about the probability of developing loss of response to anti-TNF drugs according to specific genetic variants have shown promising results (76, 77). Those patients carrying HLA-DQA1*05 are at a higher risk of immunogenicity during infliximab or adalimumab therapy in patients with CD. Currently there are no published data on the possible influence of genetic factors in the response to UST, but results on this topic are awaited.

## Microbial Markers

Gut microbiota plays an essential role in the pathogenesis of IBD. Hence, it would be plausible to find a relationship with treatment response and it may even be useful as a predictive factor of response to some medical therapies. In CD, data about the influence of specific components of the fecal microbiota on treatment outcomes are still scarce (78). In a subset of patients with moderate-severe CD refractory to TNF antagonists participating in the phase 2b clinical trials of UST, Doherty et al. found that microbial signatures were associated with treatment response or remission (CDAI decrease $\geq$100 points or below 150 points, respectively) (79, 80). Interestingly, the predictive model performed better than clinical data alone, and the combination of both data sets did not improve significantly the area under the curve over the microbiome data by itself. Responders at week 6 had significantly different baseline $\alpha$ and $\beta$-diversity than subjects with active CD. *Bacteroides* and *Faecalibacterium* were the two more abundant genus in those subjects with a better treatment response. The presence of *Faecalibacterium*, *Blautia*, *Clostridium* XIVa, *Ruminococcaceae*, and *Roseburia* was also associated with in clinical remission at week 6.

## Pharmacokinetics

Anti-TNF trough and anti-drug antibody concentrations are associated with improved outcomes in IBD (81–83). Indeed, therapeutic drug monitoring has been evaluated in multiple clinical trials and observational studies in the management of patients showing a loss of response to anti-TNF agents (84–86). Despite the increasing evidence toward the utility of drugs levels with these agents, data on the optimal drug concentrations and anti-drug antibodies thresholds with novel biologics have been less extensively explored (87). In fact, there is currently scarce comprehensive data about UST pharmacokinetics and exposure-response data in CD from large, randomized, controlled trials. **Table 3** summarizes the evidence of the influence of pharmacokinetics on UST response. The UNITI-1, UNITI-2, and IM-UNITI trial pharmacokinetics are the main sources exploring the relationship between trough levels and efficacy at 1 year (20, 90). A *post-hoc* analysis of the IM-UNITI cohort demonstrated an area under curve of 0.64 ($p < 0.003$) for clinical remission and UST concentrations, with an optimal cut-off of 0.8 ug/mL (90). In addition, UST concentrations >1.1 ug/mL were associated with an increased probability of C-reactive protein normalization at week 24 (52 vs. 25%, $p < 0.0001$).

The relationship between UST trough concentrations has also been investigated in a real-world setting, including anti-drug antibodies and clinical outcomes (94). Battat et al. conducted a prospective study in 62 patients with refractory CD, demonstrating a relationship between serum C-reactive protein and endoscopic improvement with UST trough concentrations >4.5 μg/mL at week 26 or beyond (94). Moreover, a recent prospective open-label cohort study including 86 patients, showed that UST concentrations $\geq$4.2 μg/mL at week 8 were associated with a 50% decrease in fecal calprotectin (89). Additionally, week 16 UST concentrations $\geq$2.3 μg/mL and week 24 concentrations $\geq$1.9 μg/mL were associated with endoscopic response at week 24 (89).

On the other hand, evidence regarding early UST concentrations and prediction of later outcomes in CD is limited. Recently, a prospective observational study by Hanzel et al. found that 6 of 13 patients (46%) with peak concentrations above 105 $\mu$g/mL achieved endoscopic remission, compared with only 7% among those with peak concentrations below 88 mg/mL (88). These authors concluded that therapeutic drug monitoring as early as during the first 2 weeks of initiation of UST might help stratify patients according to the probability of achieving treatment outcomes at 6 months.

In contrast to anti-TNF treatment, the immunogenicity of UST seems to be very low (<5%). The incidence of antibodies against UST was 0.2% after induction and after 1 year of treatment it was only 2.3% (using a drug-tolerant assay) in the UNITI-1, UNITI-2, and IM-UNITI trials (90). This fact suggests that combotherapy with immunomodulators may not be needed with the primary aim of reducing immunogenicity. However, as discussed above some cohorts have suggested that combination therapy could improve the clinical efficacy of UST (24–26). Nonetheless, this observation has not been confirmed in more recent cohorts and one meta-analysis (56, 57).

Finally, there are multiple factors that can influence UST trough levels in an individual patient. Higher UST exposure can be expected in patients with markers of a more limited inflammatory burden and less aggressive disease like higher albumin, lower baseline C-reactive protein, lower fecal calprotectin and no previous exposure to biological therapy (88, 89). In summary, UST concentrations have been associated with improved results in refractory patients with CD, demonstrating a favorable exposure-outcome relationship. Hence, it is expected that the increasing availability of measuring UST trough levels in clinical practice may lead to a better disease control in difficult to treat patients.

