CHALLENGES IN INFLAMMATORY BOWEL DISEASE: CURRENT, FUTURE AND UNMET NEEDS

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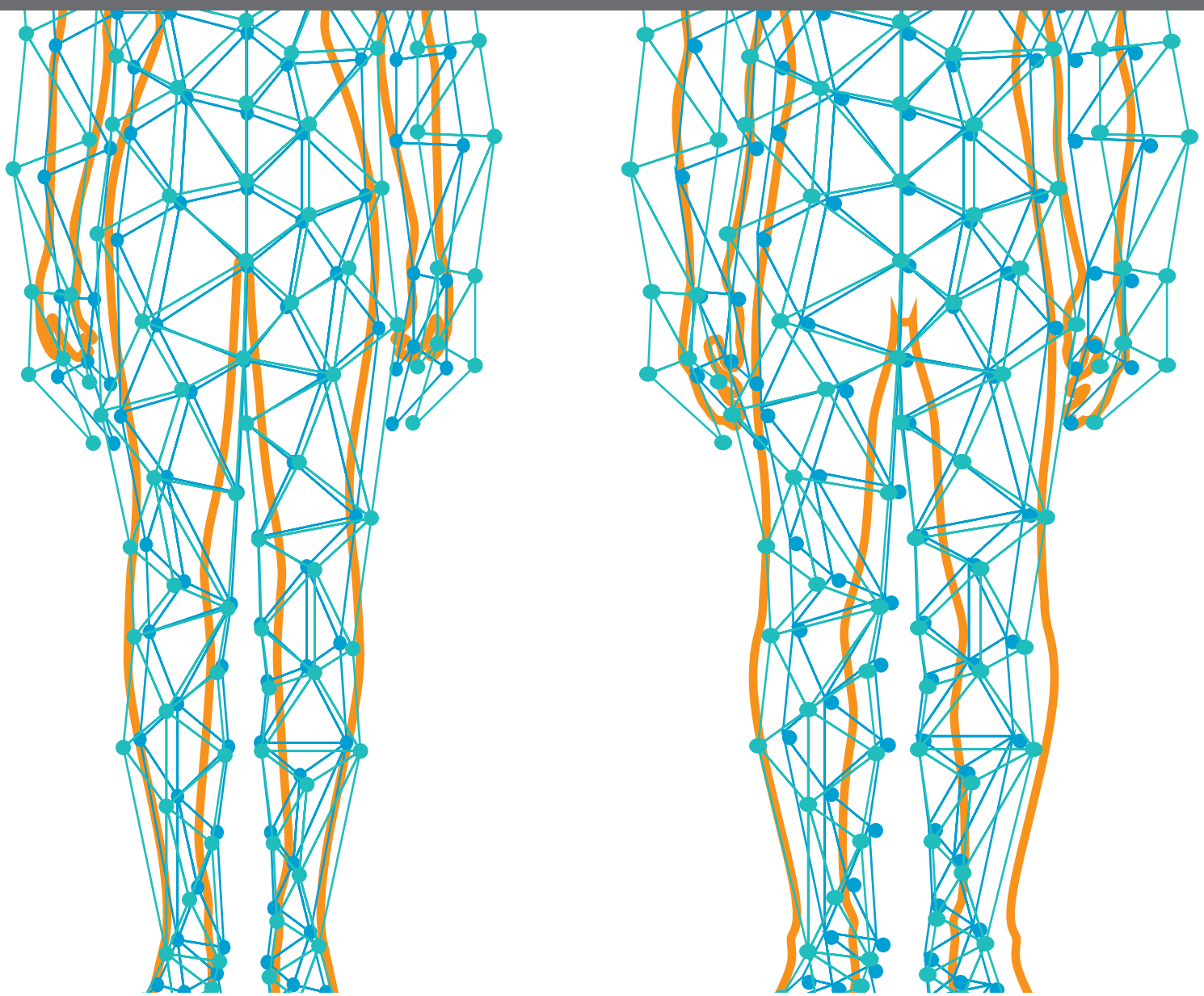
PUBLISHED IN: Frontiers in Medicine and Frontiers in Public Health



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ISSN 1664-8714
ISBN 978-2-88976-771-7
DOI 10.3389/978-2-88976-771-7

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CHALLENGES IN INFLAMMATORY BOWEL DISEASE: CURRENT, FUTURE AND UNMET NEEDS

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Citation: Gravina, A. G., and Zingone, F., eds. (2022). Challenges in Inflammatory Bowel Disease: Current, Future and Unmet Needs. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88976-771-7

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frontiers | Frontiers in Medicine

TYPE Editorial
PUBLISHED 18 July 2022
DOI 10.3389/fmed.2022.979535

OPEN ACCESS

EDITED AND REVIEWED BY
Arch Mainous,
University of Florida, United States

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SPECIALTY SECTION
This article was submitted to Family Medicine and Primary Care, a section of the journal Frontiers in Medicine

RECEIVED 27 June 2022
ACCEPTED 06 July 2022
PUBLISHED 18 July 2022

CITATION
Gravina AG, Pellegrino R and Zingone F (2022) Editorial: Challenges in Inflammatory Bowel Disease: Current, Future and Unmet Needs. *Front. Med.* 9:979535. doi: 10.3389/fmed.2022.979535

Editorial: Challenges in Inflammatory Bowel Disease: Current, Future and Unmet Needs

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KEYWORDS

Inflammatory Bowel Disease, Crohn's Disease, Ulcerative Colitis, biologic therapy, COVID-19

Editorial on the Research Topic
Challenges in Inflammatory Bowel Disease: Current, Future and Unmet Needs

Introduction

Inflammatory Bowel Diseases (IBD), to which Crohn's Disease (CD) and Ulcerative Colitis (UC) belong, are chronic digestive disorders with a natural history of remitting-recurrent course that can directly impact the patient's quality of life. UC is limited to the colon and rectum with continuous and non-transmural involvement while CD has a much more complex disease topography as it can affect the entire gastrointestinal tract from the mouth to the anus with discontinuous and transmural involvement. IBD are associated with numerous extra-intestinal manifestations (such as articular, ocular, cutaneous, urogenital, and cardiorespiratory) that greatly complicate the basic clinical picture resulting in a disease phenotype that can be extremely varied. Treatment relies on pharmacological, nutritional even surgical interventions. Often the patient, in case of intolerance or failure of conventional therapy or in case of dependence or refractoriness to steroid therapy, undergoes immunosuppressive therapy also with biologic drugs. The latter have positively revolutionized the prognosis and course of IBD. However, IBD patients on immunosuppressive therapy are exposed to a wide range of infectious agents for which appropriate preventive measures are recommended, even considering the incipient Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) pandemic. The purpose of this Research Topic was to gather new evidence on the complex needs of patients with IBD.

Current and future IBD needs

Having a non-single-perspective view of the IBD patients but looking at them from a multidimensional perspective contemplating intra-individual, inter-individual, and international differences, is certainly merit that research must constantly pursue. Some of the papers on our Research Topic have helped in this regard.

Elbadry et al., for example, studied the clinic-epidemiological characteristics of Egyptian IBD patients in a large sample of 1,104 patients, observing, among various findings, a greater presence of male patients. Always staying within the gender characteristics Shehab et al. found, in a retrospective multicenter study of more than one thousand patients, that in IBD patients on therapy with Infliximab, anti-drug antibody levels were higher in males than in females. Confirming the finding, trough levels were also lower in the male sex. The same finding was not confirmed for adalimumab.

Among the needs of IBD patients are those undergoing stoma placement, who are adapting to a new routine and new disease management with no small psychological repercussions, which have to be of extreme interest. Wang et al. observed in a stoma court of IBD patients how there is a moderate degree of perceived stigma with a low-moderate level of self-efficacy that increased in patients with higher educational status. Having a close person who accepts the stoma helps the patient in relative self-awareness and self-acceptance.

Special attention should be also paid to patients with CD at risk of common complications, including stenosis and intestinal obstruction. Xia et al. presented two clinical cases, of which in one there was endoscopic capsule retention in the small bowel of a patient with CD with concomitant bowel obstruction and, in the second case of a patient with a CD with rare intestinal obstruction from mesangial hernia and ileal stenosis. Both cases were treated with minimally invasive surgery. Thus, the authors emphasized careful evaluation of bowel patency by the clinician before performing an endoscopic video capsule.

Nardone et al. provided us with a detailed review of the role of intestinal ultrasound in the clinical management of IBD, also providing insights into the detection of post-surgical recurrence in CD. Tien et al. conducted, moreover, a retrospective study investigating the impact of IBD and specific IBD therapy on the risk of developing hyperlipidemia, comparing more than 10,000 patients with IBD with a control group. Within the IBD group, the subgroup not receiving any therapy presented an increased risk compared with patients being treated for IBD. IBD therapy appeared to downregulate the expression of hepatic lipogenic genes and, therefore, the authors stigmatized screening for hyperlipidemia in the management of IBD. Certainly, the study of non-invasive markers to monitor and assess the severity of IBD is important for our clinical practice. Lin et al. found, by examining different indices, how some of these including the C-reactive protein-to-lymphocyte ratio and C-reactive protein-to-albumin ratio can help in this field.

Khoramjoo et al., in addition, in an interesting mini-review examined the role of three proliferative pathways namely Wnt, Notch, and Hippo in IBD. This assumption draws its origin in the impairment and dysregulation of these pathways in IBD. Indeed, it emerged how these pathways are essential in maintaining an adequate bowel epithelial barrier that is impaired in inflammatory digestive diseases such as IBD. Modern techniques, such as Single Cell RNA Sequencing, have added knowledge to IBD pathogenesis. Serigado et al. have reviewed the role of this technique and found how it can provide remarkable potential in characterizing the different cytologic subpopulations involved in the pathogenesis of UC (such as BEST4+ cells, colonic M cells).

The problem of the SARS-CoV-2 pandemic

Coronavirus pandemic disease 2019 (COVID-19) has inevitably resulted in treatment delays in IBD patients, especially those on biologic agent therapies. Li et al. provided a retrospective study that examined the risk factors for delaying treatment with Infliximab in patients with CD, with a propensity-matching score, to compare the effects of the same delay on short- and long-term outcomes. They identified a treatment delay rate, in a retrospective cohort of 53 patients, of 71.7% in Xiangya Hospital, China. They found that the CD Activity Index decreased less, over the course of the study time, in the delayed-treatment patient group with a higher long-term hospital readmission rate increased by 33%.

The safety of patients with IBD, especially on immunosuppressive therapy toward infectious risk is relevant, particularly considering the current SARS-CoV-2 pandemic status.

Shehab et al. studied the safety of the vaccine by examining short- (within 3 weeks of vaccine administration) and long-term (within 24 weeks) adverse events in a sample of 408 subjects (50% allocated in the IBD group and 50% in a sample of healthy controls). A good safety profile and absence of serious adverse events emerged with some local adverse events (pain at the injection site observed in patients with IBD after the first dose and fatigue after the second dose). An additional possibility is, as described in previous evidence, that patients on immunomodulating therapies, may present an attenuated antibody responses after anti-SARS-CoV-2 vaccination. Again Shehab et al. studied, in 162 patients on combination therapy (with azathioprine) with Infliximab, the immunogenicity of the vaccine and observed how the levels of specific anti-SARS-CoV-2 IgG as well as neutralizing Ig were significantly higher in recipients of a third booster dose than in those vaccinated

with only two doses. They also observed how the booster dose increased the rate of patients who developed positive anti-SARS-CoV-2 IgG antibodies (96.5 vs. 90%) and neutralizing antibodies (100 vs. 88.9%).

Author contributions

AG and FZ contributed equally to the editorial process of this Research Topic and the drafting of this editorial. AG, RP, and FZ equally contributed to the writing of this manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgments

The editors are extremely grateful to all the authors who made contributions and participated in this Research Topic. We thank all the reviewers who engaged in deep peer-review work in a prompt, fast, effective, and constructive form. We would also like to thank Dr. Alain P. Gobert, from Vanderbilt University Medical Center, United States, who edited a manuscript that was included in this Research Topic.

Conflict of interest

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

Publisher's note

frontiers in Medicine

ORIGINAL RESEARCH
published: 23 December 2021
doi: 10.3389/fmed.2021.801532

Relationship Between Patient Sex and Serum Tumor Necrosis Factor Antagonist Drug and Anti-drug Antibody Concentrations in Inflammatory Bowel Disease; A Nationwide Cohort Study

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OPEN ACCESS

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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *25 October 2021*
Accepted: *06 December 2021*
Published: *23 December 2021*

Citation:
Shehab M, Alasfour H, Abdullah I, Alhendi G, Alhadab A, Alfadhli A, Ziyab AH and Battat R (2021) Relationship Between Patient Sex and Serum Tumor Necrosis Factor Antagonist Drug and Anti-drug Antibody Concentrations in Inflammatory Bowel Disease; A Nationwide Cohort Study. Front. Med. 8:801532. doi: 10.3389/fmed.2021.801532

Background: Anti-drug antibodies to infliximab (ATI) and adalimumab (ATA) are associated with loss of response to tumor necrosis factor antagonist (anti-TNF) therapy in inflammatory bowel disease (IBD). We evaluated the relationship between patient sex and serum TNF antagonist drug and antibody concentrations in inflammatory bowel disease.

Methods: A nationwide multicenter retrospective cohort study was conducted by evaluating patients' charts from July 2018 until September 2021. The effect of patient sex on anti-drug antibodies and serum drug concentration in patients with IBD across seven hospitals was investigated. A subgroup analysis also investigated the effect of anti-TNF combination therapy. Geometric means were calculated, and multiple linear regression was used to estimate the adjusted ratio of geometric means (RoGM) and their 95% confidence intervals (CI).

Results: In the total study sample ($n = 1093$), males receiving infliximab had higher anti-drug antibody concentrations (38.3 vs. 22.3 AU/ml; aRoGM = 1.72, 95% CI: 1.30–2.27, $p < 0.001$) compared to females. Additionally, infliximab serum drug concentrations among males were lower compared to females (2.6 vs. 4.1 ug/ml; aRoGM = 0.62, 95% CI: 0.44–0.88, $p = 0.007$). In the subgroup analysis ($n = 359$), male compared to female patients on combination therapy with infliximab and immunomodulators had similar anti-drug antibody concentrations (30.2 vs. 21.9 AU/ml; aRoGM = 1.38, 95% CI: 0.79–2.40, $p = 0.254$). There was no difference in the anti-drug antibody and serum drug concentrations among males and females on adalimumab.

Conclusion: In patients receiving infliximab, anti-drug antibodies were higher in males than females. Consistent with this, serum drug concentrations were lower in males than females on infliximab. There was no difference in anti-drug antibody and serum drug

concentrations among males and females on adalimumab. In addition, no difference in anti-drug antibodies between males and females receiving anti-TNF combination therapy was observed.

Keywords: IBD, TDM (therapeutic drug monitoring), anti-TNF agent, sex, immunogenicity

INTRODUCTION

Tumor necrosis factor antagonist (anti-TNF) therapies are commonly used for the management of moderate to severe inflammatory bowel disease (IBD) (1–4). However, about one-third of patients treated with anti-TNF therapy develop primary treatment failure (primary non-response), in which a lack of response is observed in induction therapy (5, 6). Furthermore, approximately half of patients with initial response may experience secondary loss of response by losing treatment effect during the maintenance of remission (7). One of the most common causes of treatment failure is immunogenicity, the formation of anti-drug antibodies, which is also associated with low or undetectable drug serum concentrations (8, 9). The serum drug concentrations of anti-TNF therapy might also vary depending on the severity of the disease, degree of inflammation, concurrent use of immunomodulator, patient sex, serum albumin concentration, body mass index (BMI), and genetic factors (7, 8).

Previous data show that anti-drug antibodies exist in over 20% of IBD patients treated with anti-TNF therapy (10). Additionally, patient sex and body weight significantly influence the pharmacokinetics of infliximab as its clearance has been shown to be increased in the presence of anti-drug antibodies and low serum albumin (11). On the other hand, combination therapy, the concurrent administration of an immunomodulator with an anti-TNF, has been associated with improvement in pharmacokinetics by decreasing immunogenicity and increasing serum drug concentrations (1–4). With respect to infliximab, the SONIC and UC-SUCCESS trials demonstrated that the use of infliximab combination therapy is superior to monotherapy in reducing immunogenicity and maintaining remission (12, 13). Conversely, DIAMOND trial and two other meta-analyses, by Kopylov et al. and Chalhoub et al., demonstrated that adalimumab combination therapy is associated with limited impact on maintenance of clinical remission or response (3, 14–16). When considering anti-TNF therapy for pediatric patients with IBD, the ECCO-ESPGHAN guidelines recommend the use of infliximab combination therapy, with an immunomodulator, to reduce the risk of developing anti-drug antibodies to infliximab (ATI). However, for adalimumab, it is preferred to be prescribed as a monotherapy when started as the first anti-TNF agent in children (17, 18). When combination therapy is used in pediatric patients, it is recommended to stop the concomitant use of the immunomodulator after 6–12 months, and the benefits of continuing combination therapy should be weighed against the risk of adverse events (17).

A large-scale real world studies with patient-level data are lacking on the relationship between patient sex and anti-TNF therapy and anti-drug antibody concentrations. Additionally, the impact of combination therapy on anti-TNF pharmacokinetics when accounting for sex has not been described. To address these knowledge gaps, this study utilized a large cohort with patient level data to determine the relationship between patient sex and immunogenicity of infliximab and adalimumab when accounting for important factors such as albumin and concomitant immunomodulator use.

MATERIALS AND METHODS

Study Design

A nationwide multicenter retrospective cohort study was conducted to measure the effect of patient sex on anti-TNF anti-drug antibodies and serum drug concentrations in patients with inflammatory bowel disease (IBD). A subgroup analysis was performed at an inflammatory bowel disease center, Mubarak al-Kabeer Hospital, for patients who received either infliximab or adalimumab monotherapy or in combination with an immunomodulator. This study was performed and reported in accordance with Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (19). The study protocol was reviewed and approved by the standing committee for coordination of health and medical research at the ministry of health of Kuwait (IRB 2020/1410).

We included patients that: (1) were previously diagnosed with inflammatory bowel disease (2) had been tested for an anti-drug antibody and/or serum drug trough concentrations reactively or proactively and (3) were receiving an anti-TNF therapy for at least 6 weeks at the time of measurement. (4) who received regular standard dose anti-TNF therapy. Proactive therapeutic drug monitoring (TDM) testing was at week 6 for adalimumab and at week 14 for infliximab. Reactive TDM testing was perfumed at trough levels, before next scheduled dose. Any random testing, beyond the above scheduled times, were excluded. Both serum drug and anti-drug antibody concentrations were measured at the same time. Patients who had past medical history of other autoimmune diseases, such as inflammatory arthritis, or were on immunosuppressant therapies for other non IBD medical conditions were excluded. The data were collected from patients' electronic medical records from seven different hospitals (Mubarak al-Kabeer Hospital, Alamiri Hospital, Farwaniya Hospital, Aladan Hospital, Jahra Hospital, Alsabah Hospital, and Kuwait Oil Company Hospital). Data were collected from July 22nd, 2018 until September 1st, 2021. A subgroup analysis, from the total sample, was performed at an inflammatory bowel disease center,Mubarak al-Kabeer Hospital, for patients who received either infliximab or adalimumab combination or monotherapy.

Patient's characteristics were obtained from patient's electronic medical records including age, body mass index (BMI), type and extent of IBD at the time of serum drug/antibody concentration measurements.

TABLE 1 | Characteristics of total study sample and subsample with information on combination therapy use.

Variable	Total study sample (n = 1,093)	Subsample* (n = 359)
Age (years)		
Median (interquartile range)	26.0 (19.0–36.0)	25.0 (19.0–39.0)
Age group, *n* (%)		
≤16	197 (18.0)	61 (17.00)
17–40	719 (65.8)	238 (66.3)
>40	177 (16.2)	60 (16.7)
Sex, *n* (%)		
Male	567 (51.9)	187 (52.1)
Female	526 (48.1)	172 (47.9)
Ethnicity, *n* (%)		
Middle East	1,007 (92)	330 (91.9)
Others	86 (7.9)	29 (8.1)
Median body mass index (BMI), median (IQR)	23.1 (22.0–24.2)	23.2 (23.0–24.0)
Ulcerative colitis (UC), *n* (%)	486 (44.5)	161 (44.8)
E1: ulcerative proctitis	50 10)	16 (10)
E2: left sided UC	147 (30)	48 (30)
E3: Extensive colitis	289 (60)	97 (60)
Crohn's disease (CD), *n* (%)	607 (55.5)	198 (55.2)
L1: ileal CD	280 (46)	89 (45)
L2: colonic CD	55 (9)	18 (9)
L3: ileocolonic CD	242 (40)	79 (40)
L4: upper gastrointestinal CD	30 (5)	12 (6)
B1: non-stricturing, non-penetrating CD	285 (47)	91(46)
B2: stricturing CD	158 (26)	49 (25)
B3: penetrating CD	164 (27)	58 (29)
Median infliximab therapy duration (years), median (IQR)	4.2 (4.0–4.2)	4.3 (4.0–4.4)
Median adalimumab therapy duration (years), median (IQR)	4.6 (4.0–4.6)	4.5 (4.0–4.6)
Anti-TNF drug, *n* (%)		
Infliximab	461(42.2%)	147(40.9%)
Adalimumab	632 (57.8%)	212 (59.1%)
Infliximab serum concentration, (ug/ml)		
Geometric mean (95% CI)	3.3 (2.7–4.1)	2.3 (1.6–3.3)
Sample size, (*n*)	(461)	(147)
Adalimumab serum concentration, (ug/ml)		
Geometric mean (95% CI)	8.4 (7.4–9.5)	8.4 (7.0–10.3)
Sample size, (*n*)	(565)	(201)
Anti-Infliximab antibody serum levels, (AU/ml)		
Geometric mean (95% CI)	29.0 (24.9–33.8)	25.0 (18.8–33.2)
Sample size, (*n*)	(461)	(147)
Anti-Adalimumab antibody serum levels, (AU/ml)		
Geometric mean (95% CI)	12.2 (10.9–13.7)	11.0 (9.2–13.1)
Sample size, (*n*)	(631)	(212)
Active inflammation *n* (%)		
Yes	125 (11)	43 (12)
No	965 (88)	314 (87)
Missing, (*n*)	3	2
Anti-TNF combination therapy use, *n* (%)		
Yes	NA	226 (62.9%)
No	NA	133 (37.1%)

CI, confidence interval; NA, data not available.
**Subsample includes patients with information on immunomodulators (combination therapy).*

Diagnosis of inflammatory bowel disease (IBD) was made according to the international classification of diseases (ICD-10 version: 2016). Patients were considered to have IBD when they had ICD-10 K50, K50.1, K50.8, K50.9 corresponding to Crohn's disease (CD) and ICD-10 K51, K51.0, K51.2, K51.3, K51.5, K51.8, K51.9 corresponding to ulcerative colitis (UC) (20).

Study Definitions

Patients were considered to have active inflammation if they have one of the following within 14 days from serum drug/antibody concentration measurements: (1) C-reactive protein (CRP) levels above 10 mg/L or (2) stool fecal calprotectin (Fcal) more than 250 ug/g or (3) receiving steroids. Patients were considered to be receiving steroids if they were concomitantly receiving budesonide, methylprednisolone, hydrocortisone, prednisone/prednisolone, or any steroidal agent. Moreover, patients who received an immunomodulator (such as azathioprine, 6-mercaptopurine or methotrexate) concurrently with an anti-TNF therapy were classified to be on combination therapy while patients on infliximab or adalimumab alone were classified to be on monotherapy.

Anti-drug antibody and serum drug concentration samples from all the hospitals were measured at one central immunology laboratory. A drug-tolerant, the homogeneous mobility shift assay (HMSA) was used for all study participants. Anti-drug antibodies were considered detectable at levels >5 AU/ml for infliximab or >10 AU/ml for adalimumab. Additionally, serum drug concentrations/antibody levels were collected only at trough levels, i.e., before the next scheduled dose. A serum drug level of ≥5, and ≥7.5 ug/ml was considered therapeutic for infliximab and adalimumab, respectively. Trough serum drug and antidrug antibody concentrations were performed either reactively, e.g., due to treatment failure, or proactively to optimize therapy, e.g., at week 14 for infliximab, as per each physician clinical judgment and practice.

Outcomes

The primary outcome was to compare anti-drug antibody levels between male and female patients on infliximab and adalimumab. In addition, the association between patient sex and serum drug concentration for infliximab and adalimumab was evaluated.

Secondary outcomes, in sub-analyses, evaluated the impact of combination therapy among male and female patients on infliximab or adalimumab anti-drug antibody levels. The association between serum drug concentration and combination therapy in both sexes was estimated as well. Moreover, combination therapy was compared to monotherapy in terms of anti-drug antibody levels and serum drug concentration.

Statistical Analysis

Analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC, USA). The statistical significance level was set to $\alpha = 0.05$ for all association analyses. Descriptive analyses were conducted to calculate frequencies and proportions of categorical variables in the total study sample ($n = 1{,}093$) and subsample, which was part of the main total sample, ($n = 359$). To account for skewed distribution of the continuous variables (i.e., serum drug concentrations, and anti-drug antibodies), geometric means were estimated by $\log_{10}$-transformation of the data and subsequently taking the antilog of the calculated means on the transformed scale (21). Correlations between anti-drug antibody and serum drug concentration according to anti-TNF drug type (infliximab and adalimumab) were assessed using Spearman correlation coefficients (r). In the total study sample analysis, the effect of patient sex on anti-drug antibody and serum drug concentrations of both adalimumab and infliximab was assessed. In the subsample analysis, the effect of patient sex on infliximab and adalimumab use in combination or alone on anti-drug antibody levels and serum drug concentrations was assessed.

Associations of adalimumab and infliximab with $\log_{10}$-transformed anti-drug antibody and serum drug concentration (outcome variables) were evaluated using multiple linear regression models. Associations were assessed in the total sample and stratified by sex. In the total sample analysis, associations were adjusted for the effects of sex and age at assessment. In the subgroup analysis, associations were adjusted for sex, age at assessment, and active inflammation. In the sex stratified analysis, associations were adjusted for the effects of active inflammation and age at assessment. Given that we regressed $\log_{10}$-transformed anti-drug antibody and serum drug concentration, taking the antilog of the linear regression coefficients (β) yields an adjusted ratio of geometric means (aRoGM), not the difference between geometric means. Hence, the related 95% confidence intervals (CIs) represent limits for RoGM with a null value of "1." Anti-drug antibodies and serum drug concentrations were analyzed as continuous variables while applying log-transformation to account for skewness in the data and maximize the value of the continuous measurements (see Discussion).

Additional analyses were conducted to determine whether albumin can be a confounder of the assessed associations. Albumin was categorized as normal ($\geq$40 g/L) and abnormal ($<$40 g/L). The association of albumin (normal vs. abnormal) with anti-drug antibodies to infliximab and adalimumab was assessed by applying the Wilcoxon rank sum test. This analysis allowed us to determine whether concentrations of anti-drug antibodies differ across albumin categories. Moreover, albumin was added to the multiple linear regression models to determine if confounding is present.

RESULTS

Demographics

In total, 1,093 patients [567 (51.9%) males] were included in the total study sample analysis and a total of 359 patients [187 (52.1%) males] were included in the subsample analysis. The total study sample and the subsample were similar in all characteristics investigated. Of the total study sample, 42.2% of patients were on infliximab and 57.8% were on adalimumab. Similarly, in the subsample, 40.9 and 59.1% of patients were on infliximab and adalimumab, respectively (**Table 1**). Among patients in the subsample, 62.9% used anti-TNF combination therapy.

TABLE 2 | Anti-drug antibody serum levels stratified by sex according to monotherapy and combination therapy for infliximab and adalimumab use: inter-sex comparisons.