**TABLE 3 |** Studies evaluating the influence of pharmacokinetics on ustekinumab response.

| References | Study design | No of patients | Endpoint | Cut-off trough levels | Antidrug antibodies (%) |
|---|---|---|---|---|---|
| **Week 2** | | | | | |
| Hanzel et al. (88) | Prospective observational | 41 | Biochemical and endoscopic remission week 24 | 105 $\mu$g/mL peak concentration | - |
| **Week 4** | | | | | |
| Verstockt et al. (89) | Prospective observational | 86 | 50% decrease in fecal calprotectin week 8 | >15.9 | 1 |
| **Week 8** | | | | | |
| Adedokun et al. (90) | *Post-hoc* analysis of RCT (UNITI-1, UNITI-2 and IM-UNITI) | 701 | Clinical remission week 8 | 3.3 $\mu$g/mL | 2.3 |
| Verstockt et al. (89) | Prospective observational | 86 | Biological remission week 8 | >7.2 $\mu$g/mL | 1 |
| Verstockt et al. (89) | Prospective observational | 86 | 50% decrease in fecal calprotectin week 8 | >4.2 $\mu$g/mL | 1 |
| Soufflet et al. (91) | Prospective observational | 51 | Corticosteroid-free clinical and biochemical remission week 16 | 2 $\mu$g/mL | - |
| Thomann et al. (92) | Retrospective observational | 72 | Clinical response week 16 | 2 mg/L | - |
| **Week 12** | | | | | |
| Painchart et al. (93) | Prospective observational | 72 | Biological response 6 months | 1.10 $\mu$g/mL | 0 |
| **Week 16** | | | | | |
| Soufflet et al. (91) | Prospective observational | 51 | Corticosteroid-free clinical and biochemical remission week 16 | 1.4 $\mu$g/mL | - |
| **Week 24–26** | | | | | |
| Verstockt et al. (89) | Prospective observational | 86 | Endoscopic response week 24 | 1.9 $\mu$g/mL week 24 | 1 |
| Battat et al. (94) | Prospective observational and cross-sectional cohort | 62 | Endoscopic response | 4.5 $\mu$g/mL week ≥26 | 0 |
| **Week 40** | | | | | |
| Adedokun et al. (90) | Randomized clinical trial | 1,366 | Clinical remission week 44 | 1.4 $\mu$g/mL week 40 | 2.3 |
| Liefferinckx et al. (32) | Retrospective observational | 152 | Clinical and endoscopic response week 8, 16, and 52 | None detected | - |
| **Negative studies** | | | | | |
| Rowan et al. (95) | Prospective observational | 19 | Clinical response | None detected | - |
| Murate et al. (33) | Prospective | 52 | Clinical response 24 weeks | No difference in clinical response | - |

## UST Intensification Strategies

Unlike with anti-TNF agents the optimal management of loss of response to UST is not fully established. Shortening the interval of administration and also re-induction with iv UST have been described in patients after an initial inadequate response or secondary loss of response with good results (96–101). However, data about the efficacy of both strategies in patients failing q8w dosing are still scarce. Dose escalation to q4w is able to decrease Harvey-Bradshaw index and C-reactive protein levels in refractory patients (98). In a study from the Groupe d'Étude Thérapeutique des Affections Inflammatoires du Tube Digestif (GETAID) clinical response was observed in 57% of patients

**TABLE 4 |** Summary of current evidence on predictive factors of response to ustekinumab in Crohn's disease and ulcerative colitis patients.

| Predictive factor | Crohn's disease | Ulcerative colitis |
|---|---|---|
| Age | Young | |
| Gender | Female | |
| Race | White | Caucasian, non-Asian |
| Weight | Low body weight | Lower and higher quartiles |
| Smoking habits | Active smokers | Non and prior smokers |
| Disease duration | | Shorter duration |
| Disease extent | | |
| Crohn's disease behavior | Stricturing | |
| Disease activity | More severe | More severe |
| Endoscopic severity | Lower SES-CD | |
| Concomitant steroids | | |
| Previous anti-TNF | | |
| Combination therapy | | |
| Gut microbiota | *Bacteroides*<br>*Faecalibacterium* | |
| C-reactive protein | Low | High |
| Fecal calprotectin | | >250 mg/kg |

*Green: positive correlation; red: inverse correlation; gray: no influence or insufficient data. CRP, C-Reactive protein.*

2 months after reducing UST dosing interval to q4 w (100). Kopylov et al. also recently reported a European multicenter retrospective real-world study assessing the effectiveness of dose optimization to q4w or q6w, intravenous re-induction or both. At week 16, 51, and 39% of patients achieved clinical response and remission, respectively (101). The possibility of re-induction with 6 mg/kg iv UST has been evaluated in other cohorts (42, 102). Re-induction has shown to induce a significant decrease in C-reactive protein levels, with endoscopic remission in 25% of patients (42). Patients already being intensified to 4-weekly dosing can also benefit from iv re-induction, with approximately half of patients (53%) achieving clinical remission and 67% response (99). Younger patients (98) with shorter disease duration (97), no prior surgery (97), perianal disease (96), higher clinical disease activity (96, 98) and corticosteroid use (96) have shown reduced response rates to these rescue strategies. There is an ongoing study (POWER) that will compare the efficacy of q8w 90 mg sc with re-induction with 6 mg/kg iv UST in patients with loss of response to maintenance sc UST (NCT03782376).