	Anti-drug antibody serum levels, Au/ml					
	Males		Females			
Drug	***n***	**Geometric mean* (95% CI)**	***n***	**Geometric mean (95% CI)**	**Ratio of geometric means* (95% CI) [M vs. F]**	***P*-value**
Total sample						
Infliximab	233	38.3 (31.4–46.6)	228	22.3 (18.2–27.2)	1.72 (1.30–2.27)	<0.001
Adalimumab	333	11.1 (9.4–13.1)	298	13.3 (11.2–15.9)	0.84 (0.66–1.06)	0.147
Subsample[†]						
Infliximab	14	106.5 (47.9–236.6)	19	24.2 (12.0–49.0)	4.39 (1.58–12.25)	0.005
Infliximab + IM	66	30.2 (20.0–45.6)	48	21.9 (13.8–34.9)	1.38 (0.79–2.40)	0.254
Adalimumab	47	11.8 (7.4–18.9)	53	13.7 (8.7–21.4)	0.86 (0.48–1.55)	0.621
Adalimumab + IM	60	13.5 (8.9–20.4)	52	12.0 (7.8–18.4)	1.13 (0.64–1.98)	0.677

CI, confidence interval; IM, immunomodulator; M, males; F, females.

**Adjusted for sex, active inflammation, and age at assessment.*

[+]*Includes patients with information on immunomodulators (combination therapy).*

[†]*Patients with information on immumodulator and corticosteroid use.*

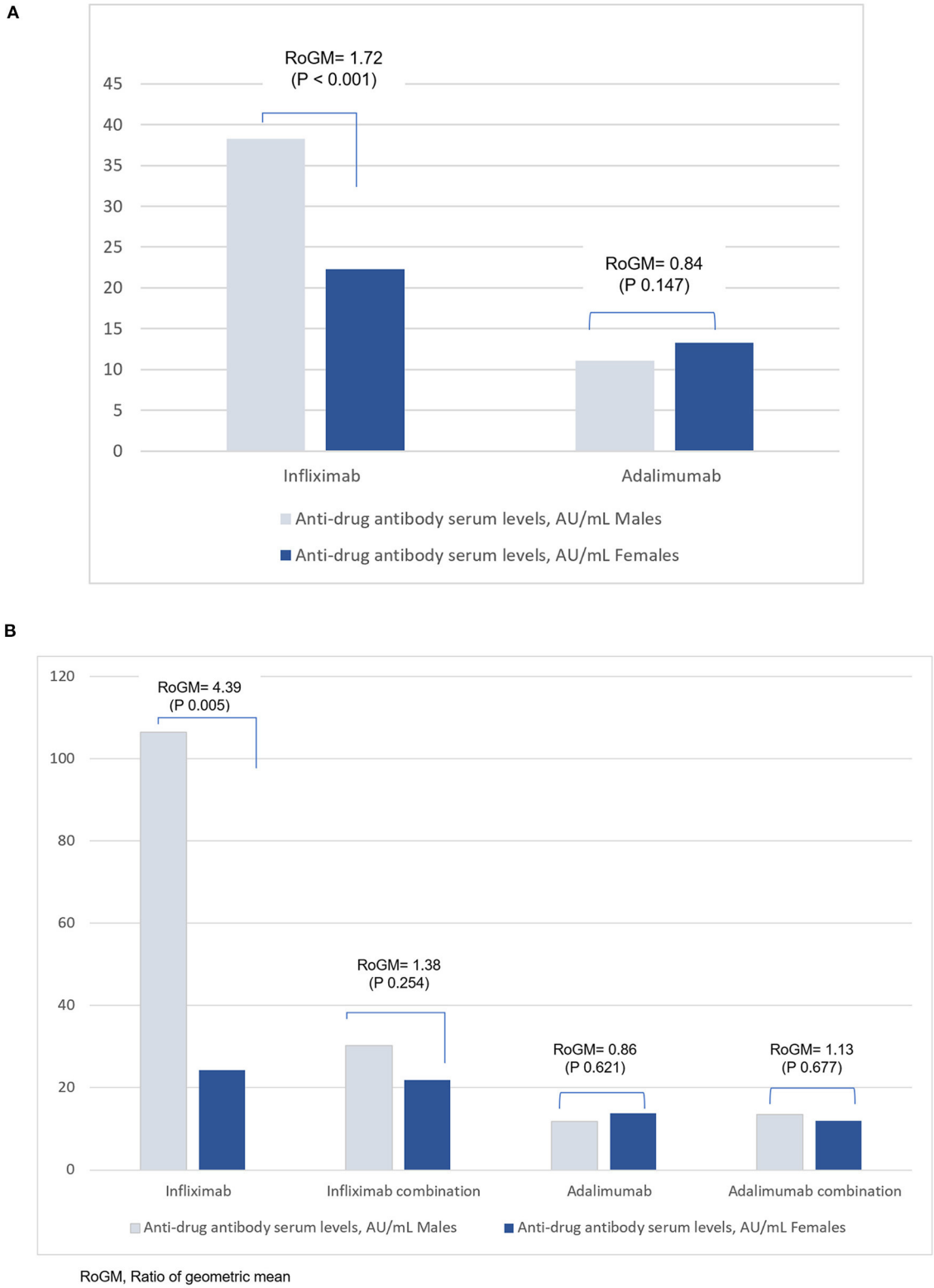

FIGURE 1 | (A) Anti-drug antibody levels in male and female patients with IBD in the total sample analysis for infliximab and adalimumab. The Y-axis corresponds to the geometric mean of the anti-drug antibodies serum levels. **(B)** Anti-drug antibody serum levels in males and females with IBD in the subgroup analysis for Infliximab and Adalimumab. The Y-axis corresponds to the geometric mean of the anti-drug antibodies serum levels.

TABLE 3 | Anti-TNF serum concentrations stratified by sex according to monotherapy and combination therapy for infliximab and adalimumab use: inter-sex comparisons.

	Anti-TNF serum concentrations, ug/ml					
	Males		Females			
Drug	***n***	**Geometric mean* (95% CI)**	***n***	**Geometric mean (95% CI)**	**Ratio of geometric means* (95% CI) [M vs. F]**	***P*-value**
Total sample						
Infliximab	233	2.6 (2.0–3.3)	228	4.1 (3.2–5.3)	0.62 (0.44–0.88)	0.007
Adalimumab	295	8.3 (6.7–10.2)	270	8.7 (7.0–10.9)	0.95 (0.70–1.29)	0.725
Subsample[†]						
Infliximab	14	0.9 (0.3–2.5)	19	1.4 (0.6–3.2)	0.71 (0.34–1.48)	0.361
Infliximab + IM	66	1.8 (1.1–3.1)	48	1.7 (1.0–3.0)	1.06 (0.54–2.10)	0.856
Adalimumab	47	5.3 (2.9–9.5)	53	7.4 (4.2–13.0)	0.67 (0.19–2.33)	0.529
Adalimumab + IM	60	4.9 (2.9–8.2)	52	8.1 (4.7–13.7)	0.61 (0.30–1.22)	0.158

CI, confidence interval; IM, immunomodulator; M, males; F, females.
**Adjusted for sex, active inflammation, and age at assessment. [†]Patients with information on immumodulator and corticosteroid use.*

Specifically, of the 147 patients on infliximab, 114 (77.6%) were on combination therapy while among the 212 patients on adalimumab, 112 (52.8%) were on combination therapy.

Outcomes

Inter-sex comparisons of anti-drug antibody within anti-TNF therapy are shown in **Table 2**. Whilst the proportion of male and female patients are similar, male patients on infliximab had higher anti-drug antibody concentrations than female patients in the total sample (38.3 vs. 22.3 AU/ml; aRoGM = 1.72, 95% CI: 1.30–2.27, $p < 0.001$) (**Table 2**, **Figure 1A**) and in the subsample sample (106.5 vs. 24.2 AU/ml; aRoGM = 4.39, 95% CI: 1.58–12.25, $p = 0.005$) (**Table 2**, **Figure 1B**). However, male and female patients on infliximab combination therapy in the subsample had similar anti-drug antibody levels (30.2 vs. 21.9 AU/ml; aRoGM = 1.38, 95% CI: 0.79–2.40, $p = 0.254$) (**Table 2**, **Figure 1B**).

In the subsample, male and female patients on adalimumab alone had similar anti-drug antibody levels (11.8 vs 13.7 AU/ml; aRoGM = 0.86, 95% CI: 0.48–1.55, $p = 0.62$). Males and females on adalimumab combination therapy had similar anti-drug antibody levels as well (13.5 vs 12 AU/ml; aRoM = 1.13, 95% CI: 0.64–1.98, $p = 0.67$, **Table 2, Figure 1B**).

Associations between anti-TNF therapy and serum drug concentrations are shown in **Table 3**. In the total study sample, male patients had lower infliximab serum drug concentrations compared to female patients (2.6 vs. 4.1 ug/ml; aRoGM = 0.62, 95% CI: 0.44–0.88, $p = 0.007$). No other between sex differences in serum drug concentrations were observed (**Table 3**). Serum drug concentrations were similar among patients on infliximab alone compared to those on infliximab combination therapy (**Supplementary Table S1**). Similarly, no difference in serum drug concentrations was observed among patients on adalimumab alone and those on adalimumab combination therapy (**Supplementary Table S1**).

Anti-drug antibody levels among patients on infliximab combination therapy were significantly lower than in patients on infliximab alone (25.7 vs. 50.8 AU/ml; aRoGM = 0.51, 95% CI: 0.28–0.91, $p = 0.023$; **Supplementary Table S2**). In addition, there was no difference in anti-drug antibody between patients on adalimumab combination therapy and those on adalimumab alone (12.7 vs. 12.7 AU/ml; aRoGM = 1.00, 95% CI: 0.66–1.51, $p = 0.995$; **Supplementary Table S2**).

In the total study sample, a negative correlation between infliximab serum concentration and anti-drug antibodies to infliximab (ATI) (spearman r = −0.388, $p < 0.001$) and adalimumab serum concentration and anti-drug antibodies to adalimumab (ATA) (spearman r = −0.415; $p < 0.001$; **Figure 2**) was observed.

In an additional analysis, we assessed the association of serum albumin [≥40 g/L (normal) vs. <40 g/L (abnormal)] with anti-drug antibodies to infliximab and adalimumab. Comparing subjects with normal and abnormal serum albumin levels, we found that average levels of anti-drug antibodies to infliximab (10.6 vs. 19.2 AU/ml, $p = 0.158$) and anti-drug antibodies to adalimumab (7.5 vs. 7.4 AU/ml, $p = 0.991$) to be similar across categories of serum albumin. Moreover, when including serum albumin in the multiple linear regression models, the observed associations between male sex and higher anti-drug antibodies to infliximab (aRoGM = 1.79, 95% CI: 1.02–3.11, $p = 0.041$) and male sex and lower infliximab serum drug concentrations (aRoGM = 0.73, 95% CI: 0.23–0.96, $p = 0.046$) remained statistically significant. Hence, these additional analyses indicate that albumin does not confound the reported associations.

DISCUSSION

In 1,093 Mediterranean patients with inflammatory bowel disease (IBD), we evaluated the effect of patient sex and combination therapy on anti-drug antibody levels and serum drug concentrations among all patients who were on infliximab

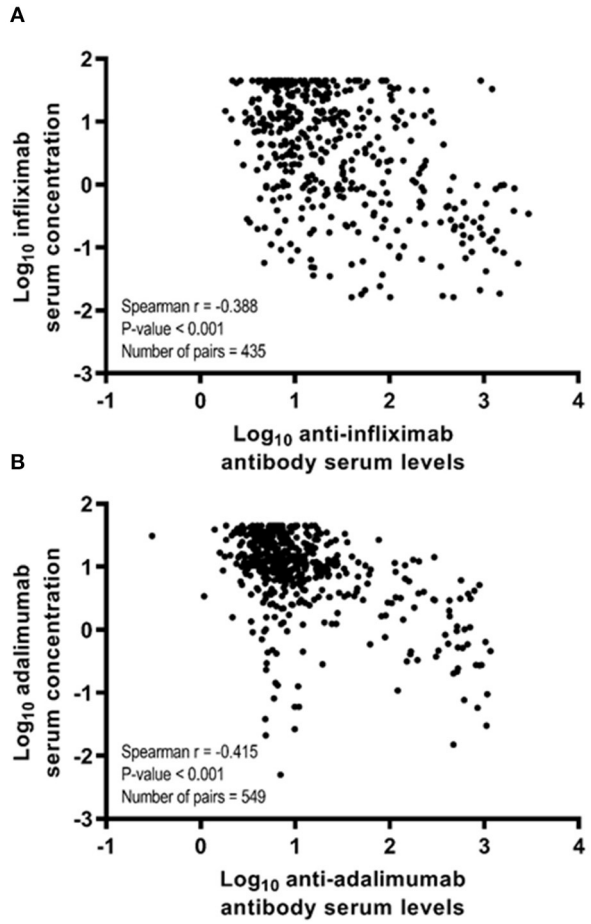

FIGURE 2 | Spearman correlation coefficients (r) between anti-drug antibody levels and anti-TNF therapy serum drug concentration according to anti-TNF therapy in the total study sample. **(A)** Spearman correlation coefficient between log_{10}-transformed infliximab serum concentration and log_{10}-transformed anti-infliximab antibody serum levels. **(B)** Spearman correlation coefficient between log_{10}-transformed adalimumab serum concentration and log_{10}-transformed anti-adalimumab antibody serum levels.

or adalimumab. The effect of the patient sex on the anti-drug antibody levels was investigated in both monotherapy and combination therapy in our subgroup analysis. The results showed that serum drug concentrations of infliximab in male patients are commonly lower than female patients. Since weight and sex are somehow correlated, where males generally weigh more than females, it is thought that male patients have higher clearance rates than female patients. These patient factors can affect the pharmacokinetics of infliximab, however; the exact mechanism of this effect is still unknown (7, 11). In our study the median BMI of all patients was within normal range. In a systematic review by Billioud et al. which evaluated loss of response to adalimumab found that male sex was associated with higher likelihood of loss of response and need for dose escalation (22). Moreover, male compared to female patients on infliximab had higher anti-drug antibody concentrations; nonetheless, there is no previously available evidence, to our knowledge, that supports the association between patient sex and presence of anti-drug antibodies. On the other hand, the presence of higher anti-drug antibody levels seems to accelerate the clearance of anti-TNF therapy, which is supported by Fasanmade et al. study that analyzed two randomized-controlled trials (23).

The available data on therapeutic drug response of patients with IBD to medications, stratified by sex, are extremely limited. A recent review by Rustgi et al. suggested the need for further investigation to the role of sex hormones on IBD, to get better therapeutic response for patients with IBD (24). Further studies needed to identify if there is a genetic factor behind a correlation between the patient sex and the anti-drug antibody levels. Two previous studies, by Wilson et al. and Sazonovs et al., identified an association between the genetic variant HLA-DQA1*05 and the formation of anti-drug antibodies, against both infliximab and adalimumab, in patients with Crohn's disease (CD) (25, 26). A study by Sazonovs et al. showed that this variant increased the anti-drug antibodies formation by 2-folds, regardless of the concurrent immunomodulator use (26). They also concluded that to minimize the risk of therapy failure, a pretreatment genetic testing for HLA-DQA1*05 might be helpful in deciding whether to use anti-TNF, or combination therapy in IBD.

As a secondary outcome of our study, the effect of combination therapy was investigated in one inflammatory bowel disease center. The results of our study were similar to the PANTS study, which showed that immunogenicity is more common in patients with CD treated with infliximab than adalimumab, and that the concomitant use of immunomodulator was associated with lower the immunogenicity (27). Moreover, higher drug concentrations and remission rates were found in patients treated with infliximab combination therapy. However, these effects were not shown in patients treated with adalimumab combination therapy, this might be influenced by lower rates of immunogenicity compared to infliximab (27). Furthermore, our study results agree with Hazlewood et al. network meta-analysis, where the effectiveness of immunosuppressants and anti-TNF were found to be comparable, and considered the combination therapy of infliximab with azathioprine and adalimumab monotherapy to be the most effective strategies for inducing and maintaining the remission of CD (28). In addition, a systematic review, by Strand et al., emphasized the role of monitoring both anti-drug antibody levels and serum drug concentrations of the used anti-TNF agent (29). This might be potentially helpful in guiding clinicians to improve anti-TNF therapy management as well as clinical outcomes. It can also reduce risks associated with immunogenicity and help in lowering costs of therapy (29).

We did not analyze anti-drug antibodies and drug concentrations as dichotomous/categorical variables (e.g., normal/low vs. abnormal/high), such categorization is helpful in clinical practice, but has some drawbacks. Loss of information, reduced statistical power, underestimating the true variability in the data, and residual confounding are the major issues with categorization of continuous variables in clinical research (30). Given the previous drawbacks, we have analyzed the

outcome variables (i.e., anti-drug antibodies and serum drug concentrations) as continuous variables.

Our study has several strengths. It is a nationwide multi center study that involved all hospitals in the country where therapeutic drug monitoring (TDM) testing is done. It is well designed with over 3 years total of all available data of eligible patients. It also addresses a gap in knowledge and encourage future research in this area.

However, there were some limitations to our study. Being a retrospective cohort, there might be some confounders, such as those on monotherapy could have been on combination therapy previously and then were discontinued due to adequate serum drug concentration and low anti-drug antibodies. In addition, patients' adherence to anti-TNF therapy at regular intervals could not be evaluated. Moreover, endoscopic and clinical targets were not studied; however, we controlled for objective inflammatory markers (CRP, Fcal, and steroids use). Combination therapy was only assessed at one center due to lack of data from other centers. Finally, the proportion of therapeutic drug monitoring (TDM) tests that were done reactively vs. proactively was not assessed.

In conclusion, anti-drug antibodies to infliximab (ATI) were higher in males than females whereas anti-drug antibodies to adalimumab (ATA) were similar in both sexes. In addition, male patients had lower infliximab serum drug concentrations compared to female patients while no sex differences was observed in adalimumab serum drug concentrations. Moreover, combination therapy was more effective than monotherapy in reducing ATI, but not better than monotherapy in reducing ATA. However, male and female patients on infliximab combination therapy had similar anti-drug antibody levels. Future studies are needed to assess the effect of patient sex, i.e., sex hormones, on anti-TNF anti-drug antibody and serum drug concentrations in patients with inflammatory bowel disease.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The study protocol was reviewed and approved by the standing committee for coordination of health and medical research at the ministry of health of Kuwait (IRB 2020/1410). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MS: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and submission of the manuscript. HA, IA, GA, and AnA: acquisition of data and drafting of the manuscript. AhA: drafting of the manuscript. AZ: statistical analysis, analysis and interpretation of data. RB: critical revision of the manuscript for important intellectual content, study supervision, and responsible for the overall work as a guarantor. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.801532/full#supplementary-material

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frontiers in Medicine

ORIGINAL RESEARCH
published: 12 January 2022
doi: 10.3389/fmed.2021.819557

Delayed Infliximab Treatment Affects the Outcomes of Patients With Crohn's Disease During the COVID-19 Epidemic in China: A Propensity Score-Matched Analysis

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Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 21 November 2021
Accepted: 20 December 2021
Published: 12 January 2022

Citation:
Li Y, Chen L, Chen S and Liu X (2022) Delayed Infliximab Treatment Affects the Outcomes of Patients With Crohn's Disease During the COVID-19 Epidemic in China: A Propensity Score-Matched Analysis. Front. Med. 8:819557. doi: 10.3389/fmed.2021.819557

Background: The coronavirus disease 2019 (COVID-19) has swept the world and led to delays in the treatment of Crohn's disease patients treated with biologics. This study aims to investigate the risk factors for delayed treatment during the epidemic and to observe the short- and long-term influences of such delays among them to provide some reference on treatments.

Methods: This study retrospectively enrolled patients diagnosed with Crohn's disease who received infliximab treatment between January 23, 2020 and April 30, 2020. Univariate and multivariate logistic regression were used to analyze the risk factors for delayed treatment. Propensity score matching was utilized to compare the effects of delayed treatment on the short- and long-term outcomes.

Result: Our cohort identified a total of 53 patients with a delay rate of 71.7%. Of these patients, 38 were in the delayed group, and 15 were in the non-delayed group. Logistic regression analysis showed that the baseline levels of C-reactive protein were an influence factor for delaying treatment (OR = 0.967, 95% CI = 0.935–1.000, $p = 0.047$). Regarding short-term effects, the delayed group had a lower decrease in the Crohn's Disease Activity Index than the non-delayed group [−43.3 (−92.7, −9.7) vs. −17.3 (−29.0, 79.9), $p = 0.038$] and significantly higher long-term readmission rates (33.3% vs. 0%, $p = 0.014$).

Conclusion: Delayed infliximab treatment could affect the short- and long-term outcomes in patients with Crohn's disease. Our study suggested that the regulated course of treatment with biological agents should be performed effectively and that education should be enhanced to minimize delays in treatment.

Keywords: COVID-19, infliximab, Crohn's disease, delayed treatment, readmission

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic originating from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was an ongoing outbreak that was more widespread than any pandemic from the past century (1). On March 11, 2020, the World Health Organization declared COVID-19 a global pandemic (2). The main route of transmission was aerosol spread by patients in the air (3).

Although COVID-19 was primarily characterized by respiratory symptoms, it also causes some gastrointestinal symptoms, such as diarrhea, nausea, vomiting and abdominal discomfort (4), which is related to angiotensin-converting enzyme 2 (ACE2) used by coronaviruses to penetrate target cells (5). The ACE2 is expressed by intestinal epithelial cells, especially in the inflamed terminal ileum and colon (6). Therefore, several gastroenterologists concerned that patients with Crohn's disease (CD) may have a higher risk for COVID-19 infection. In addition, they suggested that long-term administration of steroids and immunosuppressive agents in CD patients was also a susceptibility factor (7). According to the guidelines of the most qualified international societies and organizations for the study of inflammatory bowel disease (IBD), outpatient visits for CD patients should be postponed as well as colonoscopy and non-urgent surgery to reduce the risk of contagion (8, 9). Data were reported from an international survey with a reduction of endoscopic activities by 75–100% in the majority of IBD centers (10). Furthermore, early in the epidemic, relevant vaccines were not yet available, although current guidelines considered vaccination less impactful in patients with IBD (11, 12). These all put a tremendous strain on the mind of patients. In a series of surveys of IBD patients, the rates of anxiety and depression in them have been high as 48–62% compared to the general population, and most perceived increased vulnerability due to disease in response to the pandemic (13, 14).

Therefore, for CD patients receiving regular infliximab treatment, there is a concern that weighting the pros and cons of admission to the hospital and viral infections becomes a concerning matter. Most current studies focus on the diagnosis and management of IBD patients infected with COVID-19. A few researches showed that patients on intravenous biological treatments were more likely to stop or delay treatment (15, 16). In a recent report, ∼27.7% of patients with IBD discontinued their medication during the COVID-19 epidemic, among patients of whom, 29.3% suffered aggravated conditions (17). However, these studies did not have a long-term follow-up of patients with delayed treatment. Whether delayed treatment affects the long-term outcome of the disease and the efficacy of biological agents is currently unknown.

This study aimed to determine factors associated with delayed infliximab treatment in patients with CD during the COVID-19 crisis (from January 23, 2020 to April 30, 2020) and to investigate the short- and long-term clinical consequences of delayed treatment with infliximab infusion. It would provide some reference on biologics treatments in CD patients at present given that the epidemic has normalized.

METHODS

Patient Population

Consecutive patients with CD receiving infliximab treatment between January 23, 2020 and April 30, 2020 in the Gastroenterology Department of Xiangya Hospital of Central South University were enrolled. Patients with a diagnosis of CD according to clinical, radiological, or endoscopic evidence as suggested by the European Crohn's and Colitis Organization (ECCO) guidelines with a disease duration of more than 3 months were included. In addition, included patients aged 17–75 years should have an indication for treatment with infliximab (18). Excluded patients were allergic to or intolerant of the agent or changed to infliximab treatment during the COVID-19 epidemic. Patients lost to follow-up or without complete medical record data were not considered in our research. If combined with other drugs during the follow-up, patients would be excluded.

Study Design

All included patients received intravenous treatment with infliximab before and during the COVID-19 epidemic. The induction treatment for infliximab was 5 mg/kg at weeks 0, 2 and 6 and maintenance was 5 mg/kg every eight weeks. There were two groups (delayed group and non-delayed group) according to whether there was a delay of infliximab infusion. Data, including demographics, serological indicators [i.e., erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and hematocrit], disease activity assessed by Crohn's Disease Activity Index (CDAI) score, the change in treatment regimen, and the utilization of telemedicine, were retrospectively collected for each patient by 2 independent observers. Baseline data were recorded at the last treatment before the COVID-19 outbreak. The follow-up was performed by telephone or internet. The Pittsburgh Sleep Quality Index (PSQI) was used to evaluate the quality of patients' sleep (19). The need for surgery and readmission during the follow-up period were identified as indicators of the long-term influence.

Definition

Treatment delay was defined as more than 2 weeks after the scheduled date. This study chose 2 weeks in light of logistical challenges regarding previous studies (20). The deadline for follow-up was December 31, 2020. The secondary endpoint was the occurrence of a surgical or readmission event during follow-up. The time of first infliximab exposure during the outbreak was regarded as the time point of short-term efficacy assessment. Patients who underwent gastrointestinal surgery excluding anal fistula during the follow-up were considered to have a surgical history. A history of readmission was defined as readmission due to recurrence or exacerbation of the disease with a hospital stay longer than 2 days.

Statistical Analyses

Continuous variables expressed as medians with interquartile ranges (IQRs) or means ± standard deviations (SDs) depending on the underlying data distribution were used to describe the statistics of the cohort. Independent Student's t-test

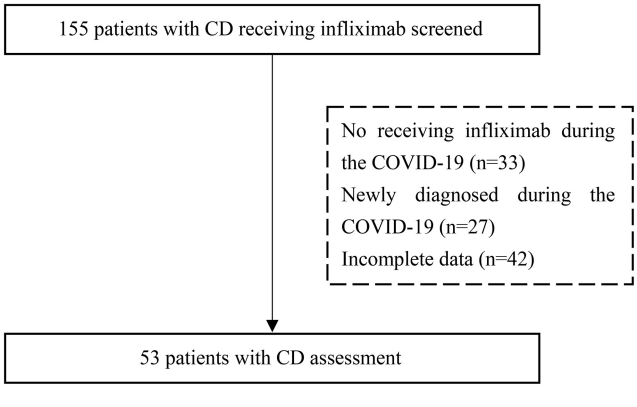

FIGURE 1 | A flow chart of the inclusion process for patients with Crohn's disease who were treated with infliximab. CD, Crohn's disease.

and Wilcoxon rank-sum test were used where appropriate. Categorical variables were described as numbers with percentages. Pearson's chi-squared test was used for independent categorical variables. Univariate analysis was performed to identify the baseline variables that were significantly different between the delayed and non-delayed groups. Multivariable logistic regression analyses were performed to determine associations with short-term influence and baseline characteristics. The factors with $p < 0.1$ in univariate analysis were included in multivariate analysis. A Kaplan-Meier curve was used to evaluate the surgical or readmission events. Propensity score matching (PSM) was applied in this study to increase the comparability between the two groups. A binary logistic regression test was performed to generate a propensity score for each patient with and without delayed treatment by the significant factors. Subsequently, a one-to-one match between both groups was obtained using the nearest-neighbor matching method with a Caliper value of 0.2 (21). All reported *P*-values are two-tailed, and $p < 0.05$ indicated statistical significance. Analyses were performed with SPSS Statistics version 26.0 software.

Ethical Considerations

The study was approved by the Xiangya Hospital of Central South University Ethics Committees, and each subject provided written, informed consent prior to study participation.

RESULTS

Characteristics of the Study Population

A total of 155 patients with Crohn's disease were included in this study. A schematic diagram of the selection process is shown in **Figure 1**. Fifty-three patients met the inclusion criteria. The patients included 34 (64.2%) males and 19 (35.8%) females with a median age of 26.0 years (IQR 18.5–36.0). Approximately three-quarters (71.7%) of patients did not receive treatment as planned with an average delay of 43.1 ± 28.3 days.

Table 1 presents the baseline data between both groups. Patients in the delayed group had significantly lower CDAI scores [89.7 (58.5, 116.9) vs. 125.9 (99.0, 200.8), $p = 0.030$] and C-reactive protein (CRP) levels [3.8 (1.5, 12.1) vs. 8.9 (2.0, 39.4), $p = 0.030$] than those in the non-delayed group. No differences in sex or age were noted between the groups. Disease duration, other serological indicators, and the utilization of telemedicine at baseline were similar in the delayed group compared with the non-delayed group ($p > 0.05$).

Comparison of the Delayed and Non-delayed Groups

On univariable analysis, the baseline characteristics that predicted delayed treatment during the COVID-19 epidemic included lower CDAI scores (OR = 0.0991, 95% CI = 0.982–1.000, $p = 0.05$) and lower CRP levels (OR = 0.965, 95% CI = 0.933–0.998, $p = 0.038$), as presented in **Table 2**. Additionally, lower CRP levels (OR = 0.967, 95% CI = 0.935–1.000, $p = 0.047$) were the only significant predictor of a delayed infusion of infliximab for CD patients in the multivariable analysis.

Characteristics of the Study Population After Propensity Score-Matched Analysis

To circumvent covariate imbalance between the two groups, a 1:1 propensity score-matched analysis was performed, resulting in 15 patients in the delayed group being matched with 15 non-delayed patients. There were no significant differences in the baseline CDAI score [135.6 (94.1, 185.2) vs. 125.9 (99.0, 200.8), $p = 1.000$] and CRP levels [10.4 (2.4, 21.5) vs. 8.9 (2.0, 39.4), $p = 0.540$] after matching, nor in the remaining variables ($p > 0.05$). The baseline characteristics after propensity score matching are indicated in **Table 3**.

Short- and Long-Term Effects of Delayed Treatment

On the short-term efficacy assessment, data after matching for details reported in **Table 4** indicated that patients in the non-delayed group experienced a greater decrease in CDAI score compared to the delayed group [−43.3 (−92.7, −9.7) vs. −17.3 (−29.0, 79.9), $p = 0.038$]. Delayed treatment appeared to have minimal impacts on ESR levels, CRP levels, hematocrit levels, PSQI score, and change in clinical decision making, whereas trends toward increased weight and BMI were noted in the delayed group ($p = 0.070$).

Considering the long-term influence, the continuous follow-up of enrolled patients after matching showed that the delayers had a higher readmission rate (33.3% vs. 0%, $p = 0.014$) and a higher surgery rate, although the difference was not statistically significant ($p = 0.068$). Outcomes evaluated for each endpoint are displayed in **Table 4** and **Figure 2**.

DISCUSSION

The advent of biologics has presented new options for the treatment of inflammatory bowel disease, and infliximab is the most widely used biological agent in the clinic (22). However,

TABLE 1 | Baseline characteristic of the enrolled patients.

Variable	Total patients ($n = 53$)	Delayed group ($n = 38$)	Non-delayed group ($n = 15$)	P^*
Male, No. (%)	34.0 (64.2)	23.0 (60.5)	11.0 (73.3)	0.381
Age, median (IQR), year	26.0 (18.5, 36.0)	27.0 (19.0, 38.0)	24.0 (16.8, 32.3)	0.243
Height, median (IQR), cm	167.0 (160.0, 170.0)	165.0 (160.0, 170.0)	170.0 (165.3, 172.5)	0.052
Weight, median (IQR), kg	52.5 (48.0, 60.0)	53.0 (48.0, 60.0)	51.3 (43.8, 63.0)	0.901
BMI, median (IQR), kg/m^2	18.9 (17.6, 21.1)	19.0 (17.7, 21.3)	18.5 (16.2, 21.1)	0.343
Disease course, median (IQR), year	2.0 (1.0, 5.0)	3.0 (1.0, 5.0)	1.0 (0.7, 3.9)	0.051
CDAI, median (IQR)	96.6 (60.1, 144.0)	89.7 (58.5, 116.9)	125.9 (99.0, 200.8)	0.030
Utilization of telemedicine, No. (%)	26.0 (49.1)	21.0 (55.3)	5.0 (33.3)	0.150
Serological parameters				
ESR, median (IQR), mm/h	34.0 (18.5, 62.5)	34.0 (19.5, 54.3)	38.0 (15.5, 77.5)	0.657
CRP, median (IQR), mg/L	4.4 (1.6, 15.2)	3.8 (1.5, 12.1)	8.9 (2.0, 39.4)	0.030
Hematocrit, median (IQR), %	38.6 (22.6, 42.1)	39.4 (36.5, 44.5)	40.6 (36.6, 44.6)	0.778

BMI, body mass index; CDAI, Crohn's Disease Activity Index score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IQR, interquartile range.
**Mann-Whitney U test for continuous variables and chi-square for proportions.*

TABLE 2 | Univariable and multivariable analyses of risk factors of delayed treatment.

Variable	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*p*	HR (95%CI)	*p*
Male	0.392 (0.094–1.634)	0.198		
Age, year	1.040 (0.980–1.103)	0.192		
Height, cm	0.904 (0.810–1.008)	0.071		
Weight, kg	1.000 (0.933–1.072)	0.998		
BMI, kg/m^2	1.131 (0.900–1.421)	0.29		
Disease course, year	1.241 (0.940–1.639)	0.127		
CDAI	0.991 (0.982–1.000)	0.05		
Utilization of telemedicine	0.476 (0.135–1.681)	0.476		
Serological parameters				
ESR, mm/h	0.993 (0.975–1.012)	0.462		
CRP, mg/L	0.965 (0.933–0.998)	0.038	0.967 (0.935–1.000)	0.047
Hematocrit, %	1.023 (0.927–1.128)	0.652		

BMI, body mass index; CDAI, Crohn's Disease Activity Index score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; HR, hazard ratio; CI, confidence interval.

there are issues with the required intravenous injection, especially with nonhuman biological agents that are prone to allergic reactions; specifically, patients have to physically come to the hospital for infusion (23). Due to the COVID-19 outbreak, patients opted to decrease their chances of contracting COVID-19 by providing home self-care rather than going to the hospital for regular care, which has caused a greater than 50% reduction in hospitalization volume (24). On the other hand, whether infliximab increased the risk of viral infection remained ambivalent early in the epidemic (25, 26). Above all, Crohn's disease patients experienced great difficulty in adhering to the infliximab schedule. In addition, the prognostic impact of a short delay in treatment on Crohn's disease remains unknown.

In this study, the final cohort of 53 patients with Crohn's disease previously treated with standard infliximab demonstrated a delayed rate of 71.7%, which is considerably increased compared with 26.4% in Khan's study (20). This finding may be related to policies in different countries at the beginning of the epidemic as well as the population's sense of self-protection. Additionally, this study suggested that delayed treatment was significantly associated with the CDAI score and CRP level at baseline. CRP is an acute-phase protein that increases with the aggravation of inflammatory reactions (27) and can also objectively respond to disease activity in CD patients (28). Therefore, the reason for this effect might be attributed to the fact that the more severe disease patients have, the more the disease is actively treated.

For the short-term effects, this study found that delayed treatment with infliximab led to a lower decrease in the CDAI score, suggesting worse control of symptoms in these patients, but this finding is not consistent with the results of serological indicators, such as CRP and ESR. However, these indicators are not specific markers of intestinal inflammation; other factors (such as infection or extraintestinal inflammation) can also

TABLE 3 | Baseline characteristic of the enrolled patients after propensity score-matched analysis.

Variable	Total patients (n = 30)	Delayed group (n = 15)	Non-delayed group (n = 15)	P*
Male, No. (%)	22.0 (73.3)	11.0 (73.3)	11.0 (73.3)	1.000
Age, median (IQR), year	24.0 (17.8, 33.8)	23.5 (18.0, 35.3)	24.0 (16.8, 32.3)	0.697
Height, median (IQR), cm	170.0 (160.0, 172.0)	165.0 (160.0, 170.0)	170.0 (165.3, 172.5)	0.131
Weight, median (IQR), kg	50.5 (44.8, 57.8)	50.5 (45.6, 55.1)	51.3 (43.8, 63.0)	0.473
BMI, median (IQR), kg/m^2	18.3 (16.2, 21.1)	18.3 (15.9, 21.2)	18.5 (16.2, 21.1)	0.822
Disease course, median (IQR), year	2.0 (1.0, 4.3)	3.0 (1.5, 4.5)	1.0 (0.7, 3.9)	0.093
CDAI, median (IQR)	130.4 (101.9, 188.9)	135.6 (94.1, 185.2)	125.9 (99.0, 200.8)	1.000
Utilization of telemedicine, No. (%)	12.0 (40.0)	7.0 (46.7)	5.0 (33.3)	0.456
Serological parameters				
ESR, median (IQR), mm/h	42.5 (23.0, 76.5)	45.0 (33.3, 74.3)	38.0 (15.5, 77.5)	0.667
CRP, median (IQR), mg/L	9.2 (2.1, 25.5)	10.4 (2.4, 21.5)	8.9 (2.0, 39.4)	0.608
Hematocrit, median (IQR), %	39.2 (36.4, 44.4)	39.1 (36.3, 41.4)	40.6 (36.6, 44.6)	0.377

BMI, body mass index; CDAI, Crohn's Disease Activity Index score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IQR, interquartile range.
**Mann-Whitney U-test for continuous variables and chi-square for proportions.*

TABLE 4 | The comparison of the effect of delayed treatment between both groups after propensity score-matched analysis.

Variable	Total patients (n = 30)	Delayed group (n = 15)	Non-delayed group (n = 15)	P*
Short-term effect				
Weight, median (IQR), kg	53.5 (46.4, 60.4)	53.5 (44.3, 57.3)	52.5 (46.3, 62.4)	0.759
ΔWeight, median (IQR), kg	0 (−1.5, 3.3)	1.5 (−0.4, 4.0)	−0.8 (−1.9, 0.6)	0.070
BMI, median (IQR), kg/m^2	18.8 (16.3, 21.4)	18.9 (16.1, 22.5)	18.7 (16.3, 21.2)	0.697
ΔBMI, median (IQR), kg/m^2	0 (−0.5, 1.2)	0.5 (−0.1, 1.4)	−0.3 (−0.7, 0.2)	0.070
CDAI, median (IQR)	103.7 (79.4, 185.2)	135.8 (85.4, 235.5)	91.7 (67.9, 121.9)	0.093
ΔCDAI, median (IQR)	−27.1 (−70.8, 34.4)	−17.3 (−29.0, 79.9)	−43.4 (−92.7, −9.7)	0.038
Change in clinical decision making, No. (%)	9.0 (30.0)	5.0 (16.7)	4.0 (13.3)	0.69
PSQI, median (IQR)	4.0 (3.0, 5.5)	4.5 (4.0, 6.8)	4.0 (2.0, 5.0)	0.156
Serological parameters				
ESR, median (IQR), mm/h	40.0 (17.0, 68.0)	35.0 (18.3, 73.3)	42.0 (12.3, 61.8)	0.886
ΔESR, median (IQR), mm/h	−2.0 (−23.3, 10.8)	−2.0 (−26.8, 8.0)	−4.5 (−22.5, 19.5)	0.667
CRP, median (IQR), mg/L	9.1 (2.0, 23.9)	10.2 (1.8, 36.0)	9.1 (5.6, 16.8)	1.000
ΔCRP, median (IQR), mg/L	0.6 (−2.4, 9.2)	1.0 (−2.1, 12.3)	−4.5 (−22.5, 19.5)	0.313
Hematocrit, median (IQR), %	40.1 (34.7, 44.2)	40 (34.9, 43.7)	41.4 (33.9, 44.8)	0.728
ΔHematocrit, median (IQR), %	1.6 (−3.4, 3.4)	1.0 (−1.9, 3.3)	2.2 (−4.5, 4.1)	0.951
Long–term effect				
Surgery, No. (%)	3 (10.0)	3 (20.0)	0 (0.0)	0.068
Readmission, No. (%)	5 (16.7)	5 (33.3)	0 (0.0)	0.014

BMI, body mass index; CDAI, Crohn's Disease Activity Index score; PSQI, Pittsburgh sleeps quality index score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IQR, interquartile range; Δ, the difference between the recent value of the variable and the baseline value.
**Mann-Whitney U test for continuous variables and chi-square for proportions.*

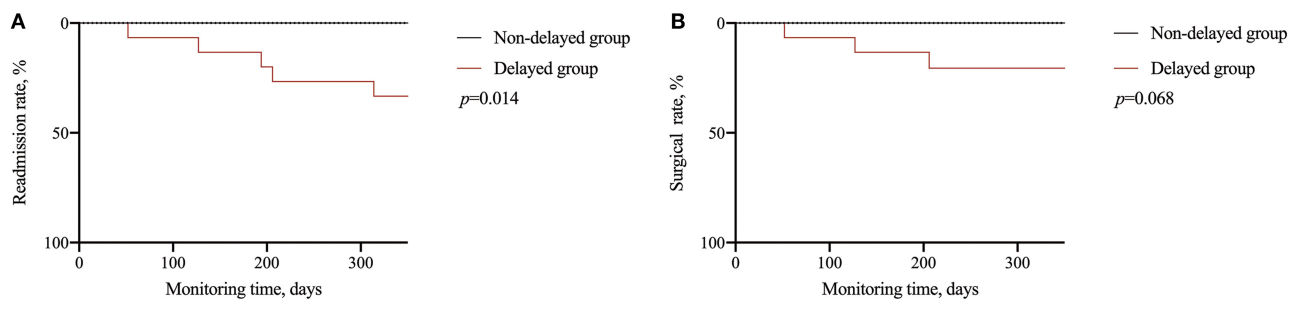

FIGURE 2 | Long-term effect of delayed treatment. **(A)** Comparison of readmission rate; **(B)** comparison of surgical rate.

affect the results (29). Indicators, such as endoscopic activity score and fecal calprotectin, should be included in further studies. For long-term prognosis, this study identified that delayed treatment with infliximab increased the readmission and operation rates of patients, which may be due to the lower infliximab concentrations in blood. Previous studies have shown that the interval of administration was closely related to the blood infliximab concentration and treatment effects. During maintenance treatment of IBD patients, the trough concentrations of infliximab in patients treated with a 4-week interval was significantly higher than that of patients treated with an 8-week interval (30). The trough concentration of infliximab was positively correlated with the remission rate of IBD (31). In addition, Scaldaferri et al. found that the noncompliance of patients during infliximab treatment could significantly increase the concentration of the antibody in serum and the rate of secondary treatment failure (32). Khan's study also showed that irregular infusion of biological agents was significantly associated with the rate of glucocorticoid-free remission during the COVID-19 epidemic (20).

There are some innovations in the study. First, previous studies mostly focused on the impact of long-term irregular delayed infliximab treatment on the prognosis of patients with Crohn's disease (33, 34), whereas this study showed that the short-term delay in infliximab treatment during the COVID-19 pandemic may also affect the long-term prognosis of patients. Second, the propensity matching method was used to eliminate possible confounding factors, thus increasing the comparability between the two groups of patients and making the results more reliable. However, this study is a single-center retrospective study with a small sample size, which needed for further verification.

CONCLUSIONS

In conclusion, the study suggested that approximately three-quarters of CD patients receiving infliximab regularly have delays in their treatment during the COVID-19 epidemic, thereby affecting which the independent risk factor was CRP levels at baseline. Delayed treatment may not only aggravate short-term disease activity but also increase the readmission rate in the future. Therefore, implementation of infliximab infusion based on an effective schedule and education programs for patients about the relative safety of these biologic medications are needed for patients and clinicians despite the COVID-19 pandemic.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The study was approved by the Xiangya Hospital of Central South University Ethics Committees, and each subject provided written, informed consent prior to study participation. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YL, LC, SC, and XL: study concept and design and critical revision of the manuscript for important intellectual content. SC and LC: acquisition of data. XL and YL: analysis and interpretation of data. YL and LC: statistical analysis and drafting of the manuscript. All authors approved the final version of the manuscript, including the authorship list.

FUNDING

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81770584 and 81700464) and Natural Science Foundation of Hunan Province (No. 2018JJ3841).

ACKNOWLEDGMENTS

We thank AJE (www.aje.com) for its linguistic assistance during the preparation of this manuscript.

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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.

frontiers in Medicine

ORIGINAL RESEARCH
published: 16 February 2022
doi: 10.3389/fmed.2022.813367

Perceived Stigma and Self-Efficacy of Patients With Inflammatory Bowel Disease-Related Stoma in China: A Cross-Sectional Study

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Background: Patients with inflammatory bowel disease (IBD)-related stoma face physical, psychological, and social adjustment challenges. However, knowledge about stigmatization and self-management, which is important for clinical care and patient education strategies, is lacking.

Objective: To evaluate the level of stigma and self-management ability of Chinese patients with IBD-related stoma using an online questionnaire.

Methods: Participants were recruited from two general hospitals in mainland China and the internet platforms of the China Crohn's and Colitis Foundation (CCCF). Participants completed a questionnaire, which gathered data on sociodemographic, clinical, and experience in online groups, self-efficacy scale, and social impact scale. The influencing factors of self-efficacy and perceived stigma were assessed by stepwise multivariate regression analyses.

Results: One hundred and seventy-six respondents were included. Most of the respondents (78/176, 44.32%) spent between 500 and 999 RMB ($77–153) on ostomy care accessories monthly. Three patients reported using homemade ostomy products. The average score on the self-efficacy scale was 75.79 ± 23.91, which reflected a moderate level of self-efficacy, and 69 (39.2%) respondents had low-level self-efficacy. The average social impact scale score was 62.76 ± 12.69, which reflected a moderate level of perceived stigma. Forty-three (24.43%) patients experienced severe levels of perceived stigma. Stepwise multivariate regression analysis revealed that self-efficacy was associated with educational level ($P = 0.007$), whereas stigma was associated with nursing privacy ($P = 0.021$) and acceptance by the closest person ($P = 0.005$). A total of 91% of respondents who participated in online peer support groups believed the groups were helpful for disease management.

Conclusions: Chinese patients with IBD-related stoma reported a moderate degree of perceived stigma; their level of self-efficacy was low to moderate. High educational level was associated with high self-efficacy. Notably, acceptance of the stoma by the closest person was an influencing factor protecting patients from perceived stigma. Interventions

OPEN ACCESS

Edited by:
Antonietta G. Gravina, University of Campania Luigi Vanvitelli, Italy

Reviewed by:
Raffaele Pellegrino, University of Campania Luigi Vanvitelli, Italy
Dennis Frohlich, Bloomsburg University, United States

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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *13 November 2021*
Accepted: *24 January 2022*
Published: *16 February 2022*

Citation:
Wang Y, Li S, Gong J, Cao L, Xu D, Yu Q, Wang X and Chen Y (2022) Perceived Stigma and Self-Efficacy of Patients With Inflammatory Bowel Disease-Related Stoma in China: A Cross-Sectional Study. Front. Med. 9:813367. doi: 10.3389/fmed.2022.813367

aimed at improving patient education, reducing economic burden, and strengthening social support should be considered to help improve the living conditions of patients with IBD-related stoma.

Keywords: inflammatory bowel disease, stoma, self-efficacy, perceived stigma, online peer support group

INTRODUCTION

Inflammatory bowel disease (IBD) is a group of diseases characterized by chronic inflammation of the digestive tract. The prevalence of IBD is increasing globally, and it is expected to continue to increase in the future, with important implications for health and the economy (1). IBD incidence in China, which includes up to 11.6 ulcerative colitis (UC) cases per 100,000 person-years and 1.4 Crohn's disease (CD) cases per 100,000 person-years, is estimated to be the highest in Asia (2–4). Ostomy is an effective and commonly used treatment option for refractory or severe IBD (5). However, post-ostomy changes and complications, such as altered body image, stool leakage, social isolation, ostomy-related dermatitis, sexual dysfunction, psychological distress, and perceived loss of control, result in decreased quality of life (6–8). Given these circumstances, patients with stoma are at risk of experiencing perceived stigma, which describes the degree to which individuals perceive discrimination directed at them or others (9). Perceived stigma is associated with decreases in self-efficacy and is a significant predictor of poorer outcomes in patients with IBD (10). According to a survey, stoma was the most embarrassing complication perceived by patients with IBD (9). A Chinese study reported 44% of participants with colorectal cancer (CRC)-related stoma experienced high levels of stigma (11). However, the level of stigma in patients with IBD-related stoma has not been accurately measured yet.

Research on chronic diseases shows that self-efficacy is an important core concept of self-management and an important predictor of stoma health care management ability (12). Improving the self-management ability of patients with stoma after ostomy is the key to their smooth recovery and adaptation to new life from the perspective of psychosocial factors. Self-management focuses on patient's ability to manage their condition rather than treatment based within the healthcare system or centered on a healthcare professional. Therefore, this management strategy seeks to restore patient's autonomy, position patients at the center of their own management process, and help them acquire and maintain competencies to enable them to efficiently manage their condition (13). From the limited data available, the self-efficacy level of patients with IBD-related stoma is unclear, whereas those with CRC-related stoma showed a moderate level (14).

Rapid development in internet technology and digital interventions (accessed via computers and mobile phones) that provide self-management information has been proposed as a promising mode of self-management intervention (13). Studies have estimated that more than 50% of patients with IBD use the internet as a source of information for disease management (15, 16). More people are participating in online peer-led social support groups, which connect people in similar circumstances and transcend geographical limitations (17–19). The function, importance, and future development strategy of online peer support groups need to be explored further.

To date, no studies have been published on stigma and self-management in patients with IBD-related stoma in China. This study seeks to address these research questions: (1) Is the extent of perceived stigma and self-efficacy in patients with IBD-related stoma consistent with those with CRC-related stoma? (2) Which social and disease factors affect perceived stigma and self-efficacy of patients with IBD-related stoma? (3) Is participating in online groups helpful for patients with IBD-related stoma in disease management?

METHODS

Study Design and Participants

A cross-sectional observational study was conducted among Chinese patients with IBD-related stoma through an electronic questionnaire in two hospitals in China from March 16 to April 23, 2021. The questionnaire was produced using Wenjuanxing (20), a professional online questionnaire tool. It was distributed to patients at the IBD clinic of the Second Affiliated Hospital of Zhejiang University Medical College and the general surgery clinic of the General Hospital of Eastern Theater Command. Additionally, the survey was posted on the internet platforms of the China Crohn's and Colitis Foundation (CCCF) to facilitate the collection of questionnaires. The inclusion criteria were patients who: (1) gave informed consent; (2) were 18 years of age or older; (3) diagnosed as CD, UC or indeterminate colitis; (4) with a stoma whether it was a colostomy or an ileostomy and was expected to be permanent or temporary; (5) being able to complete Web-based surveys in Chinese. Diagnoses of IBD were confirmed by reviewing medical records and querying treating physicians directly as needed. Identified charts were reviewed by IBD experts in detail, including clinic notes, hospitalization records, endoscopic evaluations, surgical reports, laboratory testing, microbiological testing, radiologic studies, and pathology results. Patients with questionable IBD diagnoses were excluded. The exclusion criteria included patients with a history of mental illness, cancer or other serious physical illnesses. As shown in **Figure 1**, the questionnaire was modified based on feedback from the pilot samples and was iteratively refined by IBD experts. This study was approved by the medical ethics committee of the Second Affiliated Hospital of Zhejiang University Medical College (approval number: 2021–0237).

Abbreviations: AIDS, Acquired immune deficiency syndrome; CCCF, China Crohn's & Colitis Foundation; CD, Crohn's disease; CRC, Colorectal cancer; IBD, Inflammatory bowel disease; SIS, Social impact scale; UC, Ulcerative colitis.

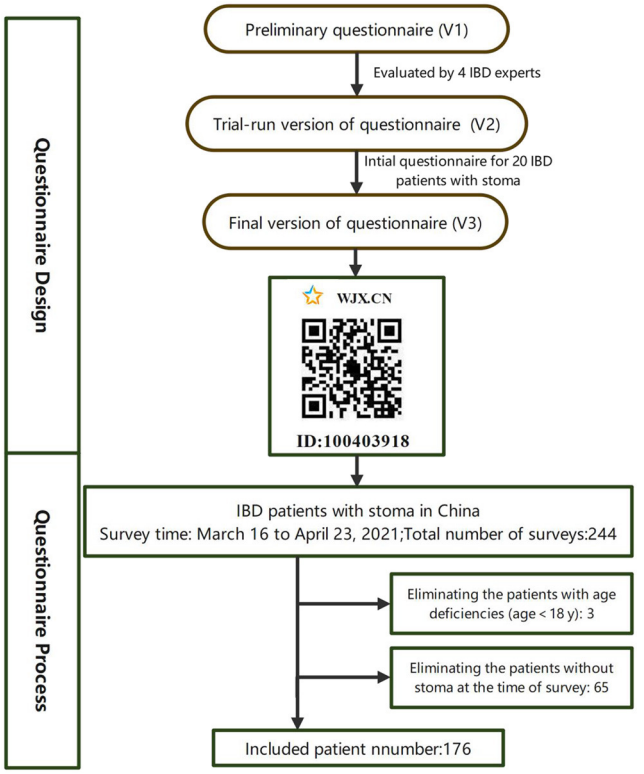

FIGURE 1 | Flow chart depicting the selection of the survey sample for analysis.

Measurements

Participants were asked to report the following sociodemographic and clinical information: age, gender, education level, employment status, place of residence, marital status, IBD diagnosis, disease duration, remission status, income, health insurance status. Study data also included type of stoma, time of ostomy, cost of stoma supplies, complications of stoma, body image, privacy of care for the stoma, ostomy leaks and difficulty of stoma care.

Information about social support was obtained, including acceptance of stoma by the closest person, and the main source of psychological support was obtained. Participants were asked to provide information on their experiences in online groups, and if so, they were asked about topics of interest, their activity state, feelings of participation in online groups and whether it's helpful. The subjects who indicated "it's helpful" were further asked about what aspects of online groups were perceived to be helpful. They could choose out of options including "increased sense of belonging," "gained confidence in the management of stoma or disease," "helped to made up my mind to undergo ostomies," "released the negativity and resonated with fellows," "drew strength from helping others" or fill in any other answers.

The self-efficacy scale by Bekkers (21) in 1996 is currently the most tested and widely used scale for measuring the level of self-efficacy of patients with stoma. The scale comprises 28 items, two dimensions (stoma care self-efficacy and social self-efficacy), and six separate items, which are scored on a 5-point scale (1 = no confidence, 2 = slightly confident, 3 = fairly confident, 4 = highly confident, and 5 = extremely confident). The total score ranges from 28 to 140, and is divided into three grades: ≤65 indicates low-level self-efficacy, 66–102 indicates moderate-level self-efficacy, and ≥103 indicates high-level self-efficacy. Wu et al. (22) from Hong Kong developed a Chinese version of the self-efficacy scale for patients with stoma, authorized this study to use the Chinese version. In this study, Cronbach's α coefficient of the scale was 0.926.

The social impact scale (SIS) was developed by Fife and Wright (23) in 2000 and translated into Chinese by Pan et al. (24) in 2007. It was originally used to assess stigma among patients with acquired immune deficiency syndrome (AIDS) and cancer. This scale contains 24 items and examines four domains of perceived stigma—social rejection, financial insecurity, internalized shame, and social isolation. The items are scored from 1 (strongly agree) to 4 (strongly disagree). Average item scores were classified into three levels: mild (1–1.99), moderate (2–2.99), or severe (3–4) stigma. Pan authorized this study to use the Chinese version. In this study, Cronbach's α coefficient was 0.949.

Statistical Analysis

All statistical analyses were performed using the SPSS software (version 25, IBM Corporation). Demographic data and stoma-related information were summarized as means and standard deviations for continuous variables after testing for normal distribution and as frequency counts (percentages) for categorical variables. The means and standard deviations of stigma and self-efficacy scores were calculated and reported as high, moderate, or low. The mean scores were compared with different characteristics using *t*-tests (for two-level variables, such as gender) or one-way ANOVA (for variables with three or more levels, such as employment status) to determine the associations between demographic data or stoma-related information and dependent variables (self-efficacy or stigma level). Least significant difference corrections were used for *post-hoc* comparisons. An α-level of 0.05 was used to statistical significance.