# ONE STEP FORWARD: ULCERATIVE COLITIS

## Clinical Efficacy

UST has been recently approved by the European Medicines Agency for the treatment of UC. Data from pivotal clinical trials have shown promising results about its efficacy and safety in naïve patients and also in those previously exposed to immunomodulators or anti-TNFs. Experience in UC is still scarce in clinical practice and it comes mainly from its use as compassionate drug therapy, therefore current evidence is obtained from the pivotal clinical trials (103, 104) and small observational cohorts in subjects with refractory disease (105, 106). The UNIFI study included 642 subjects receiving induction therapy with either 130 mg or 6 mg/kg of UST, 52–54% of them with concomitant steroids at baseline, 51% previously exposed to ≥1 TNF antagonist, and 17–18% after receiving both anti-TNF and vedolizumab. The comparisons between subjects randomized to UST and those assigned to the placebo arm revealed important baseline disease characteristics as predictors of clinical response (103). Remarkably, most of the characteristics associated with clinical remission at week 8 were observed across both treatment arms. The influence of disease duration has been extensively studied in CD and specially with anti-TNF treatment. Here, patients with disease duration ≤15 years showed improved rates of clinical remission, suggesting that early intervention could be important also with UST (**Table 4**). Additionally, some biomarkers were also found to be predictors of higher response rates, including C-reactive protein levels <10 mg/L, fecal calprotectin >250 mg/kg and fecal lactoferrin >7.24 $\mu$g/g. Clinical remission rates were also influenced by race, as Caucasian patients showed higher probability of response in both treatment arms (OR, 3.0; 95% CI, 1.63–5.56 and OR, 3.1; 95% CI, 1.68–5.70) in the 130 mg and 6 mg/kg, respectively). Similarly, non-Asian patients demonstrated better response rates. As it was previously described in CD, additional factors including weight or smoking habits seem to influence the effect of anti-interleukin therapy. Subjects in the lowest and highest weight quartiles, non-smokers or former smokers showed a similar trend toward better treatment outcomes (103). We should interpret these findings with caution, because patients recruited in this analysis may not be a representative sample of the patient profile that will be treated with UST in clinical practice, at least during our initial experience. Nevertheless, data from pivotal trials could be used as potential predictors of response at least in the short-term and they may guide further analysis in real-world studies.

Only two observational studies have described the efficacy and safety of UST for UC in clinical practice. Ochsenkühn et al. have reported their experience in 19 patients with UC, where no predictive factors of response were identified (105). A multicentric and observational cohort from France has been recently reported in 103 patients with active disease (106). In this cohort, patients with more severe disease activity—defined as partial Mayo score >6— or prior exposure to TNF antagonists and vedolizumab were associated with a lower probability of achieving steroid-free remission at week 12–16 (OR, 0.10; 95% CI, 0.01–0.90 and OR, 0.03; 95% CI, 0.01–0.42, respectively).

## Pharmacokinetics

Data about the influence of pharmacokinetics on the pivotal clinical trials in UC show similar findings to CD (107). Serum concentrations of UST correlated well with clinical and histological efficacy features, including normalization of inflammatory markers. The authors identified that a target

concentration threshold of 3.7 $\mu$g/mL at week 8 (AUC 0.65, 95% CI, 0.61–0.69) was associated with clinical response. Importantly, 5.7% of samples demonstrated anti-drug antibodies, but 44% were transient and only 28% were considered as neutralizing. Immunogenicity to UST did not seem to impact efficacy outcomes or injection site reactions. These results may help us through the treatment algorithm of UST in patients with UC, but additional data are still needed to include drug concentration of this drug in clinical practice.

## CONCLUSIONS

CD is a chronic and disabling disease that frequently leads to irreversible bowel damage. Therefore, a relevant proportion of patients receive immunosuppressants or biologics, but complete clinical or endoscopic response is achieved only in a subset. Newer biologic therapies like UST are currently used in difficult-to-treat patients, but increasing data suggest that we can identify factors associated with higher probability of response. The individualization of UST would maximize the efficacy and costs associated to this chronic and progressive condition. This is an evolving field, but data from recent years have already demonstrated many aspects that make personalized medicine with anti-interleukin biologics closer to clinical practice.

## AUTHOR CONTRIBUTIONS

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

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