Multivariate linear regressions (with stepwise variable selection) were used to explore the main factors that influenced stigma or self-efficacy among patients with stoma. Collinearity diagnostics was used to exclude correlations among independent variables. The variables for which the results of univariate linear regression analysis were statistically significant were used as independent variables. The inclusion standard was 0.05, and the removal standard was 0.10.

RESULTS

Participant Characteristics

As shown in **Figure 1**, a total of 244 questionnaires were completed. Only 176 questionnaires were valid. Respondents came from 24 provinces and cities across the country. The male-female ratio of the respondents was almost 1:1(89 males vs. 87

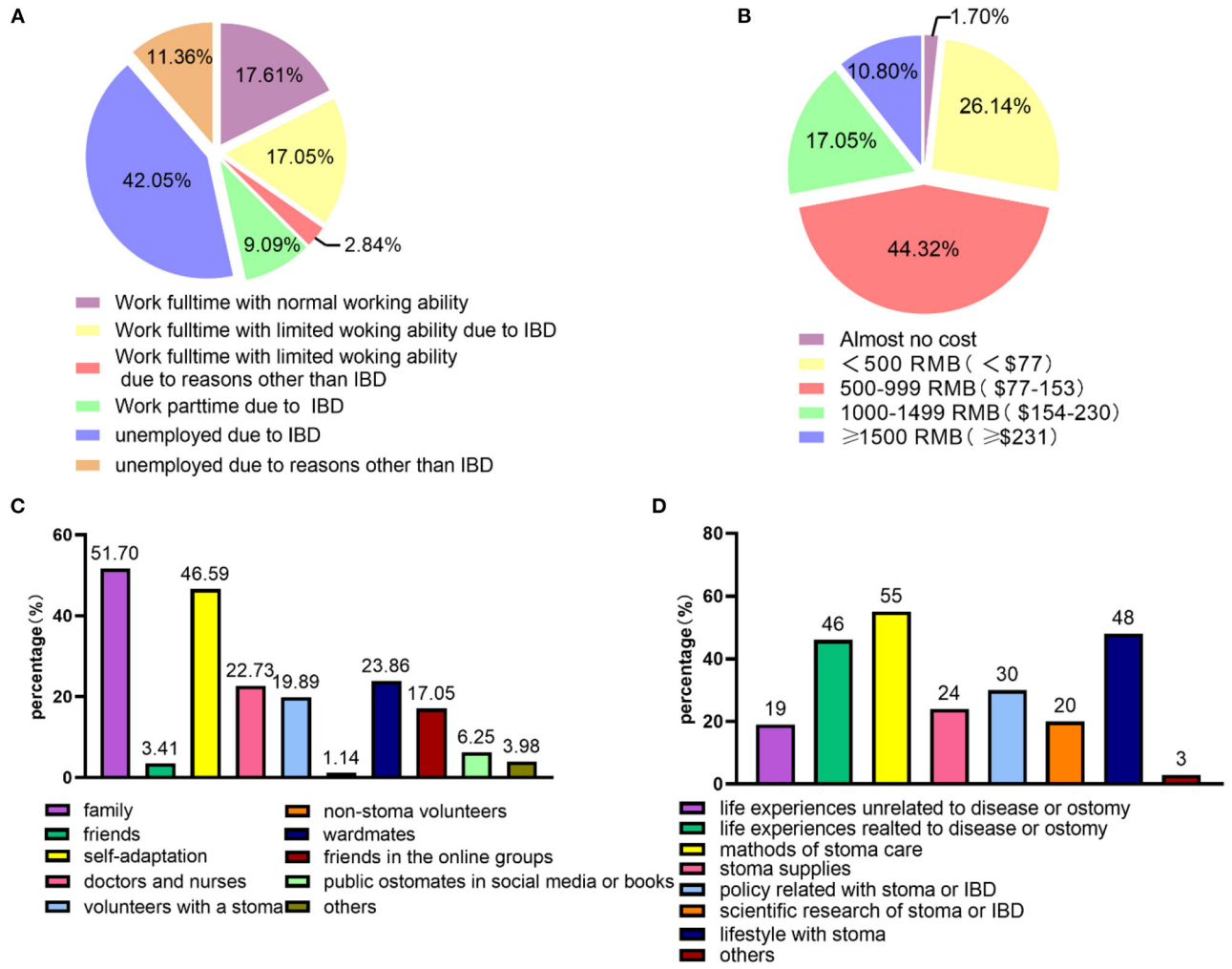

FIGURE 2 | Analysis of the status or feelings of patients with IBD stoma regarding job, costs, physiological support, and online peer support groups. **(A)** Employment status of patients with IBD-related stoma ($n = 176$). **(B)** Costs of ostomy care accessories per month (equivalent to USD, $n = 176$). **(C)** The main source of psychological support after stoma surgery ($n = 176$). **(D)** Topics of interest in online peer support groups ($n = 100$).

females). The mean age was 37.10 ± 9.98 years (range: 18–64 years). Out of 176 respondents, 156 (87.5%), 16 (9.09%), and 6 (3.41%) respondents reported a diagnosis of CD, UC, and unformed colitis, respectively. Regarding the type of stoma, 97 (55.11%) participants had a temporary stoma and planned to retract it in the future, 70 (39.77%) participants had a permanent stoma, and 9 (5.11%) participants had no idea about the type of stoma. With respect to the surgical procedure participants had undergone, 126 (71.59%), 36 (20.45%), and 5 (2.84%) participants underwent ileostomy, colostomy, and jejunostomy, respectively. Approximately 39.2% of the respondents had been diagnosed with IBD for more than 10 years, whereas, 42.6% had a stoma for 1–5 years.

Self-Efficacy of Patients With IBD-Related Stoma

Before ostomy, 67 (38.07%) participants decided the location of the stoma with their doctors, whereas 36 (20.45%) participants reported no communication with their doctors. Fifty-nine (33.52%) of participants were only informed of the location of the stoma. The ostomy affected the employment status of participants. As shown in **Figure 2A**, only 17.61% of participants worked full-time and maintained normal working ability; 9.09% of the participants worked part-time; and 42.05% of the patients were unemployed. Concerning the ostomy care accessories. most of the respondents (78/176, 44.32%) spent between 500 and 999 RMB ($77–153) monthly, whereas 19 (10.8%) of participants spent more than 1,500 RMB ($231). Three patients chose to make homemade ostomy bags (**Figure 2B**). Additionally, the reimbursement rates for these costs were assessed. Although 34.1% of the respondents indicated that estimation was difficult, the reimbursement rate of most of the remaining patients (73/176, 42.2%) was < 10%. Only 23.69% of the patients exceeded 10%.

The average score of the self-efficacy scale was 75.79 ± 23.91. Sixty-nine (39.2%) respondents had a low level of self-efficacy, 77 (43.75%) had a moderate level of self-efficacy, and 30

TABLE 1 | Summary of the associations between demographic and stoma-related characteristics and self-efficacy ($n = 176$).

Characteristic	*n*	Self-efficacy Mean ± SD	*t/F(P)*[a]	Characteristic	*n*	Self-efficacy Mean ± SD	*t/F(P)*
Gender				**Place of residence**			
Male	89	77.27 ± 23.64	0.829(0.912)	City	119	76.99 ± 23.28	0.952(0.337)
Female	87	74.27 ± 24.22		Village	57	73.28 ± 25.19	
Age (years)				**Employment status**			
18–29	32	84.42 ± 22.81	6.118(*0.003*)	Full-time	66	85.53 ± 24.09	6.268(*0.002*)
30–49	126	76.03 ± 23.85		Part-time	16	75.91 ± 14.16	
≥50	18	59.98 ± 23.76		Unemployed	94	70.33 ± 23.77	
Duration of IBD (years)				**Education level**			
<1	11	67.03 ± 20.26	2.843(*0.026*)	Primary school	8	66.98 ± 12.43	7.719(*<0.001*)
1–2.99	25	71.86 ± 21.74		Junior high school	45	61.46 ± 18.82	
3–4.99	21	90.17 ± 28.80		High/technical secondary school	38	77.64 ± 27.44	
5–9.99	50	72.32 ± 21.07		Junior college	39	85.96 ± 22.11	
≥10	69	76.74 ± 24.25		College or beyond	46	81.51 ± 22.39	
Duration of stoma (years)				**Participation in activities with other stoma patients**			
<0.25	13	64.67 ± 19.36	3.293(*0.022*)	Yes	17	81.27 ± 26.45	0.995(0.321)
0.25–0.99	42	73.04 ± 19.21		No	159	75.20 ± 23.64	
1–5	75	81.80 ± 26.30		**Joined online groups of stoma patients**			
≥5	46	71.63 ± 21.99		Yes	100	79.53 ± 23.24	2.413(*0.017*)
Monthly household income (equivalent to US$)				No	76	70.87 ± 24.03	
<2,000 (<$308)	26	70.47 ± 19.75	5.228(*<0.001*)	**Acceptance of the stoma by people closest to you**			
2,000–4,999 ($308–771)	69	69.76 ± 22.27		Not at all accepted	10	59.98 ± 14.65	16.289(*<0.001*)
5,000–9,999 ($772–1,542)	51	77.63 ± 23.29		Not accepted	22	60.07 ± 15.93	
≥10,000 (≥$1,543)	30	91.13 ± 25.57		Basically accepted	97	72.08 ± 21.36	
				Completely accepted	47	94.68 ± 23.85	

[a]*Values in italics are significant at $P < 0.05$.*

(17.05%) had a high level of self-efficacy (17.05%). **Table 1** shows the associations between demographic data or stoma-related information and self-efficacy. Significant differences in self-efficacy scores were observed among different groups regarding age, duration of illness, duration of stoma, employment status, monthly household income, education level, membership in online groups, and acceptance of stoma by the closest person.

Perceived Stigma of Patients With IBD-Related Stoma

Most patients felt that their relatives accepted the stoma (88 accepted basically and 45 accepted completely), whereas 15 (8.52%) participants reported that relatives did not accept well, 7 (3.98%) participants were certain their relatives were unaccepting of the stoma.

The average score of the SIS scale was 62.76 ± 12.69. Twelve (6.82%) patients had mild levels of perceived stigma, 121 (68.75%) patients had moderate levels of perceived stigma, and 43 (24.43%) patients had severe levels of perceived stigma. **Table 2** shows the associations between demographic data or stoma-related information and perceived stigma. Significant differences in stigma scores were observed among different groups of patients regarding age, residence in rural areas, monthly household income, privacy of stoma care, image satisfaction, education level, presence of pocket leakage, stoma complications, and acceptance of the stoma by the closest person.

Influencing Factors of Perceived Stigma and Self-Efficacy

Multivariate stepwise linear regression analysis showed that age ($\beta = -0.211$, $P < 0.001$), stigma ($\beta = -0.555$, $P < 0.001$), and educational level (college or above vs. primary school) ($\beta = 3.388$, $P = 0.007$) were the main factors affecting the self-efficacy of patients with IBD-related stoma (**Table 3**). Self-efficacy ($\beta = -0.524$, $P < 0.001$), nursing privacy ($\beta = 0.146$, $P < 0.001$), acceptance of the stoma by the closest person ($\beta = -0.178$, $P = 0.005$), and age ($\beta = -0.132$, $P = 0.029$) were the main factors affecting stigma in patients with IBD-related stoma (**Table 4**).

TABLE 2 | Summary of the associations between demographic and stoma-related characteristics and perceived stigma ($n = 176$).

Characteristic	*n*	Stigma Mean ± SD	t/F(*P*)[a]	Characteristic	*n*	Stigma Mean ± SD	t/F(*P*)
Type of stoma (classified by time)				**Place of residence**			
Temporary	97	62.31 ± 12.03	−0.446(0.656)	City	119	61.06 ± 12.18	−2.615(*0.01*)
Permanent	70	63.21 ± 14.11		Village	57	66.32 ± 13.09	
Age (years)				**Pocket leakage in past 3 months**			
18–29	32	65.93 ± 14.43	0.957(0.386)	Yes	116	64.50 ± 12.63	2.568(*0.011*)
30–49	126	62.31 ± 11.91		no	60	59.40 ± 12.21	
≥50	18	63.72 ± 13.78					
Educational level				**Monthly household income (equivalent to US$)**			
Primary school	8	68.38 ± 12.27	4.399(*0.002*)	<2,000(<$307)	26	69.50 ± 13.47	5.903(<*0.001*)
Junior high school	45	68.58 ± 11.78		2,000–4,999($308–771)	69	65.14 ± 12.33	
High/technical secondary school	38	60.32 ± 13.31		5,000–9,999($772–1,542)	51	59.71 ± 10.61	
Junior college	39	58.79 ± 10.02		≥10,000(≥$1,543)	30	56.63 ± 12.45	
College or beyond	46	61.78 ± 13.38					
Acceptance of the stoma by people closest to you				**Privacy of care for the stoma**			
Not at all accepted	10	71.00 ± 7.44	13.751(<*0.001*)	Cannot be protected at all	11	71.86 ± 16.60	8.469(<*0.001*)
Not accepted	22	73.53 ± 16.27		Mostly cannot be protected	33	70.62 ± 11.82	
Mostly accepted	97	63.17 ± 10.43		Mostly can be protected	114	60.47 ± 11.65	
Completely accepted	47	54.47 ± 11.34		Completely can be protected	18	54.46 ± 10.82	
Body image				**Have ever had Complications**			
Not at all satisfied	68	68.63 ± 14.02	11.53(<*0.001*)	Yes	108	64.51 ± 10.76	2.33(*0.021*)
Not satisfied	60	62.21 ± 9.88		no	68	59.99 ± 14.92	
Well enough	43	55.95 ± 9.98					
Satisfied	5	48.00 ± 5.00					

[a] *Values in italics are significant at $P < 0.05$.*

Social Support and Online Peer Support Groups

"The most important source of psychological support or the main driving force for you to accept the stoma" was a multiple-choice question, and respondents could choose three choices at most. As shown in **Figure 2C**, the most popular choice was "family" (91/176, 51.7%), followed by "self-adaptation" (82/176, 46.59%). Some patients received psychological support from ward mates (42/176, 23.86%), doctors and nurses (40/176, 22.73%), volunteers with a stoma (35/176, 19.89%), and friends in the online groups (30/176, 17.05%).

In this survey, 100 respondents had experiences with online peer support groups. Further investigation revealed that most of them were "diving" in group discussions, barely interacted with others but paid attention to the events happening in the group (42/100, 42%), or occasionally expressed ideas and opinions on topics of interest (41/100, 41%). Regarding which messages or discussions were of much interest, discussions on the methods of stoma care were the most popular (55/100, 55%), followed by discussions on lifestyle modifications of patients with stoma, such as diet and exercise (48/100, 48%), and discussions on disease or ostomy-related life experiences of other patients (46/100, 46%) (**Figure 2D**). Of the 100 participants, 91 participants believed that group participation was helpful for individual disease or stoma management. This finding was corroborated by the finding that group participants helped fellow patients increase their sense of belonging (65/91, 71.43%) or their confidence in the management of stoma or disease (63/91, 69.23%). For some patients (19/91, 20.88%), participating in the group helped them decide on undergoing ostomies.

DISCUSSION

According to previous studies 0.66 and 1.5% of patients with IBD in the United Kingdom and United States undergo ostomies, respectively, but no authoritative statistics on IBD are available in China (25, 26). Patients with stoma represent a minor proportion of patients with IBD in China, according to our clinical practice conditions. Additionally, the current status of such patients are poorly recognized. Psychosocial assessments of patients with stoma have been increasingly performed in recent years, but the study population predominantly included patients with CRC (11, 14). Few studies have been conducted exclusively on patients with IBD-related stoma. To our knowledge, this study is the first to assess the levels of self-efficacy and stigma in patients with IBD-related stoma.

TABLE 3 | Factors influencing self-efficacy among patients with IBD-related stoma.

Model	B	β	*T*-value	*P*-value[a]
Constant	156.238		14.824	*<0.001*
Stigma	−1.046	−0.555	−9.329	*<0.001*
Educational level (Junior high school and high/technical secondary school)	6.158	0.105	1.558	0.121
Educational level (college or above)	9.310	3.388	2.748	*0.007*
Age	−0.551	−0.211	−3.569	*<0.001*

[a] *$F = 35.254$; $R^2 = 0.452$; values in italics are significant at $P < 0.05$.*

TABLE 4 | Factors influencing stigma among patients with IBD-related stoma.

Model	B	β	*T*-value	*P*-value[a]
Constant	93.998		20.228	*<0.001*
Self-efficacy	−0.278	−0.524	−7.955	*<0.001*
Nursing privacy	4.208	0.146	2.324	*0.021*
Acceptance of the stoma by the closest person	−5.567	−0.178	−2.840	*0.005*
Age	−0.183	−0.132	−2.207	*0.029*

[a] *$F = 34.191$; $R^2 = 0.444$; values in italics are significant at $P < 0.05$.*

In this study, the stigma level of patients with IBD-related stoma was consistent with that of patients with CRC-related stoma in other studies evaluated with the same SIS scale (11, 27). However, the average self-efficacy score seems to be slightly lower than the measurement level of the same scale for patients with CRC (75.79 ± 23.91 vs. 81.03 ± 16.30) (14). One obvious difference was that patients with IBD-related stoma were younger than those with CRC-related stoma (37.10 ± 9.98 vs. 60.95 ± 7.38) (14). Moreover, patients with IBD-related stoma usually have a longer course before surgery than patients with CRC-related stoma. Additionally, ostomy may only be a clinical remission rather than a cure; therefore, they need to prepare mentally to cope with the disease for life. Therefore, the present situation of patients with IBD-related stoma should be understood.

Our previous study revealed that economic burden is a major difficulty faced by Chinese patients with IBD, and low-income patients have worse disease control (28). Another Chinese study showed that 30.6% of patients with IBD spent over half of their income on medical costs, which is an independent factor that impairs their quality of life (29). Consistent with these results, 96.02% (169/176) of participants thought that a certain degree of economic burden is caused by disease and treatment in this study. Further analysis found higher self-efficacy ($P < 0.01$) and lower stigma scores ($P < 0.01$) among participants with monthly family income more than 10,000 RMB ($1,545) compared with those with family monthly income less than this value. The economic burden to patients with IBD-related stoma is substantial and cannot be ignored. Majority of the participant (108/176, 61.36%) in our survey experienced a stoma complication, which means more additional associated costs, not to mention that re-operation is needed in some cases. Ostomy products are consumables and require regular replacement, but the highest proportion of respondents (73/176, 42.2%) in this study reported the reimbursement ratio for cost is < 10%. Three respondents reported using their homemade ostomy products made of polythene bags and rubber rings to save money.

Educational level is one of the key factors that influence self-efficacy. Understanding that patients with higher educational levels are more likely to accept various issues in daily life, obtain pertinent medical knowledge and information, and use their knowledge and skills to solve problems, is not difficult. Economic and educational factors may partly explain why rural residents have a stronger sense of stigma than urban residents in this survey ($P = 0.01$). Compared with urban residents, rural populations have a lower proportion of medical insurance payments, have more restricted access to health care, and are relatively lacking in educational resources (30). Due to IBD (74/176, 42.05%) or other reasons (20/176, 11.36%), more than half of the respondents were unemployed. The self-efficacy score of full-time patients with stoma was higher than that of unemployed patients ($P < 0.01$). On the one hand, employment status directly affects economic resources and the ability to fund one's lifestyle establishes confidence and self-worth, as these patients can undertake social and family responsibilities. On the other hand, employment status affects their participation in community life, affecting their sense of belonging and ability to address challenges.

In this survey, the main source of postoperative psychological support for patients with stoma was family (91/176, 51.7%), which is related to the strong traditional family concept in China. Family members are highly interdependent, take care of each other, and fulfill their family relationship obligations. Social support mainly refers to emotional and material help and assistance from family, relatives, friends, and other members of society, such as colleagues, organizations, groups, and communities, which reflects the closeness and quality of one's social connections (31). Studies have shown that social support has an irreplaceable role in maintaining the stability of life for patient with stoma (31), and the ability to take care of stoma is related to the level of support received and emotional "readiness" (32). Our study confirmed that acceptance of stoma by the closest person remained as a significant factor for reduced stigma in the multivariate regression analysis.

Additionally, peer support is a form of social support, which is provided by specific groups with the same experience or similar demographic characteristics by sharing experiences or transmitting information (33). Online social networking represents a prominent form of communication in many people's lives. For individuals with stigmatized illnesses or difficulties in in-person interpersonal communication, social media makes connection with others who share similar health conditions to seek or disclose health information without revealing one's personal identity possible (34). As in this survey, 42% of the respondents were fully participating in the internet group's activities. Compared with spontaneous in-person encounters, social media users maintain greater control, meaning that they can choose their own level of engagement and the extent to which

they interact with others. Studies have confirmed that identifying with a social group can improve self-esteem and self-efficacy and reduce uncertainty (35). Consistent with these findings, the self-efficacy of patients who joined the online peer support group in this survey was higher than that of patients who did not ($P < 0.05$).

Among the respondents who joined the online support group, 91% of participants found the experience helpful to the management of disease or stoma by increasing the sense of belonging (65/91, 71.43%) and enhancing confidence in the stoma management (63/91, 69.23%). This finding is consistent with other results indicating that peer support promotes positive emotional support, allows venting of negative emotions, and helps patients find strategies to deal with challenging environments (36, 37). The fear and worry about ostomy is common among patients with IBD (26), but 20.88% of patients in this study agreed that the online peer support group gave them the courage and determination to undergo surgery. Among the online group discussion topics, the most popular were stoma nursing methods (55/100, 55%), lifestyle (48/100, 48%), and peer experience (46/100, 46%), which suggested that teaching and resources from medical staff are practical for stoma management, but they may be far inferior to peer patients in emotional value.

Stoma care is not a simple task, as patients need to adjust their diet, clothing, exercise, and other daily life activities. Additionally, they need to learn to change stoma pouches, handle excrement, prevent complications, and adapt their social lives. Few patients with IBD have stoma; therefore, regarding enterostomy, patients with CRC are more considered than patients with IBD. This is the first nationwide survey of patients with IBD-related stoma to assess patient's level of stigma and self-efficacy in China. Our findings reflect the current situation of Chinese patients with IBD stoma, and specialist physicians and nurses need to understand their specific situations. The results of this study are helpful in identifying patients prone to low self-efficacy or severe stigma in the clinic for timely adjustment of medical measures and provide individualized care. In addition, this study investigated the participation and interaction characteristics of patients with IBD stoma in online peer support groups to deepen our understanding of the role of online peer support. The study findings may be significant to the future planning of CCCF online group projects.

This study had few limitations. First, the cross-sectional study design precluded the possibility of ascertaining causality between risk factors and self-efficacy or stigma. Second, few patients with IBD have stoma; therefore, the study could not gather a large sample size. Third, the questionnaire was completed online; therefore, patients with limited access to the internet could not be included in our study, and these patients were likely to have poor economic power and disease control. Therefore, selection bias could be present in this study.

CONCLUSIONS

Chinese patients with IBD-related stoma reported a moderate sense of stigma and a low-to-moderate level of self-efficacy. Patients with stoma who completed higher education (college and above) had higher levels of self-efficacy. Additionally, this survey revealed that financial burden may have a direct effect on quality of stoma care. Therefore, strengthening patient education and reducing economic burden may be the keys to improving this situation. Patients whose relatives were not accepting of their condition experienced high levels of stigma. Online peer support groups are worthy of further promotion, as social support may have a significant impact on stigma among patients with IBD-related stoma.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of the Second Affiliated Hospital of Zhejiang University Medical College. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YC and YW conceived and designed the project. SL, JG, LC, and XW collected the data. YW and QY analyzed the data. YW, DX, and YC wrote the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

We would like to thank all of the patients who participated in the survey and thank the CCCF staff Jie Zhao for her help with recruiting participants. We also acknowledge Professor Helen Kit-Man Wu and Professor Ay-Woan Pan for authorization to use the scales.

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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.

frontiers in Public Health

ORIGINAL RESEARCH
published: 10 March 2022
doi: 10.3389/fpubh.2022.851295

Inflammatory Indexes for Assessing the Severity and Disease Progression of Ulcerative Colitis: A Single-Center Retrospective Study

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OPEN ACCESS

Edited by:
Antonietta G. Gravina, University of Campania Luigi Vanvitelli, Italy

Reviewed by:
Raffaele Pellegrino, University of Campania Luigi Vanvitelli, Italy
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Specialty section:
This article was submitted to Family Medicine and Primary Care, a section of the journal Frontiers in Public Health

Received: 09 January 2022
Accepted: 07 February 2022
Published: 10 March 2022

Citation:
Lin H, Bai Z, Wu Q, Chu G, Zhang Y, Guo X and Qi X (2022) Inflammatory Indexes for Assessing the Severity and Disease Progression of Ulcerative Colitis: A Single-Center Retrospective Study. Front. Public Health 10:851295. doi: 10.3389/fpubh.2022.851295

Background: Active and severe ulcerative colitis (UC) and non-response to 5-aminosalicylic acid (5-ASA) are related to poor outcomes and should be accurately identified. Several integrated inflammatory indexes are potentially useful to assess the disease severity in patients with acute or critical diseases but are underexplored in patients with UC.

Methods: Patients with UC consecutively admitted to our hospital between January 2015 and December 2020 were retrospectively grouped according to the activity and severity of UC and response to 5-ASA. The neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), neutrophil-to-platelet ratio (NPR), platelet-to-albumin ratio (PAR), C-reactive protein-to-albumin ratio (CAR), and C-reactive protein-to-lymphocyte ratio (CLR) were calculated. The areas under receiver operating characteristic curves (AUC) were calculated.

Results: Overall, 187 patients with UC were included, of whom 151 were active, 55 were severe, and 14 were unresponsive to 5-ASA. The active UC group had significantly higher NLR, PLR, SII, and PAR levels. SII had the greatest predictive accuracy for active UC, followed by PLR, PAR, and NLR (AUC = 0.647, 0.641, 0.634, and 0.626). The severe UC group had significantly higher NLR, PLR, SII, PAR, CAR, and CLR levels. CLR had the greatest predictive accuracy for severe UC, followed by CAR, PLR, SII, NLR, and PAR (AUC = 0.732, 0.714, 0.693, 0.669, 0.646, and 0.63). The non-response to the 5-ASA group had significantly higher CAR and CLR levels. CAR had a greater predictive accuracy for non-response to 5-ASA than CLR (AUC = 0.781 and 0.759).

Conclusion: SII, CLR, and CAR may be useful for assessing the severity and progression of UC, but remain not optimal.

Keywords: ulcerative colitis, inflammatory indexes, activity, severity, 5-aminosalicylic acid

INTRODUCTION

Ulcerative colitis (UC) is a relapsing and remitting mucosal inflammation often restricted to the colon and rectum, which may be associated with dysregulated immune response (1). The highest incidence and prevalence of UC is 4.6 per 100,000 person-years and 57.3 per 100,000 persons in Eastern Asia, respectively (2). Patients with active UC always suffer from embarrassing and painful symptoms, such as fecal incontinence, abdominal pain, bloody diarrhea, arthritis, and fatigue, and tend to develop poorer psychosocial outcomes than those with inactive UC (3). Active UC is usually classified into mild, moderate, and severe according to the recommendation by the international guideline (4). Generally, severe UC can bring more negative impact on the patient's quality of life, social and psychological wellbeing, healthcare resource utilization (5), and prognosis than mild-moderate UC (6, 7). Early medications can avoid the progression from mild-moderate to severe UC (7). 5-aminosalicylic acid (5-ASA) is the first-line choice of medication for patients with UC diagnosed within the first year (8). Usually, patients with severe UC are not well responsive to 5-ASA, leading to the use of corticosteroids, immunosuppressants, and biologics (9–12). Therefore, it is a clinical priority to identify patients who require more aggressive treatment to reach clinical remission.

In 1955, Truelove and Witts (13) established their criteria to explore the efficacy of cortisone medications in patients with UC. Currently, the Truelove and Witts criteria have been the cornerstone of assessing the severity of UC. However, it still has several potential limitations. The most critical limitation is that the definitions of improvement and worsening are ambiguous (14), as well as that of moderate UC. In 1987, Schroeder et al. (15) further developed the Mayo score by combining clinical symptoms, laboratory tests, and endoscopic findings. Despite one of the most commonly used scores for the severity of UC, it contains endoscopic procedures, which may be invasive, expensive, and time-consuming. Recently, the partial Mayo score (PMS) has been increasingly recognized, because it can properly discriminate this disease based on stool frequency, rectal bleeding, and physician's global assessment, but does not contemplate endoscopic data (16–18). However, the requirement of clinician's subjective evaluation of patient's symptoms remains its potential drawback (19).

Erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) are two traditional serologic biomarkers and are usually used for monitoring the disease course of UC, but their sensitivity and specificity are unsatisfactory (20). Recently, several integrated indexes have been used for the assessment of infective, acute, and critical diseases, including neutrophil-to-lymphocyte ratio (NLR) (21), platelet-to-lymphocyte ratio (PLR) (22), systemic immune-inflammation index (SII) (23), neutrophil-to-platelet ratio (NPR) (24), platelet-to-albumin ratio (PAR) (25), CRP-to-albumin ratio (CAR) (26), and CRP-to-lymphocyte ratio (CLR) (27). Notably, they are non-invasive and easier to use at a low cost. Thus, the purpose of this study is to determine the accuracy of inflammatory indexes for diagnosing active UC and severe UC and identifying the response to 5-ASA medication.

METHODS

Patient Selection

This is a single-center retrospective, cross-sectional study. We extracted the medical records of all UC patients who were consecutively admitted to the General Hospital of Northern Theater Command between January 1, 2015 and December 31, 2020 from the inpatient information system. The exclusion criteria were as follows: (1) medical records cannot be reviewed in detail; (2) patients were diagnosed as suspected UC and unclassified inflammatory bowel disease; (3) routine blood tests were missing; (4) history of colectomy; (5) co-existing conditions that potentially influence inflammatory indexes (i.e., severe trauma, pregnancy, liver cirrhosis, uremia, and malignancy); and (6) other autoimmune diseases (i.e., psoriasis, Behcet's disease, urticarial vasculitis, and rheumatoid arthritis). The study protocol was approved by the Ethical Committee of General Hospital of Northern Theater Command [Y (2021) 078] and conformed to the ethical guidelines of the 1975 Declaration of Helsinki. The requirement of informed written consent was waived because only the data from the inpatient's electronic medical records were extracted.

Data Collection

The patient's demographics, history of surgery, comorbidities, duration of UC, history of UC-related medication treatments, clinical symptoms of UC (i.e., abdominal pain, diarrhea, hematochezia, and fever), endoscopic reports, blood tests at admission, extra-intestinal manifestations, UC-related complications, UC-related medications during hospitalization, and length of stay (LOS) were manually extracted from the inpatient's electronic medical records. Fever was defined, if the body temperature, which was measured on the first day of admission according to the electronic medical records, was $> 37.3^{\circ}$C. The Montreal classification for disease extent of UC (28) and Mayo endoscopic subscore (29) were also assessed through endoscopic reports. Several inflammatory indexes, including NLR, PLR, SII, NPR, PAR, CAR, and CLR, were calculated. NLR was calculated as the neutrophil counts (10^9/L) divided by the lymphocyte counts (10^9/L) (21). PLR was calculated as the platelet counts (10^9/L) divided by the lymphocyte counts (10^9/L) (22). SII was calculated as the neutrophil counts (10^9/L) multiplied by the platelet counts (10^9/L) and divided by the lymphocyte counts (10^9/L) (23). NPR was calculated as the neutrophil counts (10^9/L) multiplied by 1,000 and divided by the platelet counts (10^9/L) (24). PAR was calculated as the platelet counts (10^9/L) divided by the albumin levels (g/L) (25). CAR was calculated as the CRP levels (mg/L) divided by the albumin levels (g/L) (26). CLR was calculated as the CRP levels (mg/L) divided by the lymphocyte counts (10^9/L) (27).

Groups

The patients were grouped according to the activity of UC, the severity of active UC, and response to 5-ASA. First, patients were classified into active and remission UC groups according to the modified Mayo score, which has different definitions of clinical activation and remission of UC as compared to

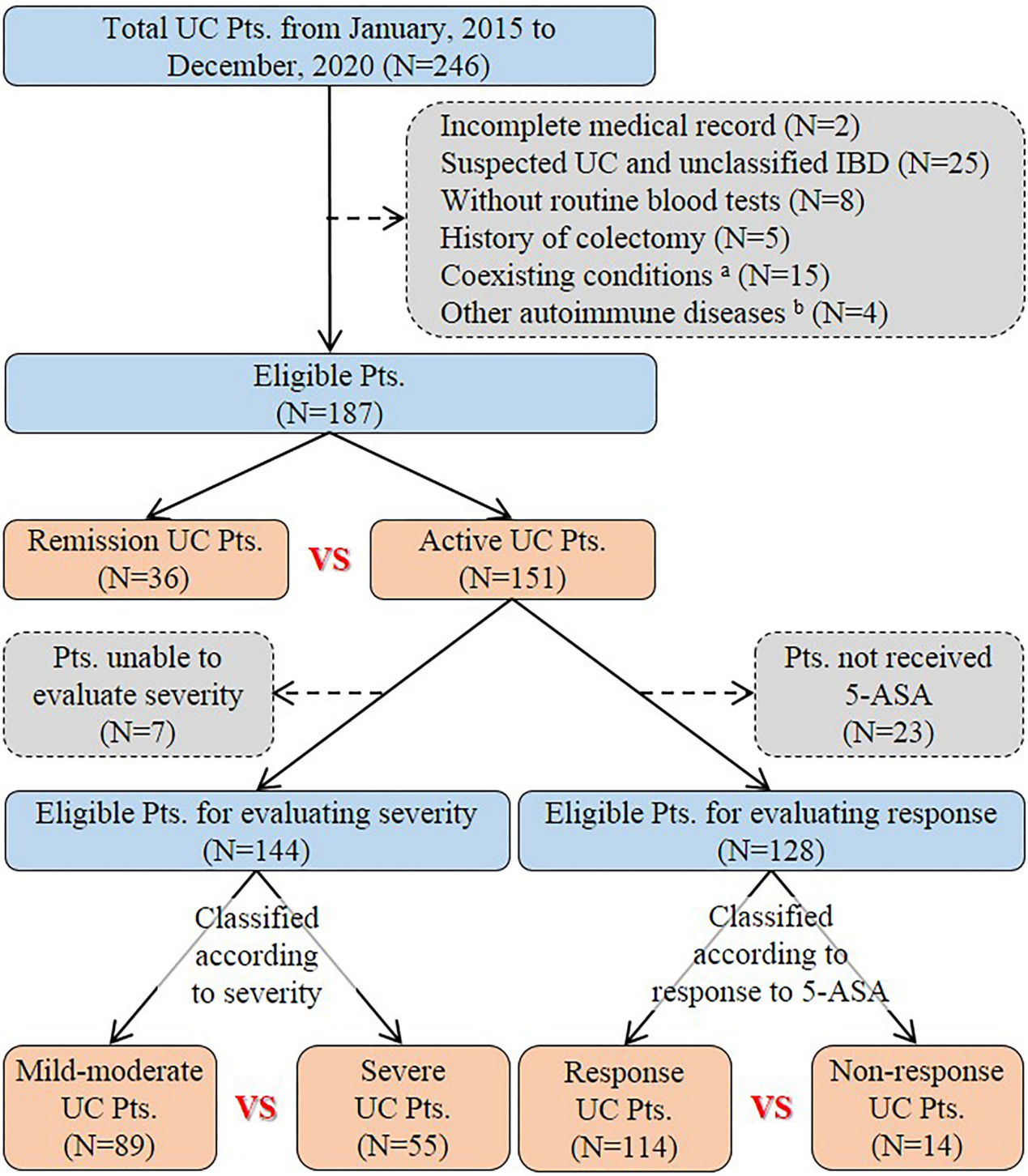

FIGURE 1 | Flowchart of patient selection. [a]Patients had coexisting conditions that potentially influence inflammatory indexes, including severe trauma ($n = 1$), pregnancy ($n = 1$), liver cirrhosis ($n = 2$), uremia ($n = 1$), and malignancy ($n = 10$). [b]Four patients had other autoimmune diseases, including psoriasis ($n = 1$), Behcet's disease ($n = 1$), urticarial vasculitis ($n = 1$), and rheumatoid arthritis ($n = 1$). UC, Ulcerative colitis; Pts, Patients; IBD, Inflammatory bowel disease; 5-ASA, 5-aminosalicylic acid.

TABLE 1 | Baseline characteristics of ulcerative colitis (UC) patients.

Variables	No. Pts	Mean ± SD, Median (Range) or Frequency (Percentage)
Age (years)	187	47.4 ± 16.3 49 (13.3–89.3)
Male (%)	187	100 (53.5%)
History of smoking (%)	187	38 (20.3%)
History of alcoholism (%)	187	17 (9.1%)
History of surgery (%)	187	52 (27.8%)
Comorbidities		
Hypertension/Diabetes/CHD (%)	187	14/8/10 (7.5%/4.3%/5.3%)
Duration of UC (years)	187	3.6 ± 6.5 0.5 (0–40)
History of UC related medication treatments		
5-ASA (%)	187	97 (51.9%)
Corticosteroids (%)	187	15 (8%)
Immunosuppressants (%)	187	1 (0.5%)
Biological agents (%)	187	1 (0.5%)
Traditional Chinese medicine (%)	187	48 (25.7%)
Probiotics (%)	187	28 (15%)
Clinical symptoms of UC		
Abdominal pain/Diarrhea/Hematochezia/Fever (%)	187	125/135/121/7 (66.8%/72.2%/ 64.7%/3.7%)
Activity of UC		
Active/Remission	187	151/36 (80.7%/19.3%)
Severity of UC		
Severe/Mild and moderate/Cannot be evaluated (%)	151	55/89/7 (36.4%/58.9%/4.6%)
Montreal classification for disease extent		
E3/E2/E1/None/Cannot be evaluated (%)	187	53/24/32/12/66 (28.3%/12.8%/17.1%/ 6.4%/35.3%)
Mayo endoscopic subscore of UC		
3/2/1/0/Cannot be evaluated (%)	187	26/49/7/15/90 (13.9%/26.2%/3.7%/ 8%/48.1%)
Extra-intestinal manifestations		
Cholelithiasis (%)	187	9 (4.8%)
Fatty liver (%)	187	10 (5.3%)
Pyoderma gangrenosum (%)	187	1 (0.5%)
Peripheral arthritis (%)	187	2 (1.1%)
UC-related complications		
Intestinal stenosis (%)	187	5 (2.7%)
Intestinal obstruction (%)	187	3 (1.6%)
Intraepithelial neoplasia (%)	187	15 (8%)
Massive gastrointestinal bleeding (%)	187	7 (3.7%)
Parenteral nutrition (%)	187	9 (4.8%)
Central venous catheterization (%)	187	2 (1.1%)
UC related medication treatments during hospitalization		
5-ASA (%)	187	141 (75.4%)
Corticosteroids (%)	187	14 (7.5%)
Biological agents (%)	187	1 (0.5%)
Traditional Chinese medicine (%)	187	69 (36.9%)
Probiotics (%)	187	107 (57.2%)
Length of stay (days)	187	13.2 ± 10.1 11 (1–64)

UC, Ulcerative colitis; SD, Standard deviation; CHD, Coronary heart disease; 5-ASA, 5-aminosalicylic acid; E3, Extensive colitis; E2, Left-sided colitis; E1, Proctitis.

TABLE 2 | Comparison of inflammatory indexes between active and remission UC groups.

Indexes	Active group		Remission group		*P*-value
	No. Pts	Mean ± SD, Median (Range) or Frequency (Percentage)	No. Pts	Mean ± SD, Median (Range) or Frequency (Percentage)	
NLR	151	3.53 ± 3.16 2.63 (0.63–20.33)	36	2.23 ± 1.06 2 (0.88–5.3)	**0.019**
PLR	151	181.17 ± 103.53 153.08 (46.25–632.22)	36	133.75 ± 47.45 136.84 (63.55–240)	**0.009**
SII	151	1117.49 ± 1428.01 676 (105–10558.11)	36	552.34 ± 397.31 517.76 (150–2400)	**0.006**
NPR	151	19.33 ± 10.66 17.06 (5.25–83.82)	36	17.13 ± 6.21 15.87 (7.58–31.36)	0.457
PAR	140	8.02 ± 4.39 6.54 (1.57–29.03)	29	6.37 ± 3.33 5.4 (2.86–19.65)	**0.023**
CAR	85	0.98 ± 2.01 0.21 (0.003–14.79)	16	0.33 ± 0.59 0.04 (0.006–2.14)	0.091
CLR	89	21.46 ± 41.9 3.89 (0.06–322)	16	5.9 ± 10.72 0.78 (0.08–38.95)	0.064

UC, Ulcerative colitis; Pts, Patients; SD, Standard deviation; NLR, Neutrophil-to-lymphocyte ratio; PLR, Platelet-to-lymphocyte ratio; SII, Systemic immune-inflammation index; NPR, Neutrophil-to-platelet ratio; PAR, Platelet-to-albumin ratio; CAR, C-reactive protein-to-albumin ratio; CLR, C-reactive protein-to-lymphocyte ratio.

TABLE 3 | Correlation analyses of inflammatory indexes with the activity of UC, the severity of UC, and non-response to 5-aminosalicylic acid (5-ASA).

Indexes	Activity of UC		Severity of UC		Non-response to 5-ASA	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
NLR	0.172	**0.02**	0.245	**<0.001**	0.137	0.123
PLR	0.193	**0.01**	0.324	**<0.001**	0.097	0.277
SII	0.201	**0.01**	0.284	**<0.001**	0.148	0.096
NPR	0.055	0.459	0.042	0.618	0.12	0.176
PAR	0.175	**0.02**	0.219	**0.01**	0.159	0.083
CAR	0.169	0.091	0.369	**<0.001**	0.353	**0.002**
CLR	0.182	0.063	0.401	**<0.001**	0.321	**0.004**

UC, Ulcerative colitis; 5-ASA, 5-aminosalicylic acid; NLR, Neutrophil-to-lymphocyte ratio; PLR, Platelet-to-lymphocyte ratio; SII, Systemic immune-inflammation index; NPR, Neutrophil-to-platelet ratio; PAR, Platelet-to-albumin ratio; CAR, C-reactive protein-to-albumin ratio; CLR, C-reactive protein-to-lymphocyte ratio.

the original Mayo score (15, 29). Briefly, the modified Mayo score is calculated based on the stool frequency, rectal bleeding, endoscopic findings, and physician's global assessment. Clinical

activation was defined as a total modified Mayo score of ≥3 points. Clinical remission was defined as a total modified Mayo score of ≤2 points without a sub-score of >1 point. Second, patients with active UC were classified into severe and mild-moderate UC groups according to the modified Truelove and Witts criteria (13). Severe UC was defined as a bloody stool frequency ≥6 per day along with at least one sign of systemic toxicity, including pulse rate >90 bpm, temperature > 37.8°C, hemoglobin level < 10.5 g/dl, ESR > 30 mm/h, and/or CRP > 30 mg/l. Third, patients with active UC receiving 5-ASA during their hospitalizations were classified into response and non-response to 5-ASA groups. Response to 5-ASA was defined as corticosteroid-free clinical remission, considering that corticosteroids are alternatives in our patients who did not achieve clinical remission after 5-ASA.

Statistical Analyses

All statistical analyses were conducted using the SPSS 20.0 (SPSS Inc., Chicago, IL, United States of America), the MedCalc 20.0 (MedCalc Software bvba, Ostend, Belgium), and the GraphPad Prism 8.0.2 (GraphPad Software Inc., San Diego, CA, United States of America). Categorical data were summarized as the frequency with percentage. Differences between groups were assessed using the chi-squared test. Continuous data were summarized as mean ± SD and median with range. Differences between groups were assessed using the non-parametric Mann–Whitney U test. Spearman's correlation coefficients were used to analyze the correlation of inflammatory indexes with the activity of UC, the severity of UC, and response to 5-ASA. Correlation coefficients (r) were reported as follows: $0 < r < 1$, positive correlation; $-1 < r < 0$, negative correlation; and $r = 0$, no correlation. The diagnostic accuracy of the inflammatory indexes for active UC, severe UC, and non-response to 5-ASA medications was identified by receiver operating characteristic (ROC) curves (30). Their optimal cut-off values, area under the curve (AUC) with 95% CI, sensitivity, and specificity were calculated. The optimal cutoff value was determined in the case that the summation of sensitivity and specificity values was the highest, maximizing Youden's index (31). A two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of Patients

A total of 246 patients with UC were initially identified and reviewed for potential inclusion. Finally, 187 patients were eligible for the final analysis in the study (**Figure 1**). The mean age was 47.4 ± 16.3 years. The percentage of males was 53.5%. The mean disease duration was 3.6 ± 6.5 years. Nearly half of the eligible patients (51.9%) had already received 5-ASA medications for UC before admission. Diarrhea (72.2%), abdominal pain (66.8%), and hematochezia (64.7%) were the most common clinical symptoms at admission. Extensive colitis was observed in 53 (28.3%) patients through endoscopy. The Mayo endoscopic sub-score of 3 points was observed in 26 (13.9%) patients. One hundred and forty-one (75.4%) patients received 5-ASA medications for UC at our hospitals. The mean length of hospital stay was 13.2 ± 10.1 days (**Table 1**).

TABLE 4 | Comparison of inflammatory indexes between severe and mild-moderate UC groups.

Indexes	Severe group		Mild-moderate group		*P*-value
	No. Pts	Mean ± SD, Median (Range) or Frequency (Percentage)	No. Pts	Mean ± SD, Median (Range) or Frequency (Percentage)	
NLR	55	4.48 ± 4.02 3.27 (1–20.33)	89	2.92 ± 2.35 2.14 (0.63–15.5)	**0.003**
PLR	55	221.57 ± 124.25 183 (81.76–632.22)	89	156.73 ± 80.9 131.69 (46.25–488.89)	**<0.001**
SII	55	1587.16 ± 1973.78 910 (212.59–10558.11)	89	844.4 ± 909.58 552 (105–6045)	**0.001**
NPR	55	19.91 ± 12.63 18.1 (6.62–83.82)	89	18.78 ± 9.46 16.11 (5.25-50.99)	0.616
PAR	51	9.27 ± 4.77 7.42 (3.7–29.03)	83	7.44 ± 4.1 6.17 (1.57–27.74)	**0.012**
CAR	37	1.64 ± 2.69 0.76 (0.003–14.79)	45	0.51 ± 1.04 0.06 (0.01–5.13)	**0.001**
CLR	40	36.72 ± 55.78 20.08 (0.06–322)	46	9.51 ± 18.9 1.22 (0.09–87.06)	**<0.001**

UC, Ulcerative colitis; Pts, Patients; SD, Standard deviation; NLR, Neutrophil-to-lymphocyte ratio; PLR, Platelet-to-lymphocyte ratio; SII, Systemic immune-in?ammation index; NPR, Neutrophil-to-platelet ratio; PAR, Platelet-to-albumin ratio; CAR, C-reactive protein-to-albumin ratio; CLR, C-reactive protein-to-lymphocyte ratio.

TABLE 5 | Comparison of inflammatory indexes between non-response and response to 5-ASA groups.

Indexes	Non-response group		Response group		*P*-value
	No. Pts	Mean ± SD, Median (Range) or Frequency (Percentage)	No. Pts	Mean ± SD, Median (Range) or Frequency (Percentage)	
NLR	14	4.99 ± 4.85 2.94 (1.13–18.56)	114	3.24 ± 2.57 2.56 (0.63–15.5)	0.122
PLR	14	213.15 ± 134.4 154.04 (109.41–632.22)	114	180.99 ± 102.05 163.44 (54.62–618.75)	0.275
SII	14	1953.09 ± 2716.53 841.01 (304–10558.11)	114	990.99 ± 1025.29 659.97 (105–6045)	0.096
NPR	14	20.95 ± 8.99 19.72 (8.03–41.97)	114	18.14 ± 8.86 16.28 (5.25–50.99)	0.175
PAR	14	10.89 ± 6.65 8.47 (4.87–29.03)	106	7.97 ± 4.11 6.57 (2.73–27.74)	0.083
CAR	12	3.21 ± 4.16 2.71 (0.01–14.79)	65	0.61 ± 1.07 0.14 (0.003–5.13)	**0.002**
CLR	12	65.78 ± 88.51 56.63 (0.29–322)	68	14.21 ± 23.26 3.18 (0.06–115)	**0.004**

5-ASA, 5-aminosalicylic acid; Pts, Patients; SD, Standard deviation; NLR, Neutrophil-to-lymphocyte ratio; PLR, Platelet-to-lymphocyte ratio; SII, Systemic immune-inflammation index; NPR, Neutrophil-to-platelet ratio; PAR, Platelet-to-albumin ratio; CAR, C-reactive protein-to-albumin ratio; CLR, C-reactive protein-to-lymphocyte ratio.

Inflammatory Indexes and Activity of UC

Of the 187 included patients, 151 and 36 were diagnosed with active UC and remission UC, respectively. The mean NLR, PLR,

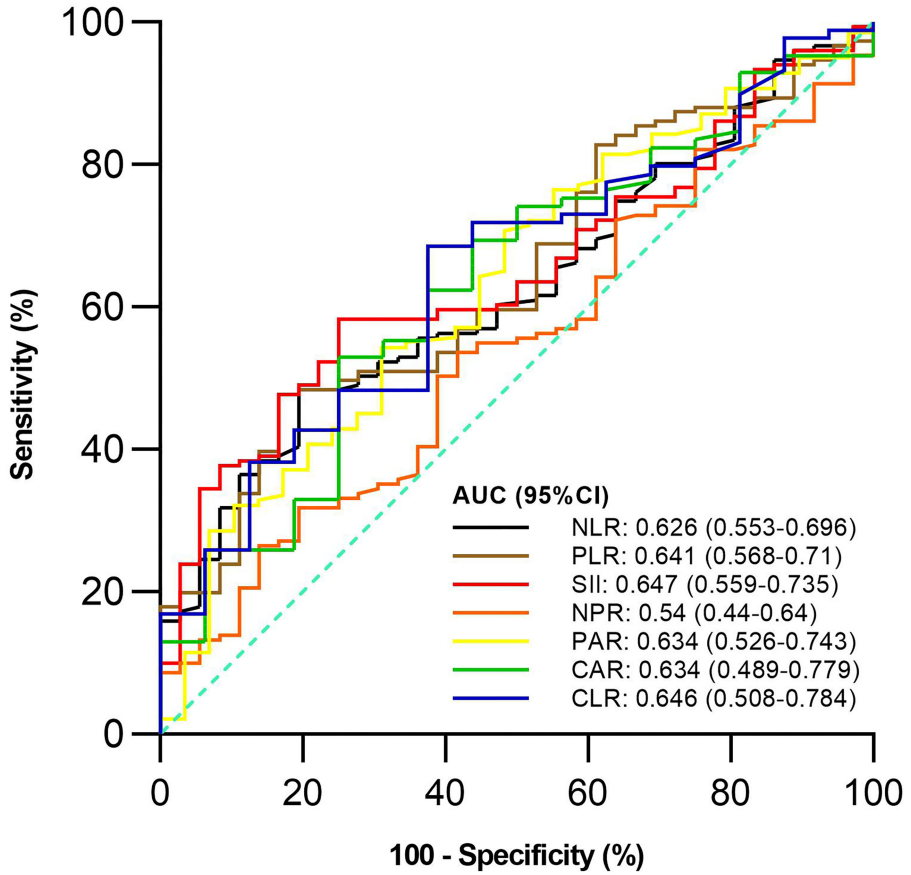

FIGURE 2 | Comparison of predictive performance of inflammatory indexes for active ulcerative colitis (UC). UC, Ulcerative colitis; AUC, Area under the curve; CI, Confidence interval; NLR, Neutrophil-to-lymphocyte ratio; PLR, Platelet-to-lymphocyte ratio; SII, Systemic immune-inflammation index; NPR, Neutrophil-to-platelet ratio; PAR, Platelet-to-albumin ratio; CAR, C-reactive protein-to-albumin ratio; CLR, C-reactive protein-to-lymphocyte ratio.

SII, and PAR ($P = 0.019$, 0.009, 0.006, and 0.023), but not NPR, CAR, or CLR ($P = 0.457$, 0.091, or 0.064), were significantly higher in the active group than in the remission group (**Table 2**). NLR, PLR, SII, and PAR were significantly correlated with the activity of UC ($P = 0.02$, 0.01, 0.01, and 0.02) (**Table 3**). SII had the largest AUC (AUC = 0.647), followed by CLR, PLR, PAR, CAR, NLR, and NPR (AUC = 0.646, 0.641, 0.634, 0.634, 0.626, and 0.54) (**Figure 2**). The optimal cut-off value of SII for active UC was 595.47×10^9/L with a sensitivity and specificity of 58.28 and 75%, respectively (**Supplementary Table 1**). The AUC of SII was significantly different from that of NPR ($P = 0.0428$), but not NLR, PLR, PAR, CAR, or CLR ($P = 0.3506$, 0.8508, 0.8233, 0.5929, or 0.6023).

Inflammatory Indexes and Severity of UC

Of the 144 active UC patients, 55 and 89 were diagnosed with severe UC and mild-moderate UC, respectively. The mean NLR, PLR, SII, PAR, CAR, and CLR ($P = 0.003$, <0.001, 0.001, 0.012, 0.001, and <0.001), but not NPR ($P = 0.616$), were significantly higher in the severe group than in the mild-moderate group (**Table 4**). NLR, PLR, SII, PAR, CAR, and CLR were significantly correlated with the severity of UC ($P =<$ 0.001, <0.001, <0.001, 0.01, <0.001, and <0.001) (**Table 3**). CLR had the largest AUC (AUC = 0.732), followed by CAR, PLR, SII, NLR, PAR, and NPR (AUC = 0.714, 0.693, 0.669, 0.646, 0.63, and 0.525) (**Figure 3**). The optimal cut-off value of CLR for severe UC was 7 mg/10^9 with a sensitivity and specificity of 65% and 73.91%, respectively (**Supplementary Table 2**). The AUC of CLR was significantly different from that of SII, NPR, and PAR ($P = 0.0473$, 0.043, and 0.0138), but not NLR, PLR, or CAR ($P = 0.0562$, 0.0723, or 0.1886).

Inflammatory Indexes and Non-Response to 5-ASA

Of the 128 active UC patients who received 5-ASA, 14 were not responsive to 5-ASA. The mean CAR and CLR ($P = 0.002$ and 0.004), but not NLR, PLR, SII, NPR, and PAR ($P = 0.122$, 0.275, 0.096, 0.175, and 0.083), were significantly higher in the non-response group than in the response group (**Table 5**). CAR and CLR were significantly correlated with non-response to 5-ASA ($P = 0.002$ and 0.004) (**Table 3**). CAR had the largest AUC (AUC = 0.781), followed by CLR, PAR, SII, NLR, NPR, and PLR (AUC = 0.759, 0.643, 0.637, 0.627, 0.611, and 0.59) (**Figure 4**). The optimal cut-off value of CAR for non-response to 5-ASA

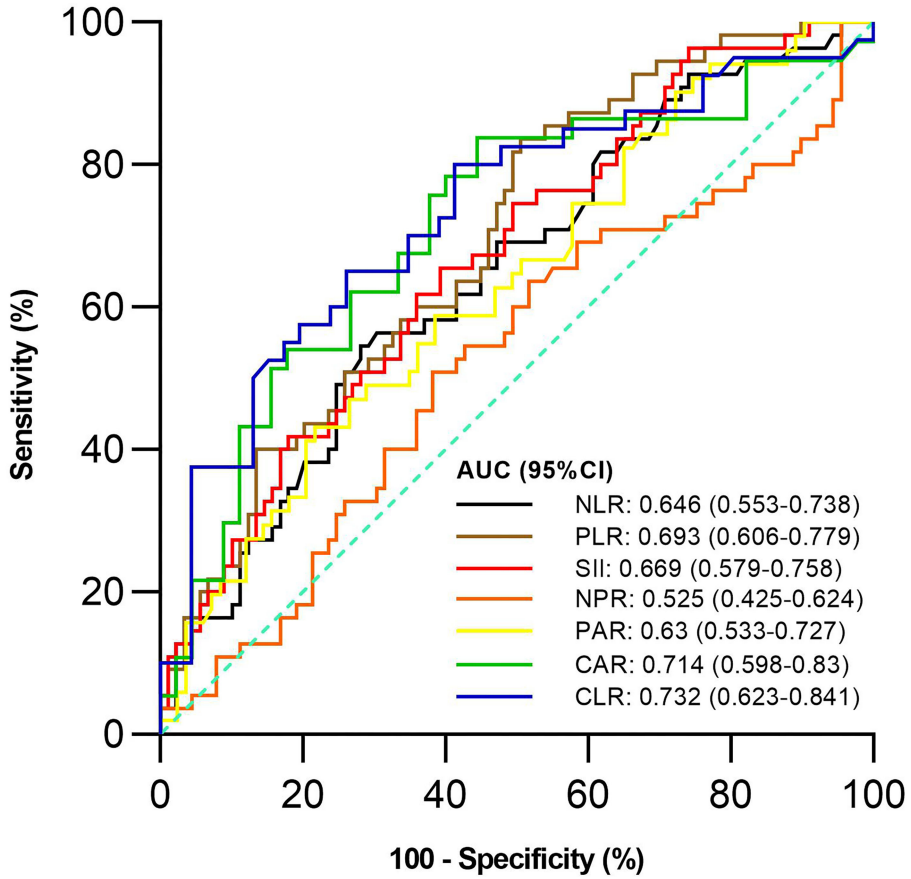

FIGURE 3 | Comparison of the predictive performance of inflammatory indexes for severe UC. UC, Ulcerative colitis; AUC, Area under the curve; CI, Confidence interval; NLR, Neutrophil-to-lymphocyte ratio; PLR, Platelet-to-lymphocyte ratio; SII, Systemic immune-inflammation index; NPR, Neutrophil-to-platelet ratio; PAR, Platelet-to-albumin ratio; CAR, C-reactive protein-to-albumin ratio; CLR, C-reactive protein-to-lymphocyte ratio.

was 2.41mg/g with a sensitivity and specificity of 58.33% and 95.38%, respectively (**Supplementary Table 3**). The AUC of CAR was significantly different from that of PLR ($P = 0.0014$), but not NLR, SII, NPR, PAR, or CLR ($P = 0.055$, 0.0673, 0.1451, 0.1529, or 0.3271).

DISCUSSION

The present study has compared the value of seven inflammatory indexes, including NLR, PLR, SII, NPR, PAR, CAR, and CLR, at baseline for assessing the activity of UC, the severity of UC, and response to 5-ASA, and found that SII had the greatest predictive value for active UC, CLR for severe UC, and CAR for non-response to 5-ASA.

Systemic immune-inflammation index (SII) was originally developed as an independent predictor of recurrence and survival for patients with hepatocellular carcinoma after surgery (23). Recently, it has also been demonstrated that SII level was higher in patients with UC than in healthy control groups and that SII level was positively associated with disease activity in patients (32) with UC. Similarly, our study found that the SII level was significantly correlated with the activity of UC. Moreover, SII had a better diagnostic capability for active UC than other inflammatory indexes. This finding may be attributed to the fact that immunity and inflammation are crucial for the occurrence of UC (1) and SII is calculated as neutrophil counts multiplied by platelet counts and divided by lymphocyte counts (23).

First, in a healthy human body, although 1-2 $\times$ 10^{11} neutrophils are generated every day in the bone marrow (33), chemokine receptors, including CXCR4 and CXCR2, may maintain a delicate balance of neutrophil counts by mediating the retention of neutrophils in the bone marrow and their mobilization to peripheral blood (34, 35). In patients with active inflammatory bowel disease (IBD), there are significantly increased interleukin-17A (IL-17A) levels in inflamed mucosa that promote the transcription of granulocyte colony-stimulating factor in bone marrow (36), thereby inhibiting and activating the expression of CXCR4 binding ligands and CXCR2 binding ligands, respectively. As a result, increased neutrophils are released from the bone marrow into the peripheral blood (37). Therefore, patients with active IBD often have peripheral neutrophilia (38).

Second, lymphocytes, such as Th1 cells, Th17 cells, and B cells, can produce pro-inflammatory cytokines and activate intestinal

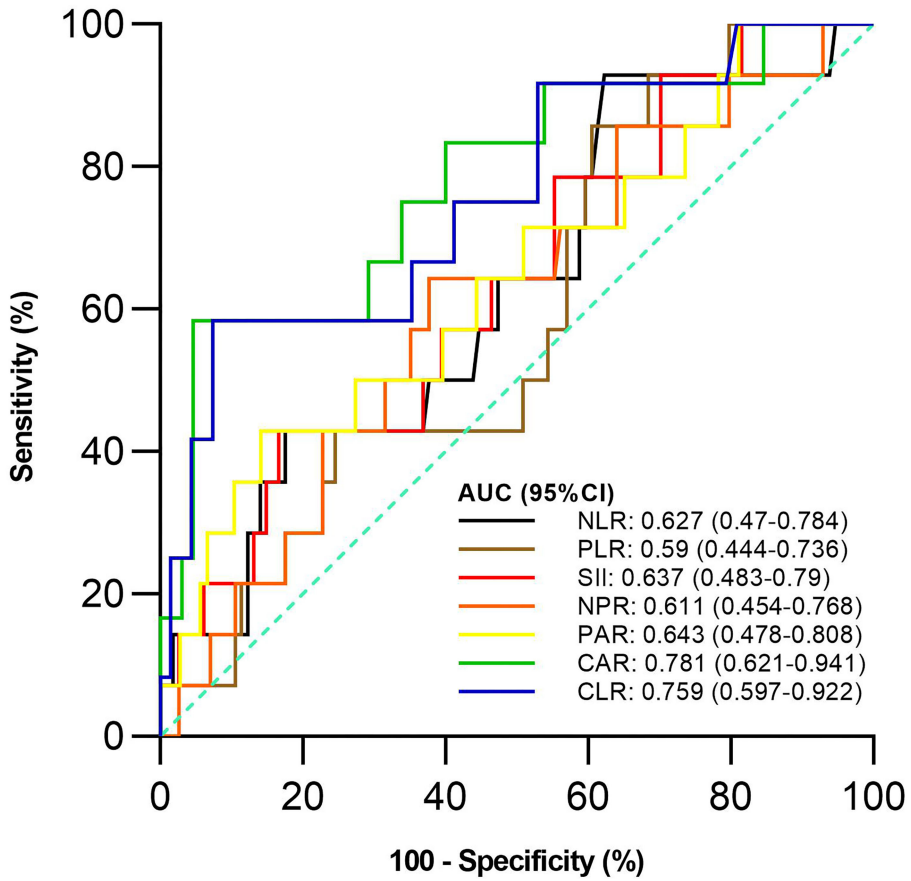

FIGURE 4 | Comparison of the predictive performance of inflammatory indexes for non-response to 5-aminosalicylic acid (5-ASA). 5-ASA, 5-aminosalicylic acid; AUC, Area under the curve; CI, Confidence interval; NLR, Neutrophil-to-lymphocyte ratio; PLR, Platelet-to-lymphocyte ratio; SII, Systemic immune-inflammation index; NPR, Neutrophil-to-platelet ratio; PAR, Platelet-to-albumin ratio; CAR, C-reactive protein-to-albumin ratio; CLR, C-reactive protein-to-lymphocyte ratio.

proteases leading to mucosal damage (39), and are accumulated in the inflamed lamina propria of IBD (40, 41). In patients with active IBD, lymphocytes are accumulated from the peripheral blood into the inflamed intestine, which eventually results in peripheral lymphopenia (39).

Third, platelets are produced from long cytoplasmic processes fragmentation of megakaryocyte in the extravascular marrow space (42). Patients with IBD have elevated levels of thrombopoietin and IL-6, which are involved in megakaryocytic maturation (43). Moreover, in patients with IBD, the platelets in the peripheral blood are active and can spontaneously aggregate with increased susceptibility to proaggregating agents (44). Thus, patients with active UC often have peripheral thrombocytosis (45). Additionally, platelet counts are more strongly associated with the activity of UC than the severity of UC. Platelet counts in peripheral blood are markedly increased in patients with active UC compared with inactive UC (45), but not correlated with the Ulcerative Colitis Colonoscopic Index of Severity, which has an excellent overall assessment of endoscopic severity (46).

C-reactive protein-to-lymphocyte ratio (CLR) was originally developed as a novel index to predict the major morbidity after esophagogastric cancer resection (27). To our knowledge, only Con et al. explored the role of CLR for assessing the dynamic response to infliximab salvage treatment and predicting the risk of subsequent colectomy in patients with UC (47). Similarly, our study found that CLR had a better capability to predict the severity of UC than other inflammatory indexes. This finding may be attributed to the fact that CLR is calculated as CRP levels divided by lymphocyte counts (27). CRP, the most important acute-phase protein, is produced almost exclusively by hepatocytes in response to stimulation by IL-6, IL-1β, and tumor necrosis factor α. In the presence of an acute-phase inflammation or infection, CRP levels are increased dramatically. Contrarily, CRP levels are quickly decreased when inflammation is effectively treated (48). CRP alone can be used to predict the severity of active UC (49). Additionally, CRP levels are more strongly associated with the severity of UC than the activity of UC (50).

C-reactive protein-to-albumin ratio (CAR) was originally developed to predict the outcome of patients from acute medical ward (26). Gibson et al. (51) detected the predictive value of CAR to steroid response in patients with acute severe UC aiming to select patients who need early rescue treatment (51). Similarly, our study found that CAR had a better capability to predict the response to 5-ASA than other inflammatory indexes. This finding may be attributed to the fact that CAR is calculated as CRP levels divided by albumin levels (26). First, CRP levels are significantly correlated with the proportion of corticosteroid use in patients (52) with Crohn's disease. Corticosteroids, as rescue medications, are usually used in patients who are not well responsive to 5-ASA (9, 10). Second, albumin levels can reflect the nutritional status of patients (53) with UC and are correlated significantly with the clinical severity of UC (54). Moreover, an *in vitro* study suggested a strong interaction between 5-ASA and human serum albumin (55). In a mouse model of UC, 5-ASA conjugated with human serum albumin was found to show a significant therapeutic effect (56). Thus, albumin levels may influence the therapeutic effect of 5-ASA for UC.

The present study had several limitations. First, it was conducted at a single center with a relatively small sample size of total patients and a very small sample size of patients in the non-response group, probably compromising the statistical analyses presented. Second, the external validity of our findings was lacking. Third, endoscopy was not performed by the same expert at our hospital, so endoscopic assessment might be a bit inconsistent. Fourth, this study was retrospective and cross-sectional, where CRP and albumin levels were not routinely tested in our patients. Moreover, there were some damages in the inpatient information system, leading to the lack of details of two patient's medical records. Fifth, only a few patients were treated with corticosteroids or biologics in our study. Therefore, we cannot evaluate the correlation between inflammatory indexes and response to corticosteroids or biologics.

In conclusion, SII, CLR, and CAR have higher diagnostic performance than other inflammatory indexes for active UC, severe UC, and response to 5-ASA, respectively. Dynamic changes of these inflammatory indexes along with activity and severity of UC should be further explored. Moreover, future studies should also evaluate the association between these inflammatory indexes and mucosal severity of UC.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee of General Hospital of Northern Theater Command. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

XQ: conceptualization. HL and XQ: methodology. HL, ZB, and XQ: formal analysis and writing–original draft. HL, ZB, GC, and XQ: data curation. HL, ZB, QW, GC, YZ, XG, and XQ: writing–review and editing. XG and XQ: supervision. All authors have made an intellectual contribution to the manuscript and approved the submission.

FUNDING

YZ received funding supported by the Natural Science Foundation of Liaoning Province (No. 2019-ZD-1058).

ACKNOWLEDGMENTS

The abstract was published in the 5th Korea Digestive Disease Week (KDDW 2021) Conference as a poster presentation, please see the following link: http://people-x.com/kddw2021/KDDW2021_abstract.pdf.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.851295/full#supplementary-material

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frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 19 April 2022
doi: 10.3389/fmed.2022.867293

Clinico-Epidemiological Characteristics of Patients With Inflammatory Bowel Disease in Egypt: A Nationwide Multicenter Study

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Background and Aims: Ulcerative colitis (UC) and Crohn's disease (CD) are the most common types of Inflammatory bowel disease (IBD), with variable responses to traditional therapies and unpredicted prognosis. In Egypt and most developing countries, the lack of recent epidemiological and prognostic data adversely affects management strategies. We collected and analyzed data of patients with IBD from multiple centers across Egypt to evaluate patients' clinical and epidemiological characteristics.

Methods: This retrospective multicenter study included patients diagnosed with IBD between May 2018 and August 2021, at 14 tertiary gastroenterology units across Egypt. Record analysis addressed a combination of clinico-epidemiological characteristics, biochemical tests, stool markers, endoscopic features, histological information, and different lines for IBD treatment.

OPEN ACCESS

Edited by:
Antonietta G. Gravina, University of Campania Luigi Vanvitelli, Italy

Reviewed by:
Ivana Trivic, Children's Hospital Zagreb, Croatia
Boldeanu Mihail Virgil, University of Medicine and Pharmacy of Craiova, Romania

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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *31 January 2022*
Accepted: *21 March 2022*
Published: *19 April 2022*

Citation:
Elbadry M, Nour MO, Hussien M, Ghoneem EA, Medhat MA, Shehab H, Galal S, Eltabbakh M, El-Raey F, Negm M, Afify S, Abdelhamed W, Sherief A, Abdelaziz A, Abo Elkasem M, Mahrous A, Kamal G, Maher M, Abdel-Hameed O, Elbasuny A, El-Zayyadi I, Bassiony A, Moussa A, Bedewy E, Elfert A and El Kassas M (2022) Clinico-Epidemiological Characteristics of Patients With Inflammatory Bowel Disease in Egypt: A Nationwide Multicenter Study. Front. Med. 9:867293. doi: 10.3389/fmed.2022.867293

Results: We identified 1104 patients with an established diagnosis of IBD; 81% of them had UC, and 19% showed CD. The mean age of onset was 35.1 ± 12.5 years ranging from 5 to 88 years, the mean duration of illness at inclusion was 13.6 ± 16.7 years, gender distribution was almost equal with a significant male dominance (60.4%, $p = 0.003$) among patients with CD, 57% were living in rural areas, and 70.5% were from Delta and Coastal areas. Two hundred nineteen patients (19.8%) displayed comorbid conditions, primarily associated with CD. The most frequent complaints were diarrhea (73.2%), rectal bleeding (54.6%) that was significantly higher among patients with UC (64%, $p < 0.001$), and 46.8% with abdominal pain (more often with CD: 71%, $p < 0.001$). Conventional therapy was effective in treating 94.7% of patients. The main lesion in patients with CD was ileal (47.8%); patients with UC mainly exhibited proctosigmoiditis (28.4%). Dysplasia was detected in 7.2% of patients, mainly subjects with UC.

Conclusions: To our knowledge, our effort is the first and largest cohort of Egyptian patients with IBD to describe clinical and epidemiological characteristics, and diagnostic and management approaches. More extensive prospective studies are still needed to fully characterize disease distribution, environmental factors, and pathological features of the disease.

Keywords: ulcerative colitis, Crohn's disease, multicenter study, Egypt, inflammatory bowel disease

INTRODUCTION

In 2017, 6.8 million Inflammatory bowel disease (IBD) cases were reported globally. The prevalence of IBD has increased substantially in many regions of the world, creating a substantial social and economic burden on health systems (1). Ulcerative colitis (UC) and Crohn's disease (CD) are major chronic IBD conditions in addition to indeterminate colitis that cause varying degrees of gastrointestinal (GI) tract inflammation. This classification was addressed by Montreal working party and commonly used in clinical practice and also for future serological and genetic studies in IBD (2). Data from several studies report a relationship between smoking and CD. However, quitting smoking may be accompanied by an increased risk of UC (3). Dietary fiber (particularly vegetables and fruits), saturated fats, sleep disorders, depression, and low vitamin D levels have been associated with increased IBD incidence. Also, stress, microbiota, some medications, such as NSAIDs, and early antibiotic exposure during infancy are factors that might increase the risk for IBD incidence, especially in genetically susceptible individuals (4, 5). Therefore, some studies concluded that, modification of one or more of these environmental factors has a bidirectional effect on the disease activity (6). Disease presentation includes signs and symptoms such as diarrhea, rectal bleeding, and abdominal pain. Fever, weight loss, extraintestinal symptoms, and fatigue may be observed (7). A serious complication of IBD is the malignant transformation of colonic mucosa that increases the incidence of colorectal carcinoma. Further, psychiatric health is negatively affected by IBD, especially in young patients (8). Also, extraintestinal manifestations (EIMs) can occur in some patients and affect joints, the hepatobiliary system, skin, and eyes (9).

Assessment of patients with IBD includes physical examination with a focus on extraintestinal complications, laboratory evaluation [C-reactive protein (CRP), erythrocytes sedimentation rate (ESR), and fecal calprotectin (FC)], endoscopy, and different imaging modalities (10). All these clinical examinations are used to confirm the diagnosis, gauge disease extent, and evaluate severity. Treatment depends on disease extent and severity (7).

The disease is a worldwide concern, but the incidence is highest in the United States, Sweden, and United Kingdom (11–13). IBD has a growing incidence in the Middle East and North Africa; however, a lack of accurate registry and epidemiological cohort studies are still obstacles to evaluating the current situation (14–16). UC is more common than CD in various parts of the world. In Egypt, few data regarding the epidemiology of IBD are available; however, some studies suggest the relative incidence ratio of UC and CD is 6:1 (17).

We conducted this retrospective cross-sectional multicenter study to represent all of Egypt. We collected clinical information to evaluate epidemiological characteristics, laboratory and imaging findings, colonoscopy results, and different treatment strategies. We aimed to assess updated data to draw a real map of IBD in Egypt in order to help us introduce scientific recommendations to optimize IBD diagnosis and management strategies.

METHODS

Study Design, Settings, and Inclusion Criteria

This retrospective multicenter study was conducted on patients diagnosed with IBD at 14 tertiary GI units. These centers

are affiliated with universities, distributed across Egypt, and represent the main geographical areas within the country where most of the Egyptian population is concentrated along the banks of the Nile River and on the river's delta including (1) Greater Cairo (Helwan, Cairo, Ain Shams, Al-Azhar, and the National Hepatology and Tropical Medicine Research Institute), (2) Delta region (Mansoura, Tanta, Zegazeg, and Kafr Elsheikh), (3) Coastal region (Alexandria and Damietta), (4) Upper Egypt (Sohag, Assiut, and Aswan). Patients from remote areas in Egypt, Sinai Peninsula, and Oases are largely referred to these tertiary centers. Data were collected both manually and electronically from available medical records between May 2018 and August 2021. Medical records of patients of all ages and both sexes with a confirmed diagnosis of IBD during the study period were enrolled.

Study Variables

Demographic features recorded were age of onset, gender, residence, geographic area, duration of illness, history of smoking, associated comorbidities, and other autoimmune diseases. Disease characteristics, including the severity of symptoms, presence of EIMs, type of medical treatment, and history of surgical intervention, were tabulated. Remission was defined as complete resolution of signs and symptoms, and endoscopic and histological healing of colon mucosa.

Diagnosis of IBD

The diagnosis of CD or UC was based on a combination of clinical manifestations, biochemical tests, stool markers, endoscopic features, and histological evaluation.

The recorded biochemical information included complete blood count (CBC), CRP, ESR, liver, and kidney function tests. Also, FC as a stool marker of intestinal inflammation was noted. Reports of abdominal ultrasonography and upper endoscopy were retrieved for all enrolled patients.

Endoscopic features consistent with UC were continuous and confluent colon inflammation with clear demarcation and rectal involvement. Endoscopic features consistent with CD were discontinuous lesions, mucosal nodularity, ulceration (both aphthous and linear), and strictures. Disease distribution and activity of UC were evaluated using the Montreal classification and Mayo score, respectively. While patients with CD were evaluated according to the severity of the onset of the disease using Harvey-Bradshaw score and Phenotypic distribution according to Montreal classification (2, 18).

Treatment of IBD

All recorded data about treatment approach either step up or step down, type of treatment either topical or systemic or biological in addition to antibiotic and steroid therapy were extracted from the patients' medical records and statistically analyzed.

Ethical Considerations

All procedures involving human participants were carried out according to the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments, or comparable ethical standards. The study was approved by the Research Ethics Committee for human subject research at the Faculty of Medicine, Helwan University (Serial: 76-2021). The study data set was fully anonymized.

Statistical Analysis

Analysis used SPSS version 25.0 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp., USA). Mean ± SD was used for quantitative variables, and frequency and percentage were used for qualitative variables. Mann-Whitney and Wilcoxon tests were used to assess the differences in means of quantitative non-parametric variables. Chi-square and Fisher's Exact tests were used to assess differences in the frequency of qualitative variables. The statistical methods assumed a significance level of $p < 0.05$ and a highly significant level of $p < 0.001$.

RESULTS

The study included 1,104 patients with an established diagnosis of IBD. Ulcerative colitis was diagnosed in 81.3% of the study population, while −18.7% exhibited CD. The mean age of onset was 35.1 ± 12.5 years ranging from 5 to 88 years with mean duration of illness at inclusion of 13.6 ± 16.7 years for the cohort. The gender distribution was almost equal; however, a significant male dominance (60.4%, $p = 0.003$) was observed among patients with CD. The residence of the recruited patients was found to be in rural areas in 57% of cases. These patients displayed a higher prevalence of UC (59.2%, $p = 0.002$). Further, 70.5% were from North of Egypt. About of patients suffered from comorbid conditions, mainly hypertension (HTN) (9.1%) and diabetes (DM) (8.4%). Most comorbidities were more frequent among patients with CD, except for DM and other autoimmune diseases (**Table 1**).

Clinical Presentations at Diagnosis

The most frequent manifestations, at the time of diagnosis, among all patients was diarrhea (73.2%) which was similar for both UC and CD. Bowel movements/day was 4.7 ± 2.2 ranging from 0 to 20. -Rectal bleeding was reported by 54.6% of patients, and it was significantly higher among UC subjects, while 48.6% -of patients complained from abdominal pain. Abdominal pain was higher among patients with CD, but the difference was insignificant. Weight loss was seen in 15.3% of patients, and 11.7% displayed EIM, mainly arthropathy. Fever was the least frequent symptom (8.5%) and was significantly higher among subjects with CD (**Table 2**).

Laboratory Investigations at Time of Diagnosis

Laboratory characteristics at the time of diagnosis showed significantly improved HB, CRP, ESR, WBC, and FC levels after treatment in both UC and CD patients (**Table 3**). PLT and AST were decreased in CD and UC patients, respectively. No notable change was observed in serum levels of total protein, albumin, and ALT.

TABLE 1 | General characteristics of patients with inflammatory bowel disease in Egypt (May 2018 to August 2021).

Variables		Total *n* = 1,104 (%)	UC group*n* = 897 (%)	CD group *n* =207 (%)	P-value
Age of onset (years)	Mean ± SD Min–Max	35.1 ± 12.55–88	35.3 ± 12.5 5–88	34.1 ± 12.49–70	0.250
Gender	Male	561 (50.8)	436 (48.6)	125 (60.4)	0.003*
	Female	543 (49.2)	461 (51.4)	82 (39.6)	
Residence	Urban	475 (43.0)	366 (40.8)	109 (52.7)	0.002*
	Rural	629 (57.0)	531 (59.2)	98 (47.3)	
Geographic area	Greater Cairo	154 (13.9)	115 (12.8)	39 (18.8)	0.001*
	Delta & Coastal region	778 (70.5)	626 (69.8)	152 (73.4)	
	Upper Egypt	172 (15.6)	156 (17.4)	16 (7.7)	
Disease duration at inclusion (years)	Mean ± SD	13.6 ± 16.7	15.1 ± 17.9	12.8 ± 14.7	0.086
Smoking		194 (17.6)	149 (16.6)	45 (21.7)	0.085
Comorbidities[a]		219 (19.8)	176 (19.6)	43 (20.8)	0.700
Diabetes mellitus		93 (8.4)	87 (9.7)	6 (2.9)	0.001*
Other autoimmune disease[b]		36 (3.3)	35 (3.9)	1 (0.5)	0.008*
Cardiac diseases		33 (3.0)	15 (1.7)	18 (8.7)	<0.001*
Hepatic diseases		35 (3.2)	12 (1.3)	23 (11.1)	<0.001*
Renal diseases		24 (2.2)	4 (0.4)	20 (9.7)	<0.001*
Hypertension		101 (9.1)	73 (8.1)	28 (13.5)	0.022*
Others[c]		23 (2.1)	20 (2.2)	3 (1.4)	0.598

UC, Ulcerative colitis; CD, Crohn's disease.

[a]Some patients have more than one comorbid condition.

[b]For Ulcerative colitis; 6 hypothyroidism, 5 SLE, 5 thyroiditis, 5 rheumatoid arthritis, 3 ankylosing spondylitis, 2 vitiligo, 2 autoimmune hepatitis, panhypopituitarism, axial arthralgia, scleroderma, myositis, polyarthritis, Behcet disease, and autoimmune hemolytic anemia.

For Crohn's disease; Eosinophilic gastritis.

[c]For Ulcerative colitis; 3 TB, 2 hemorrhoids, 2 ulcers, perforation/sigmoid colon mass, steroid-dependent, fistula (colonic), depression, FMF, 1ry infertility, hyperthyroidism, epilepsy, chest disease, pyoderma gangrenosum, G6PD deficiency, pityriasis versicolor, and conjunctivitis.

For Crohn's disease; osteoporosis, TB, DVT, and Marjolin ulcer.

[*]Significant.

Imaging, Endoscopic, and Histopathological Features of the Rectruited Patients

patients with patients with The lesions in CD subjects at diagnosis were mainly ileal (47.8%) and ileocolonic (38.6%). Lesions were mainly non-stricturing and non-penetrating (61.4%) and less commonly stricturing (20.8%) (**Figure 1**).

At diagnosis, CT/MRI revealed fistulization in about 11.1% of patients, and no abnormality was detected in about 18.4%. Colonoscopy showed proctosigmoiditis in 28.4% of patients with UC, proctitis in 25.1%, and pancolitis in 22.9%. Mild, moderate, and severe lesions were detected in 44.7, 35.8, and 19.5%, respectively (**Figure 2**).

Dysplasia was detected histologically in 80 patients (7.2%), mainly associated with UC (75 patients). Low-grade dysplasia was predominant, seen in 68 patients (6.2%). Sixty-four patients were diagnosed with UC ($p = 0.012$).

Lines of Management for the Recruited Patients

Most patients (94.7%) were managed using a step-up approach. The main antibiotics used were quinolone and metronidazole (40–50%). Topical therapies available in Egypt are (5-ASA suppositories and budesonide enema, and they) were used in 21–31% of cases. Unfortunately, other forms of topical therapies (5-ASA enemas, 5-ASA foams, 5-ASA gels, Corticosteroids foams, tacrolimus suppositories, and cyclosporin enemas) are not present in Egypt. Systemic treatment was prescribed for 50–86%, mainly with 5-ASA. About one-fourth received biological treatment, and only 2.6% were referred for coloniccolectomy or intestinal resection and anastomosis (**Table 4**).

DISCUSSION

This study is the largest cohort evaluated for IBD in Egypt to the best of our knowledge. We found that the prevalent cases of UC are about four-fold greater than CD. This ratio is higher than reported elsewhere. In 2009, census data estimated that 1,171,000 Americans exhibited IBD (565,000 CD and 593,000 UC) (19). In 2018, the overall prevalence of IBD, CD, and UC in the UK were 725, 276, and 397 per 100 000 people, respectively (20). The occurrence of bleeding, which is more common in UC, is a potent stimulant for seeking medical advice, and so UC cases can be identified more frequently than CD cases. Furthermore, the diagnosis of CD may require exhaustive workup including detailed history taking, laboratory investigations, imaging modalities, and colonoscopy with ileal intubation or even enterostomy. These diagnostic facilities are expensive (not fully covered by insurance) and not available in all centers, particularly that more than half of our study population

TABLE 2 | Clinical characteristics of patients with inflammatory bowel disease in Egypt (May 2018 to August 2021).

Variables		Total *n* = 1104 (%)	UC group*n* = 897 (%)	CD group *n* =207 (%)	*P*-value
Bowel motions /day	Mean ± SD Min–Max	4.7 ± 2.20–20	4.6 ± 2.0 0–12	5.0 ± 2.81–20	0.162
Pulse	Mean ± SD Min–Max	83.7 ± 12.560–133	84.0 ± 12.8 60–133	83.0 ± 11.666–120	0.234
Diarrhea		808 (73.2)	655 (73.0)	153 (73.9)	0.862
Rectal bleeding		603 (54.6)	574 (64.0)	29 (14.0)	<0.001*
Abdominal pain		517 (46.8)	370 (41.2)	147 (71.0)	<0.001*
Fever		94 (8.5)	66 (7.4)	28 (13.5)	0.008*
Extraintestinal manifestations[a]		129 (11.7)	107 (11.9)	22 (10.6)	0.719
Significant weight loss		169 (15.3)	130 (14.5)	39 (18.8)	0.133

UC, Ulcerative colitis; CD, Crohn's disease.

[a]For Ulcerative colitis (some patients have more than one condition); 43 arthropathy, 10 pyoderma gangrenosum, 9 oral aphthous ulcers, 7 sacroiliitis, 7 primary sclerosing cholangitis, 6 episcleritis, 4 gall bladder stones, 4 uveitis, 3 erythema nodosum, 3 skin lesions, 3 cholangitis, 3 ankylosing spondylitis, Celiac disease, sickle cell anemia, Budd-Chiari syndrome, DVT and thrombosis, iridocyclitis, renal stones, and fistula.

For Crohn's disease (some patients have more than one condition); 14 arthropathy, 3 skin lesions, 2 erythema nodosum, 2 sacroiliitis, 2 oral aphthous ulcers, gall bladder stones, ankylosing spondylitis, Celiac disease, retroperitoneal fibrosis, and retinitis.

**Significant.*

TABLE 3 | Laboratory characteristics of patients with inflammatory bowel disease in Egypt (May 2018 to August 2021) before and after treatment.

Variables	UC group		P-value	CD group		P-value
	Before	after		Before	After	
WBC	7.9 ± 3.2	6.5 ± 2.2	<0.001*	7.7 ± 3.3	6.2 ± 2.0	<0.001*
HB	11.2 ± 1.9	11.9 ± 1.6	<0.001*	11.8 ± 1.8	12.3 ± 1.4	0.003*
PLT	314.4 ± 118.7	308.3 ± 119.0	0.199	284.5 ±125.9	231.6 ± 125.2	0.001*
Total Protein	6.97 ± 0.79	7.09 ± 0.70	0.514	7.08 ± 1.13	7.24 ± 1.24	0.361
Albumin	3.76 ± 0.59	3.65 ± 0.55	0.404	3.73 ± 0.56	3.72 ± 0.45	0.449
ALT	28.2 ± 19.7	29.8 ± 26.1	0.433	29.0 ± 17.8	24.8 ± 8.8	0.084
AST	30.2 ± 18.6	27.3 ± 15.0	<0.001*	25.9 ± 12.1	26.5 ± 10.4	0.465
CRP	21.2 ± 29.9	8.2 ± 20.4	<0.001*	34.1 ± 35.7	4.6 ± 6.9	<0.001*
ESR	46.2 ± 28.8	25.7 ± 21.7	<0.001*	52.9 ± 28.5	21.8 ± 18.5	<0.001*
Fecal calprotectin	584.5 ± 652.1	220.1 ± 311.0	<0.001*	505.1 ±712.2	176.5 ± 240.3	<0.001*

WBC, White blood cells; HB, Hemoglobin; PLT, Platelets; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; CRP, C reactive protein; ESR, erythrocyte sedimentation rate.
**Significant.*

are from rural areas. However, such differences may be due to many factors that should be studied in community-based research. Key issues may be socioeconomic status, environmental conditions, and access to diagnostic and treatment facilities.

Our study's mean age of onset was 35 and 34 years for UC and CD subjects, respectively. The onset of IBD in adults was reported as 31–34 years in North America, Western Europe, and Oceania (21–25). In Asia, the median age at diagnosis of CD was 34 years, and for UC was 42 years. UC was also found to start at more advanced ages, up to 79 years (26, 27).

The development of UC in our cohort was independent of gender, and however, CD was more common in males. These findings are consistent with epidemiological data from Europe, North America, and Oceania (21–25). However, UC was more common in males in data from Asia (28).

More than half of the study population was from rural areas, inconsistent with the available literature on IBD demographics. The urban population in Egypt has been almost stable since 2010 and represents 42.8% of the Egyptian population (29). However, the lifestyle in rural areas has been urbanized, and this change should be studied selectively. Zuo et al. (30) indicated that rapid urbanization in the developing world is associated with an increasing incidence of several autoimmune diseases, including IBD. Urbanization impacts gut microbiota through westernization of diet, raised pollution levels, increased usage of antibiotics, and better hygiene status. A westernized diet is low in carbohydrates and high in animal proteins and fats. This diet will alter gut microbiota.

The clinical presentation of IBD in our cohort is consistent with globally published data. The predominant manifestations of UC were diarrhea, rectal bleeding, and mucous discharge from the rectum (31). Conversely, CD was characterized by prolonged intermittent diarrhea with abdominal pain (32–35).

Ileal (47.8%) and ileocolonic (38.6%) regions were predominant sites of CD lesions, Colonic lesions were seen in (12.1%), while isolated upper GI CD was detected in only 1.4% of our study population. Slightly less than two-thirds of our patients (61.4%) showed non-complicated disease. Twenty-point

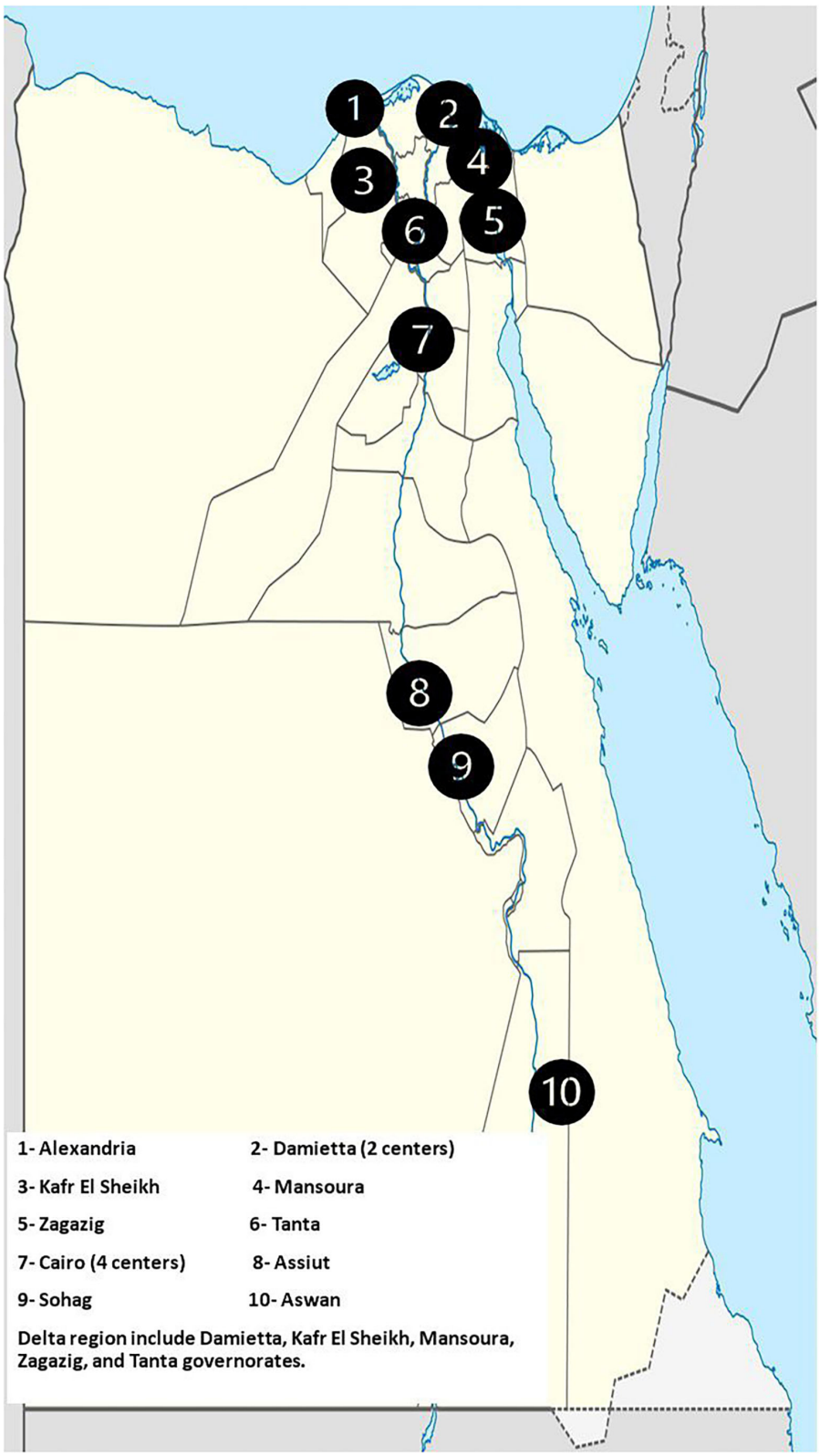

FIGURE 1 | Distribution of the participating centers in the study across Egypt.

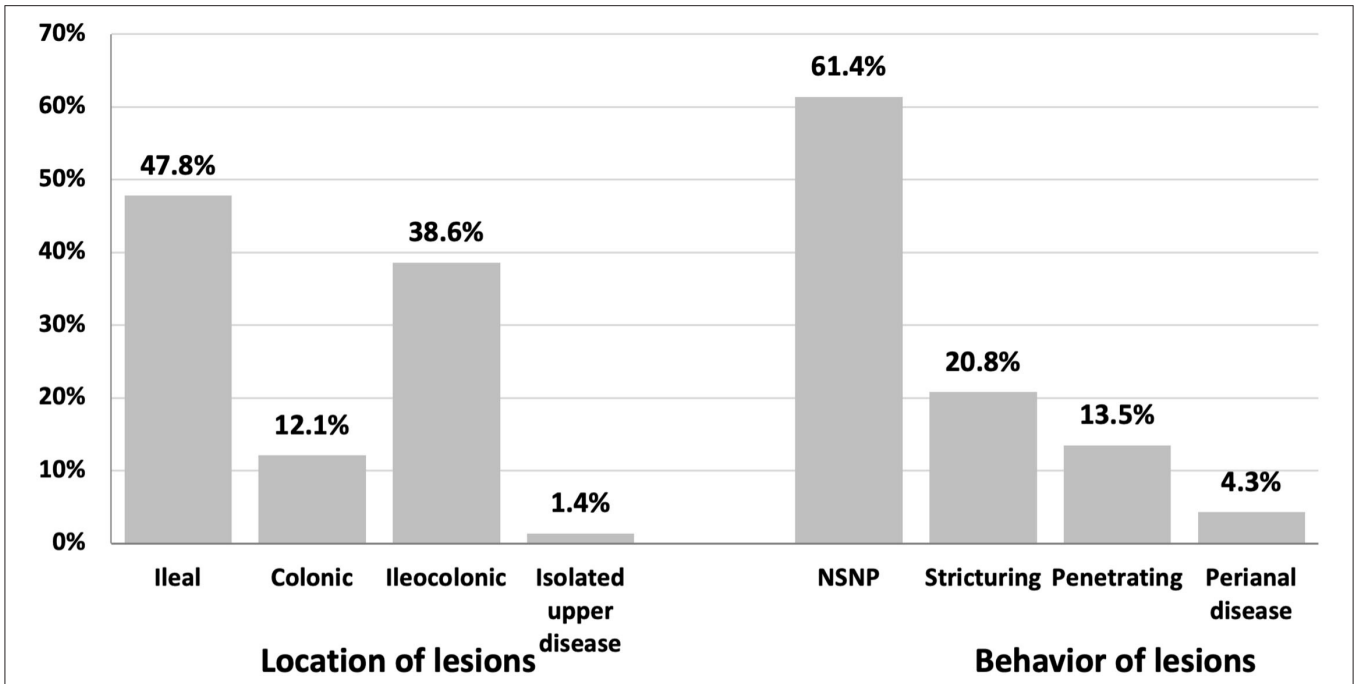

FIGURE 2 | Location and behavior of lesions by colonoscopy in patients with Crohn's disease in Egypt (May 2018 to August 2021) at the time of diagnosis.

TABLE 4 | Treatment characteristics of patients with inflammatory bowel disease in Egypt (May 2018 to August 2021).

Treatment[a]		Total *n* = 1104 (%)	UC group*n* = 897 (%)	CD group *n* =207 (%)
Approachfor treatment	Step-up	1045 (94.7)	864 (96.3)	181 (87.4)
	Step-down	59 (5.3)	33 (3.7)	26 (12.6)
Antibiotics				
Metronidazole		442 (40.0)	366 (40.8)	76 (36.7)
Quinolone		556 (50.4)	478 (53.3)	78 (37.7)
Others		60 (5.4)	40 (4.5)	20 (9.7)
Local				
5-ASA		344 (31.2)	335 (37.3)	9 (4.3)
Steroids		239 (21.6)	230 (25.6)	9 (4.3)
Systemic				
5-ASA		955 (86.5)	797 (88.9)	158 (76.3)
Steroids		691 (62.6)	582 (64.9)	109 (52.7)
Azathioprine		553 (50.1)	438 (48.8)	115 (55.6)
Biological[b]		281 (25.5)	221 (24.6)	60 (29.0)
Surgical (resection)		29 (2.6)	14 (1.6)	15 (7.2)

UC, Ulcerative colitis; CD, Crohn's disease; 5-ASA, 5-aminosalicylic acid.

[a] *Some patients received more than one treatment regimen and this treatment represented the cumulative frequency of drug usage.*

[b] *Some patients received more than one type of biological treatment.*

eight percent exhibit stricturing CD, and 13.5% show penetrating CD. These data are consistent with data from Europe and North America, where 27–42% of patients with CD have ileal lesions at the time of diagnosis, 23–33% exhibit ileocolonic disease, 28–35% show colonic lesions, and only 1–6% of patients present with upper GI CD (24, 36–39). Data from stricturing CD is estimated to occur in 29% and 19% of patients with CD in Europe (38) and North America (36), respectively. Conversely, Asian cohorts reported that more than half of patients with CD have an ileocolonic disease (40–42).

Multiple serum biomarkers were evaluated for their ability to confirm the IBD disease type and to predict the disease course. The atypical antineutrophil cytoplasmic antibody (atypical pANCA) and anti-Saccharomyces cerevisiae antibody (ASCA) are the most studied antibodies in IBD patients. is most often expressed by patients with ulcerative colitis. Atypical pANCA is

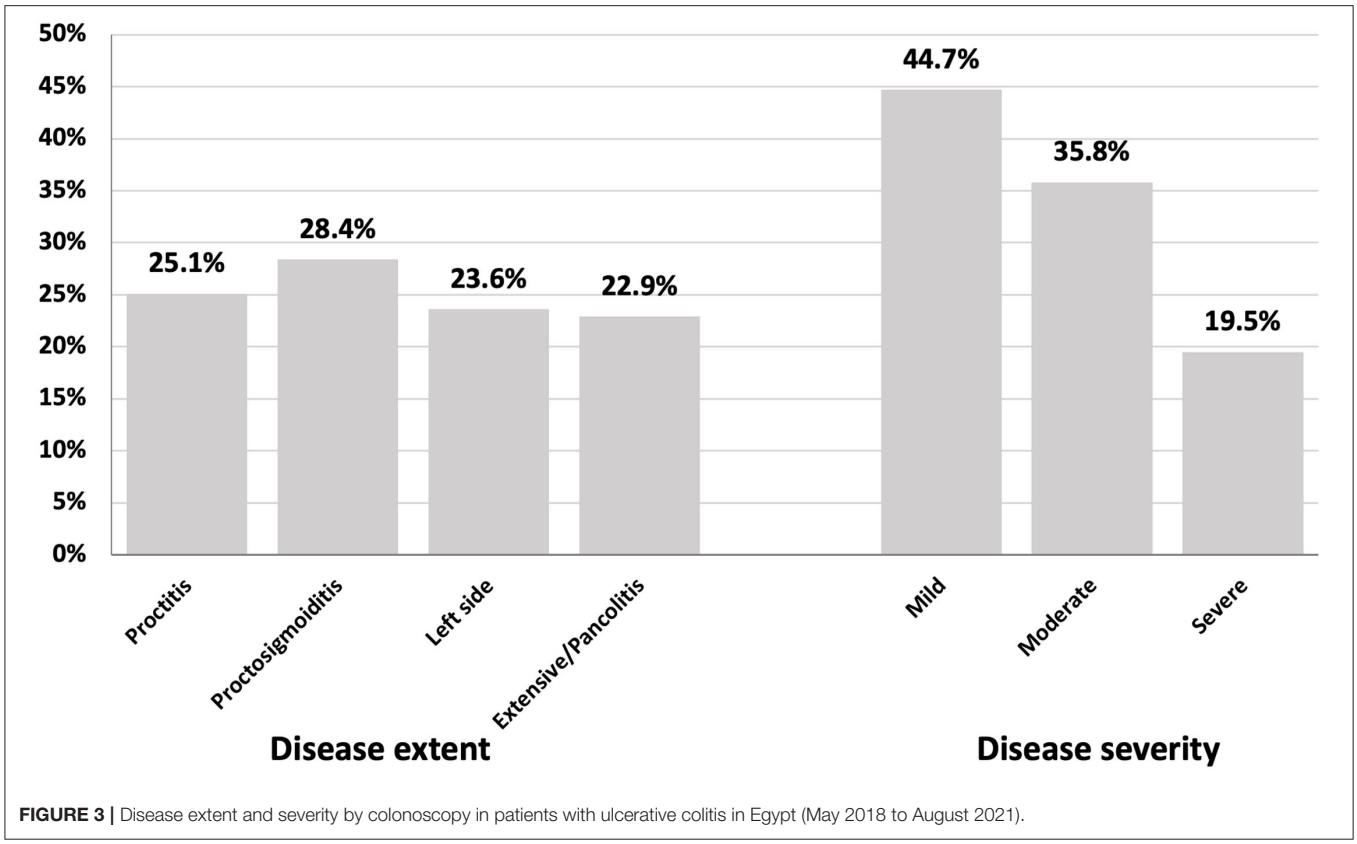

FIGURE 3 | Disease extent and severity by colonoscopy in patients with ulcerative colitis in Egypt (May 2018 to August 2021).

more common patients in patients with UC while ASCA in more frequent in CD patients (43). However, these tests are expensive and covered by health insurance, so they are commonly tested in Egypt.

Proctosigmoiditis was the most common disease site in UC cases in our cohort. Proctitis, left-sided colitis, and pancolitis were also seen in order of descending incidence. Most cases were mild to moderate at diagnosis. Disease extent and severity by colonoscopy in patients with ulcerative colitis are shown in **Figure 3**. Other cohorts showed that left-sided colitis was the most common disease site in Europe (39, 44).

Most of our patients (96.3% of patients with UC and 87.4% of patients with CD) received conventional medical therapy as a first-line treatment even if they were indicated for biological therapy. The high costs of biological therapy likely limit its use, which may not be sustainable. However, health insurance and government-sponsored treatment have started to cover the costs of biological therapy at many centers in Egypt. Infliximab and adalimumab began to be used in 2013, and new biological therapies, such as ustekinumab have been used more recently (45).

Fortunately, conventional step-up therapy is a practical approach for treating Egyptian patients with IBD. Significant improvement in inflammatory markers, including CRP, ESR, and FC, were observed after treatment of both UC and CD.

Colorectal cancer is the most serious complication for patients with IBD, and dysplasia usually precedes the development of colorectal cancer (46). In our study, cases with confirmed dysplastic lesions constituted 7.2% of the total population. Most were low-grade and discovered on the first diagnosis.

Available evidence suggests that IBD incidence in Africa is increasing. Data are still lacking to understand the disease pattern across this continent. A few reports from North and South Africa are available (47).

The major strengths of our study are its large sample size and geographic diversity of patients, and collected data provide a comprehensive, updated picture of IBD in Egypt. Data describe differences in some clinical, epidemiological aspects of the disease. However, the study has some limitations. First, the distinction between UC and CD was not always clear. We depended on the most likely diagnosis from the treating physician. Lacking the distinction between IBD subtypes is a global problem. In a recently published population-based cohort study in European countries including 1,289 patients with IBD, the confirmation of IBD subtype was impossible in 7% of the study population, even after 5 years of follow-up (48). Second, the incomplete data for the follow-up of some patients did not allow us to track the changes in the disease patterns or to do a statistical analysis to see the histological features at time diagnosis will affect the outcome of our patients. Third, we may have missed early mild cases of IBD, especially in the private sector. Fourth, risk factors and environmental determinants of IBD were not discussed. Finally, certain socioeconomic factors, such as income and education, in addition to the yearly incidence of new cases could not be assessed.

CONCLUSION

To our knowledge, our study is the first Egyptian cohort study from multiple highly specialized GI centers from all over the country. It examines the largest cohort of Egyptian patients with IBD and describes the clinical, epidemiological presentation, diagnostic procedures, disease behavior, and prognostic implications along with available therapeutic options. The number of IBD cases was higher in rural than urban areas despite limited resources and relatively poor facilities in rural areas. More effort should be directed toward screening patients with IBD in rural areas for early detection and proper management of the disease. Such effort may relieve the burden of unexpected serious maladies. Step-up conventional therapy for patients with IBD is still recommended and effective, especially in countries with limited resources. More extensive prospective epidemiological studies in Egypt, other countries of the Middle East, and Africa are still needed to fully characterize disease distribution, environmental factors, and pathological features of IBD. Such data can be compared with other parts of the world to complete the global map of IBD and produce worldwide guidelines for managing this severe expanding disease.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee for human subject research at the Faculty of Medicine, Helwan University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MElb and MEK: study design. MON: data analysis. MElb, MON, MH, EG, MAM, HS, SG, MElt, FE-R, MN, SA, WA, AS, AA, MA, AMa, GK, MM, OA-H, AE, IE-Z, AB, AMo, EB, and AE: patient recruitment, data collection, and writing up of the first draft of the paper. All authors revised and approved the final version of the manuscript.

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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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REVIEW
published: 20 April 2022
doi: 10.3389/fmed.2022.868508

Ulcerative Colitis: Novel Epithelial Insights Provided by Single Cell RNA Sequencing

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Edited by:
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *02 February 2022*
Accepted: *14 March 2022*
Published: *20 April 2022*

Citation:
Serigado JM, Foulke-Abel J, Hines WC, Hanson JA, In J and Kovbasnjuk O (2022) Ulcerative Colitis: Novel Epithelial Insights Provided by Single Cell RNA Sequencing. Front. Med. 9:868508. doi: 10.3389/fmed.2022.868508

Ulcerative Colitis (UC) is a chronic inflammatory disease of the intestinal tract for which a definitive etiology is yet unknown. Both genetic and environmental factors have been implicated in the development of UC. Recently, single cell RNA sequencing (scRNA-seq) technology revealed cell subpopulations contributing to the pathogenesis of UC and brought new insight into the pathways that connect genome to pathology. This review describes key scRNA-seq findings in two major studies by Broad Institute and University of Oxford, investigating the transcriptomic landscape of epithelial cells in UC. We focus on five major findings: (1) the identification of BEST4 + cells, (2) colonic microfold (M) cells, (3) detailed comparison of the transcriptomes of goblet cells, and (4) colonocytes and (5) stem cells in health and disease. In analyzing the two studies, we identify the commonalities and differences in methodologies, results, and conclusions, offering possible explanations, and validated several cell cluster markers. In systematizing the results, we hope to offer a framework that the broad scientific GI community and GI clinicians can use to replicate or corroborate the extensive new findings that RNA-seq offers.

Keywords: Ulcerative Colitis, single cell RNA sequencing, intestinal epithelium, goblet cells, colonic microfold cells, stem cells

INTRODUCTION

Ulcerative Colitis (UC) is a chronic and debilitating inflammatory disease of the colon, and is a distinct condition within a broad group of pathologies termed inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and indeterminate colitis. The etiology of UC remains uncertain, but increasing evidence implicates complex genetic and environmental contributions. Early genomic and immunologic studies were crucial to identifying therapeutic targets and avoiding surgical intervention. Development of infliximab and other monoclonal antibodies against TNFα, and the biologics targeting cytokines (IL-12 or IL-23) that significantly reduce inflammation and induce macroscopic/endoscopic healing, were major breakthroughs in the management of UC (1).

Newer drugs such as anti-integrin agents (vedolizumab and ustekinumab) and JAK inhibitors also yielded endoscopic improvements (1–4).

With the development of biologic therapies, the goal of UC treatment extended beyond symptomatic improvement to include both histologic and molecular healing. Several observational studies have shown that histological remission, including epithelial tissue restoration, is associated with lower rates of disease-related complications such as hospitalization, corticosteroid use, and colectomy compared to either resolution of symptoms or endoscopic improvement alone (5–7). Recognizing that histologic improvements are now included in UC clinical trials of multiple novel therapeutic agents (8), markers of active disease, therapeutic response and remission are urgently needed.

There are numerous UC-related pathologies that contribute to inadequate epithelial maintenance and regeneration. Cell turnover is increased due to autophagy and apoptosis, yet colonocyte differentiation is reduced (9–11). Barrier defects manifest as abnormal glycosylation and sulfation of mucins, loss of the protective mucus layer, and increased tight junction permeability (12–16). Microbial dysbiosis also contributes to altered epithelial function, in which reduced butyrate oxidation yields energy-deficient epithelium, and increased exposure to microbial signals stimulates inflammatory cytokine secretion (17–20). It is not clear if the many recognized pathologies initiate the disease or are consequences that fuel further development. Overall, the spectrum of pathologies highlights the challenges in defining molecular markers of health, UC damage, and epithelial restoration.

Single cell RNA sequencing (scRNA-seq) allows for in-depth transcriptional characterization of epithelial pathologies in UC and identification of possible pharmacologically fruitful targets. In this review, we discuss findings from two independent scRNA-seq datasets generated by the Broad Institute (21) and the University of Oxford (22) that compared subsets of epithelial cell types in healthy and UC colonic tissue. Our goal is to highlight novel markers for epithelial UC disease that, when further validated, may lead to identification of pharmacologic targets to promote epithelial healing in UC patients.

EPITHELIAL CELL DIVERSITY REQUIRES SINGLE CELL RESOLUTION TO CAPTURE DISEASE MARKERS

The heterogeneity of cells that compose the normal colonic epithelium originates from a common intestinal stem cell through the actions of multiple niche factors. As cells migrate from the crypt to surface epithelium, they mature into specialized cells for molecular transport, sensing, secretion, and barrier formation. Each epithelial cell type is critical to sustain organ function, and perturbations in the proportion or phenotype of a cell lineage can contribute to disease. Thus, approaches to study epithelial cell identity and function must distinguish among unique populations within the epithelium.

Transcript analyses using mRNA microarrays that preceded RNA-seq analysis allowed for the simultaneous detection of thousands of genes at one time (23) and was essential to demonstrate the presence of a persistent inflammatory state in patients with histologic remission (24, 25). A significant disadvantage of the microarray is that they require prior knowledge of the gene sequence under investigation. In contrast, RNA-seq has a wider dynamic range, allows for sequencing of transcripts without prior information, and can detect gene fusions, indels, and single nucleotide polymorphisms (26). scRNA-seq captures detailed molecular snapshots of individual cells that can be compared between healthy and diseased states to identify altered cell populations within non-homogeneous tissues. scRNA-seq quantifies the expression of each individual gene at a single cell level, offering high sensitivity for low abundance targets that may be masked in a bulk sequencing approach. This technology unveils the heterogeneity within major cell types, describes cell clusters that contribute to the pathogenesis of disease, and allows for a detailed description of the pathways that connect genome and transcriptome to pathology (27). scRNA-seq might be ideal to understand epithelial pathologies associated with UC in relation to healthy cells.

The technical aspects of scRNA-seq are described in great detail in several excellent reviews (28–30). Typical elements of scRNA-seq analysis include single-cell dissociation, isolation, library construction, and single cell sequencing (27, 31, 32). In general, the datasets generated by this technique are large and highly complex requiring robust bioinformatic analysis to be fully interpretable (29, 33).

The complexity of genetics and environmental factors that fuel UC make it a particularly good candidate for investigation through single cell transcriptomics. scRNA-seq potentially allows for detailed molecular snapshots of the different cell types composing the intestinal mucosa in health and UC. In particular, this technology facilitated the identification of previously unrecognized cell types and their potential transcriptional shift in disease that correlated to disease severity. These new findings could have direct implications in the management of UC. By stratifying patients on the basis of underlying transcriptional and genetic variability, scRNA-seq opens new ways for the development of targeted therapies.

A comparison of parameters and outcomes in the Broad and Oxford scRNA-seq studies is highlighted in **Table 1**. Notably, the number of subjects sampled and the epithelial cell types, or clusters identified by the two studies, differs. We focus on cell clusters that exhibited most dramatic transcriptional changes with potential relevance to inflammation, host stress response, immune regulation, and epithelial regeneration: BEST4$^+$ cells, colonic M-like cells, goblet cells, colonocytes and stem cells.

BEST4$^+$ CELLS

Both data sets discovered a novel differentiated absorptive surface colonocyte cluster characterized by expression of a $Ca2^+$-sensitive chloride channel (BEST4) and proton-conducting ion

TABLE 1 | Summary of RNA-seq studies in Ulcerative Colitis.

	Broad study	**Oxford study**
Study subjects	18 patients with UC and 12 healthy controls	3 patients with UC and 3 healthy individuals
Biopsy samples	One non-inflamed and one inflamed region or Two adjacent non-inflamed and two adjacent inflamed biopsies	Endoscopically inflamed distal areas of the colon and proximal clinically non-involved regions
Number of cells yielded	366,650 cells	11,175 cells
Single cell suspension	Dissociation with TrypLE Express Enzyme	Dissociation with TrypLE Express Enzyme
RNA-seq methodology	Droplet-based scRNA-seq technique	Droplet-based scRNA-seq technique
Library preparation	SMART-Seq2 protocol	SMART-Seq2 protocol
Differential gene expression	MAST; AUC $\geq$ 0.75	Seurat; AUC $\geq$ 0.7
Cells identified	Identified fifteen distinct groups of epithelial cells: - Stem cells - TA 1 - TA 2 - Cycling TA - Immature Enterocytes 1 - Immature Enterocytes 2 - Enterocyte Progenitors - Enterocytes - Microfold cells - BEST4 + Enterocytes - Secretory TA - Immature Goblet cells - Goblet cells - Tuft cells - Enteroendocrine cells	Identified seven distinct groups of epithelial cells*: - Undifferentiated #1 - Undifferentiated #2 - Enteroendocrine cells – Colonocytes - Crypt Top Colonocytes - BEST4 + /OTOP2 cells - Goblet cells

TA: transit amplifying.
**Which were further subdivided into 5 clusters based on their location along crypt-surface axes.*

channel (OTOP2) proposed to regulate luminal pH. These cells were also enriched in the paracrine hormones guanylin (GUCA2A) and uroguanylin (GUCA2B), which can stimulate fluid and electrolyte transport through action on the receptor guanylate cyclase. Among 46 transcripts significantly elevated in healthy BEST4 + cells in the Broad study, 29 were also detected as significantly elevated in the Broad study (**Figure 1**). However, several transcripts were not exclusive to BEST4 + cells and thus do not serve as unique markers.

We performed STRING interactome analysis (human Protein-Protein Interaction Networks and Functional Enrichment Analysis using[1]) of the characteristic transcripts of BEST4 cells which confirmed across both studies that in healthy tissue, BEST4 cell function includes metallothionein expression (HSA-5661231), guanylate cyclase/dehydratase activity (GO:0030250), and proton channel activity (GO:0015252). Surprisingly, proteomic analysis of these BEST4 cells, conducted in the Oxford study, identified several highly upregulated proteins as BAG1 and APOO. These transcripts were not identified as "characteristic" for this cell cluster in Oxford scRNA-seq dataset (the exceptions were BEST4 and CTSE proteins which were only slightly upregulated in comparison to the rest of epithelial cells). These data indicate that further validation at the protein levels are necessary to reliably identify these BEST4 + cells in tissue and better define their role in colonic physiology.

UC-associated changes in the BEST4 + cell transcriptome did not completely agree between the studies. The Broad study found the number of BEST4 + cells were substantially reduced in UC, and 16 significantly upregulated transcripts ($\log_2$FC $\geq$ 4.0; **Figure 1**) were putative disease-associated BEST4 cell markers. In contrast, the Oxford study observed stable numbers of BEST4 + cells and listed 28 upregulated transcripts (fold increase for each transcript was not provided) of which only two (CCL20 and S100P) overlapped with the Broad results (**Figure 1**). MT1E was the only transcript elevated in BEST4 + cells in both studies regardless of disease state. Other UC-associated BEST4 + upregulated transcripts, such as MT1H and MUC1 (Oxford) or TNFAIP2 and NCEH1 (Broad), did not correlate between the studies. Results of the Oxford study suggest that BEST4 + cells maintain host protective functions in UC [metallothioneins (HSA-5661231), antibacterial (HSA-6803157) and antiviral (CL:4665) pathways], whereas no specific function was identified in the elevated transcripts from Broad study.

Because most of the upregulated transcripts in UC-associated BEST4 + cells were elevated in other cells types, we cross-referenced the single cell results with an earlier bulk RNA-seq data set generated from three treatment-naïve UC patients (34). Markers of healthy BEST4 + cells, including BEST4, OTOP2, CA7, MT1H, and MT1G, were also downregulated in active UC analyzed by bulk RNA-seq. Elevation of UC-associated transcripts not specific to BEST4 + cells, including CCL20, S100P and PLA2G2A, were also detected in the bulk RNA-seq study.

[1]https://string-db.org/

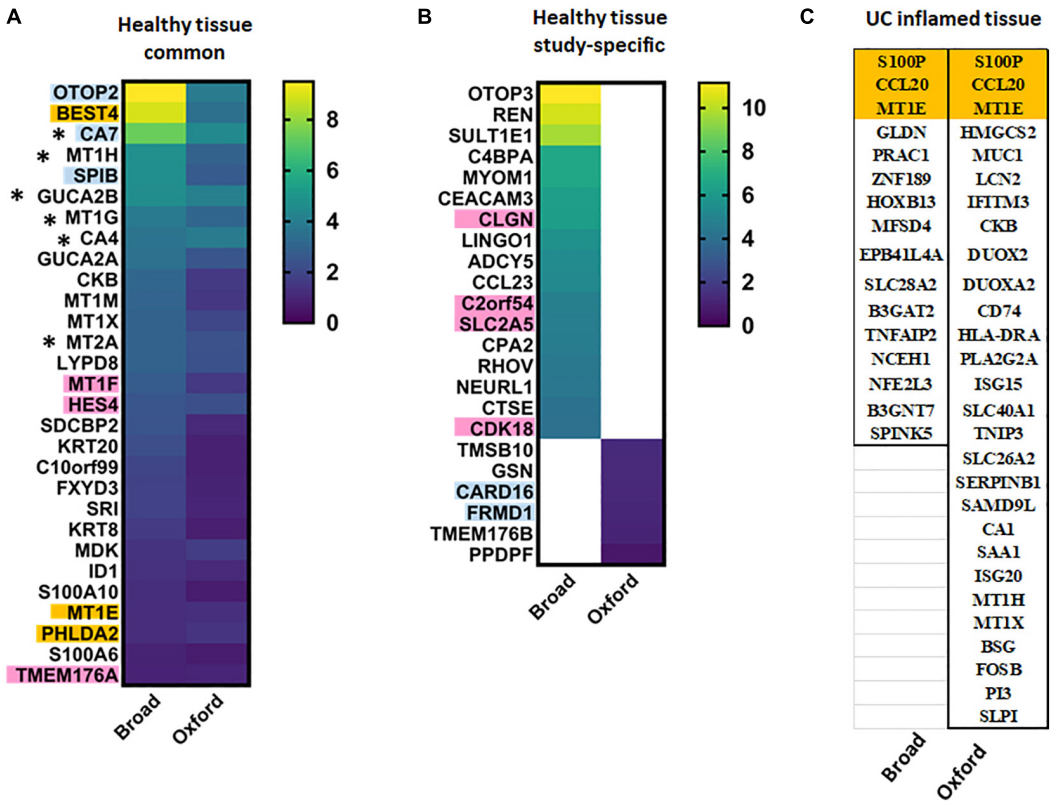

FIGURE 1 | BEST4$^+$ cell characteristic transcripts in health and UC inflammation. Heat maps of transcripts significantly elevated in BEST4$^+$ cells in **(A)** both studies or **(B)** one of the studies in healthy epithelium presented as $\log_2$[fold change (FC)]; **(C)** transcripts characteristic for BEST4$^+$ cells in UC inflamed epithelia. Transcripts in orange are BEST4$^+$ cell-specific in both studies and thus might serve as cluster markers in health or disease. Transcripts in blue are specific to healthy BEST4$^+$ cells in Oxford study; transcripts in pink are specific to healthy BEST4$^+$ cells in Broad study. * – transcripts are elevated in healthy BEST4 cells in both studies, but are downregulated in active UC in the Broad study.

Upregulated PLA2G2A expression was further confirmed by qRT- PCR and immuno-histochemistry in tissue samples from UC patients and healthy controls, further supporting the bulk RNA-seq and scRNA-seq data (34).

Additional scRNA-seq study of colon biopsies from UC patients and healthy controls of Chinese Han ancestry (35) complemented the Broad and Oxford studies that were conducted in individuals of primarily European ancestry (21, 22). In this study, 43,218 cells representing UC-affected sigmoid colon, unaffected proximal colon from UC patients, and sigmoid colon from healthy controls with an average of 1053 genes per cell were mapped into 21 clusters, six of which were epithelial cells (enterocytes, enterocyte progenitors, goblet cells, goblet progenitors, LGR5 + stem cells, and TRPM5 + tuft cells). A BEST4 + cell cluster was not uniquely identified but was found among transcripts in the enterocyte cluster.

COLONIC MICROFOLD (M)-LIKE CELLS

Microfold (M) cells are part of an integrated system of immunosurveillance in the intestinal mucosa, with a principal function of transporting luminal antigens to gut-associated lymphoid tissue (36, 37). This activity has led to the idea that M-like cells could contribute to a "leaky gut" (38). There are several types of small intestinal M cells that display a common set of morphologic and functional features but differ in their specific gene expression patterns. Our knowledge of M-cell morphologic, molecular and functional features are mainly based on small intestinal M cells. M-like cells in the colon have been described to emerge under inflammatory conditions in mouse models, but relatively little is known about M-like cells in human colon (39).

According to the Broad study, specific markers of colonic M-like cells in healthy tissue were quite different from M cells in ileal Peyer's patches. Glycoprotein 2 (GP2), the M cell-specific marker that functions as a bacterial uptake receptor in the Peyer's patches, was not reported in colonic M-like cells, and neither were genes such as PGLYRP2, CLEC7A (Dectin-1), nor JAG1. M cells from Peyer's patches and colon shared only two transcripts, CCL20 and SPIB, which are known to initiate M cell differentiation (41). CCL20 is a UC GWAS gene that may serve as a marker of colonic M-cells in healthy tissue (40). Transcripts for both SPIB and TNFSF11, the RANKL receptor, in colonic M-like cells indicate similarities between the pathway of M cell differentiation in ileum and colon. However, SPIB was also present in BEST4 + and Tuft cells, precluding this gene as a

specific M-like cell marker. SPIB transcription in BEST4 + cells was confirmed by the Oxford study (22).

We analyzed differences between M-like cells in healthy controls and UC (**Table 2**). Not only were M-like cell numbers significantly elevated in inflamed mucosa, these cells also had increased transcription of CCL20 and CCL23, implicating them in the recruitment of other immune cells and propagating inflammation. Only two transcripts, CCL20 and SPINK5, were elevated in M-like cells in healthy colon as well as in UC. However, both transcripts were highly upregulated in UC in other absorptive and secretory cell clusters, thus precluding them from being specific M-like cell markers. Notably, SOX8 did not appear upregulated in any other cell cluster in UC (**Table 2**). We conclude that SOX8 could be used to identify inducible colonic M-like cells and study their function in health and UC.

GOBLET CELLS

Goblet cells are critical in maintaining a protective mucus layer that provides separation between epithelia and luminal content. Both studies identified undifferentiated and differentiated transcriptional clusters of goblet cells (GCs) in relationship to the colonic crypt-surface axis. GCs were sub-classified into immature or mature populations (Broad), or grouped into five transcriptionally distinct clusters (Oxford). Among the 64 major GC markers identified by the Oxford study, 35 were also found elevated in GC clusters in the Broad study. Twelve transcripts highly elevated ($\log_2$FC $\geq$ 4.0) in both, mature and immature GCs in Broad study were also elevated in this cell type in Oxford study (**Figure 2**). Both studies are concordant in that there is increasing expression of MUC2 and ZG16 along the crypt-surface differentiation axis, while TFF3, ITLN1, SPINK4, CLCA1, and WFDC2 expression is higher in immature GCs (**Figure 2**). Surprisingly, only BEST2 and ZG16 were GC-specific in Broad study.

Importantly, transcripts elevated in goblet cells (**Figure 2**) were in agreement with the subsequent study by Li et al. (35), including MUC2, ITLN1, REP15, LRRC26, NPDC1, TPSG1, SERPINA1, CLCA1, and SPINK4.

Of particular interest is the enrichment of mucins other than MUC2 in mature GCs. Both studies agree that transmembrane MUC1 and MUC4 are enriched in differentiated GCs, but are also detected in other cell clusters (transit amplifying (TA) cells). MUC12 is listed as elevated in GC cluster 4 in the Oxford study, but is elevated in colonocytes in the Broad study. MUC13 is enriched in mature GCs and in absorptive cells in both studies. MUC17 was not reported in GCs in any of the studies. Surprisingly, gel-forming MUC5B was elevated in mature GCs in the Oxford study but was not listed in GC clusters in the Broad study (instead, MUC5B was elevated in TA lineages).

The changes in goblet cells in UC through the prism of scRNA-seq are striking. It is widely accepted that number of GCs and the luminal mucus layer are significantly reduced in UC-damaged epithelia (41). Both studies found a significant decrease in GC numbers and significantly altered transcriptional signatures (**Figure 2**). However, the transcriptional sub-populations of spatially distinct goblet cells (crypt bottom vs. top) were preserved in active UC-derived epithelial tissues. Additionally, in the Oxford study, a novel cluster of inflammation-associated GCs was found in UC.

Overall, GC clusters in UC partially lose their secretory identity and are characterized by a mix of transcripts present in both absorptive and secretory lineages. Each GC cluster in UC exhibited highly heterogeneous transcriptional changes, and a consensus for GC markers was not evident. Among 139 transcripts listed in the Oxford study as upregulated in GCs in UC, only 15 (11%) were also found in the Broad study. Additionally, many of these transcripts were also significantly elevated in non-GCs in UC. This significant transcriptional transformation of GCs may explain their functional transformation.

In UC-affected epithelium (**Figure 2**), only SCD, RNASE1, and ASRGL1 were concordant with goblet cell findings by Li et al. (35).

STRING interactome analysis of GC-upregulated transcripts in UC from the Oxford study suggest that GCs gain properties for antigen presentation (HSA04612), stress response (GO:0006950), and immune regulation (GO:0002376). Other studies have alluded to this ability of GCs to act as antigen presenting cells,

TABLE 2 | Differential gene expression for M cells.

Broad Study	
Health	**Ulcerative Colitis**
CCL23	PI3
SOX8	FAM84A
RP11-161M6.2	CRABP2
NTRK2	KCNE3
CCL20	TMEM158
NPB	SLC6A14
MIA	ANKRD34A
SPINK5	HOXB13
SERPINF2	TCF12
KCNE2	BHLHE41
CTSE	
TNFAIP2	
SPIB	
MSLN	
TNFRSF11A	
POLD1	
SLC2A6	
AC026471.6	
TNFRSF11B	
AKR1C2	
GJB3	

	Transcript also reported in Oxford study (but not linked to M-cell)
Red	Transcript reported in at least one other cell lineage in Broad study
Blue	Transcript reported in both healthy subjects and UC in Broad study

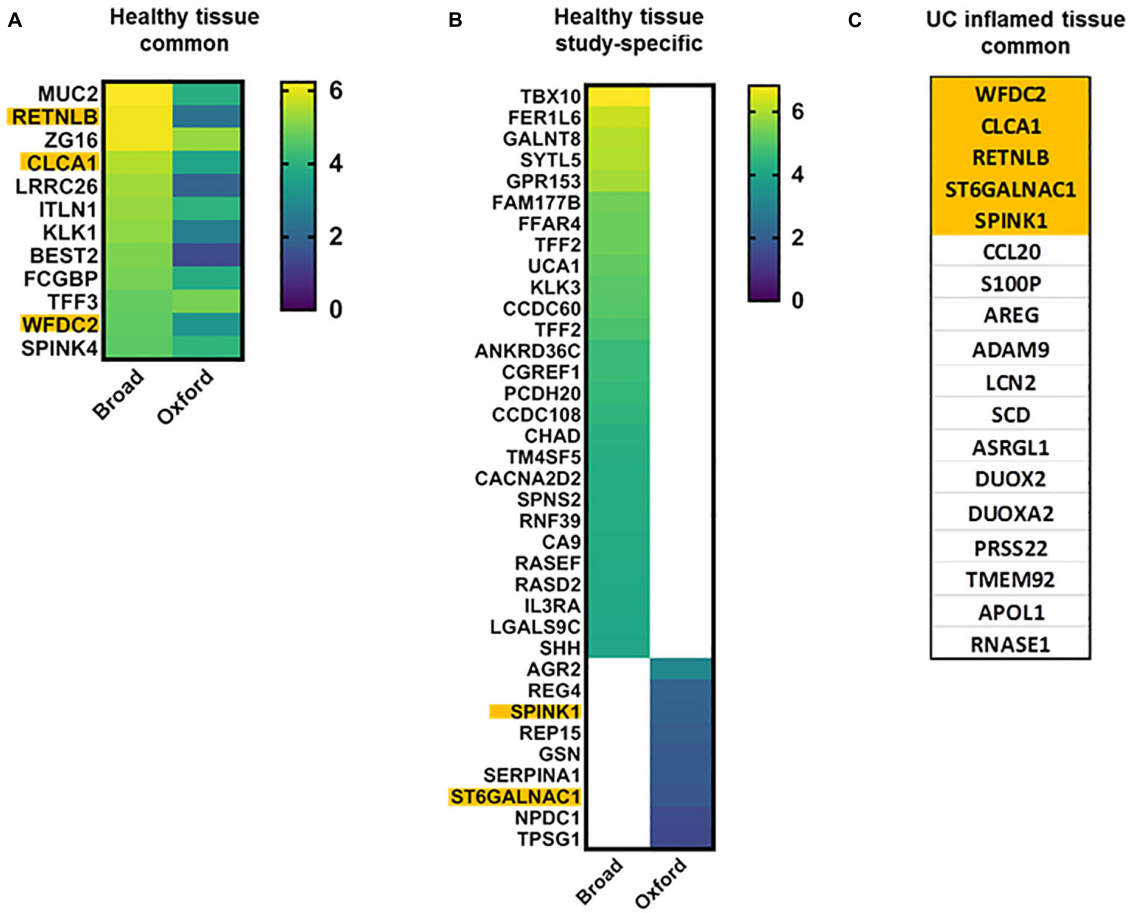

FIGURE 2 | Goblet cell characteristic transcripts in health and UC inflammation. Heap maps of transcripts characteristic for differentiated and undifferentiated goblet cells in healthy tissue [**A,B**; log_2(FC)]; transcripts characteristic for differentiated and undifferentiated goblet cells in UC **(C)** in both datasets. Transcripts in orange are common for healthy and UC-affected GCs. ITLN1 and CCL20 are GWAS genes.

including delivery of antigens to dendritic cells (42) or microbial sensing and recruitment of inflammatory cells (43).

One of the most striking results from both scRNA-seq datasets is that MUC2 transcript levels were similar between healthy and UC samples, yet MUC2 protein is substantially decreased in patients with severe UC (41, 44). The discrepancy in detected MUC2 transcripts and protein in UC prompted us to perform a similar comparison for other goblet cell markers. Proteomic analysis of mucus from healthy or UC sigmoid colon biopsies showed that major core mucus components, including MUC2, FCGBP, CLCA1, and ZG16 were significantly reduced in active UC (42). Protein levels of TFF3, MUC4, 5B, 12 and 13 and ITLN1 did not change in mucus from UC patients. These data suggest post-transcriptional regulation of several core mucus proteins in disease accounts for differences between mRNA and protein expression in active UC.

It was surprising that transcript levels of MUC5AC, a gel-forming mucin that is typically associated with surface gastric epithelia, but can be induced in the intestine during infection (45, 46), was significantly elevated in inflamed colon. A recent study also found elevated MUC5AC transcript levels in active UC, although there was no significant correlation between MUC5AC expression levels and UC disease severity (47). However, MUC5AC was not reported among the core mucus proteins in active UC (45). MUC5AC is frequently present in colorectal adenomas and colon cancers, thus it's elevation might be due to UC-induced carcinogenesis (48).

In conclusion, according to both scRNA-seq datasets, goblet cell clusters in UC lost some of their secretory hallmarks and were characterized by a mix of transcripts present in both absorptive and secretory lineages.

COLONOCYTES

Colonocytes represent the largest cell pool in colonic epithelial tissue. Numerous studies have characterized the UC inflammation-induced pathologies of colonocytes and suggested that these pathologies must be pharmacologically addressed to achieve a full and sustained remission. Colonocytes can be spatially and functionally divided into three groups: Undifferentiated cells at the bottom of crypts, cells undergoing

a transitional differentiation, and fully differentiated surface colonocytes. For the first time, scRNA-seq allows for precise characterization of the stages of cell differentiation and identification of the number of transcriptionally stable transition clusters which traverse from undifferentiated to fully differentiated. We analyzed the cluster specific markers of colonocytes in health and UC disease to gain insights into the molecular-cellular pathways driving UC disease.

Differentiated Absorptive Colonocytes in Health and Ulcerative Colitis Disease

Both studies identified a cluster of terminally differentiated colonocytes (additionally to BEST4 + cells) named Crypt Top Colonocytes (CTC) in the Oxford study or Enterocytes in the Broad study. The CTC cluster included ∼ 264 upregulated transcripts (AUC > 0.7) and Enterocyte cluster included ∼150 significantly upregulated transcripts. Seventeen of these highly elevated transcripts are identified in both studies but neither of these transcripts is cluster-specific (**Figure 3A**). However, seven less elevated transcripts are cluster-specific and overlap in both studies (**Supplementary Table 1A**), and thus may serve as markers of mature colonocytes. ABCB1, an ATP-dependent drug efflux pump for xenobiotic compounds, is one of the common transcripts, and mutations in this gene are associated with IBD. Others are interferon-regulated proteins, including dsRNA-activated antiviral enzyme OAS1 which plays a critical role in cellular innate antiviral response, ubiquitin-like modifier ISG15, and sodium-phosphate symporter SLC20A1, which plays a fundamental housekeeping role in phosphate transport.

Many transcripts highly upregulated in differentiated colonocytes in one (**Figure 3B**) or both studies were also detected in other cell clusters (**Supplementary Tables 1B,D,E**). For example, CTC-specific CDHR2, CLCA4, MS4A12, and SLC9A3R1 were also significantly elevated in differentiated colonocytes in Broad study, but were not cluster-specific. SLC26A3/DRA, the bicarbonate/Cl- exchanger is one of these transcripts and is a well-known marker of differentiated colonocytes validated in numerous studies (49). Low levels of DRA are also detected in undifferentiated colonocytes in both studies. DRA is involved in response to stimulus, immune system process, cell junction organization, bicarbonate transport and regulation of sodium transport. The expression differences in such transcripts between the two studies is likely due to large differences in the number of analyzed cells (**Table 1**). A larger number of analyzed cells, as in Broad studies, improves the detection sensitivity for low abundance transcripts. Only a few transcripts are single cluster-specific and could not be detected in other clusters. Instead, the majority of the transcripts form gradients between different cell clusters. Further validation experiments are necessary to test whether inter-cluster copy number difference is large enough to allow use of a particular transcript as the cluster marker of differentiated colonocytes in healthy tissue, as in the case of DRA (**Supplementary Tables 1B,D,E**).

Only four transcripts were discordant (**Supplementary Table 1**). These transcripts were elevated in healthy Enterocytes in the Broad study but were among the UC inflammation-elevated transcripts in the Oxford study.

In UC inflammation, only four highly elevated and specific transcripts for differentiated colonocytes were common (**Figure 3C** and **Supplementary Table 1I**); these might serve as UC disease markers of the differentiated colonocyte cluster. In the Oxford study, the UC-affected CTC cluster is characterized by ∼300 upregulated transcripts, with only 17 transcripts shared between inflamed and healthy CTC (**Supplementary Table 1D**), suggesting substantial structural and functional changes in this cell type in disease. STRING analysis of the CTC-specific transcripts in UC confirmed high representation of immune system processes. In contrast, bicarbonate and sodium transport and cell junction organization pathways were absent from the UC transcriptome. Loss of SCL26A3 was previously linked to inflammatory diarrhea in UC patients (50, 51).

Additionally, there were significant changes in the composition of claudins in CTC cells; while CLDN7, 23, and 4 were elevated in healthy epithelium, CLDN1 and eight were elevated in disease. Mutations in NOD2, one of the major UC disease susceptibility genes, have been shown to alter the composition of tight junction proteins including upregulation of CLDN1 (52). scRNA-seq results confirm previous findings and correlate with the less studied CLDN8 in UC. UC-affected CTC cells also lose expression of CDHR5, an important intermicrovillar adhesion molecule that forms calcium-dependent heterophilic complexes with CDHR2 on adjacent microvilli (53). These two proteins control the packing of microvilli at the apical membrane of colonocytes and play a central role in brush border differentiation. Taken together, the CTC transcriptional signature reflects loss of differentiation in UC-inflamed epithelium. Interestingly, transcripts related to viral entry, specifically TMPRSS2, CHMP2A, 2B, and 4B, are downregulated in the CTC cluster in active UC, which might be protective against viral infections, including SARS-CoV2, despite an elevated cytokine profile (54, 55).

In the Broad study, ∼111 protein transcripts were upregulated in the UC-inflamed Enterocyte cluster with only 16 being cluster-specific (**Supplementary Tables 1I,L**). Again, large difference between the numbers of cluster specific elevated transcripts in UC–affected differentiated colonocytes between the studies is probably due to the number of analyzed cells; the less cells are analyzed the more cluster-specific transcripts it would produce. Elevation of the proinflammatory cytokine LIF was previously reported in UC patients, confirming the scRNA-seq data. LIF stimulates cell proliferation and thus may play a critical role in tumor development in UC patients (56). Another 13 transcripts elevated in multiple clusters in UC from the Broad study were also specifically elevated in UC-affected CTC cells (**Supplementary Table 1J**). The Broad study identified a single SAA1 transcript elevated in both healthy (specifically) and UC-affected (non-specifically) Enterocytes. SAA1 expression in epithelium has been shown to serve as an important link between mucosal T cells, microbial communities, and their tissue environment in patients with IBD (57), further supporting these scRNA-seq findings.

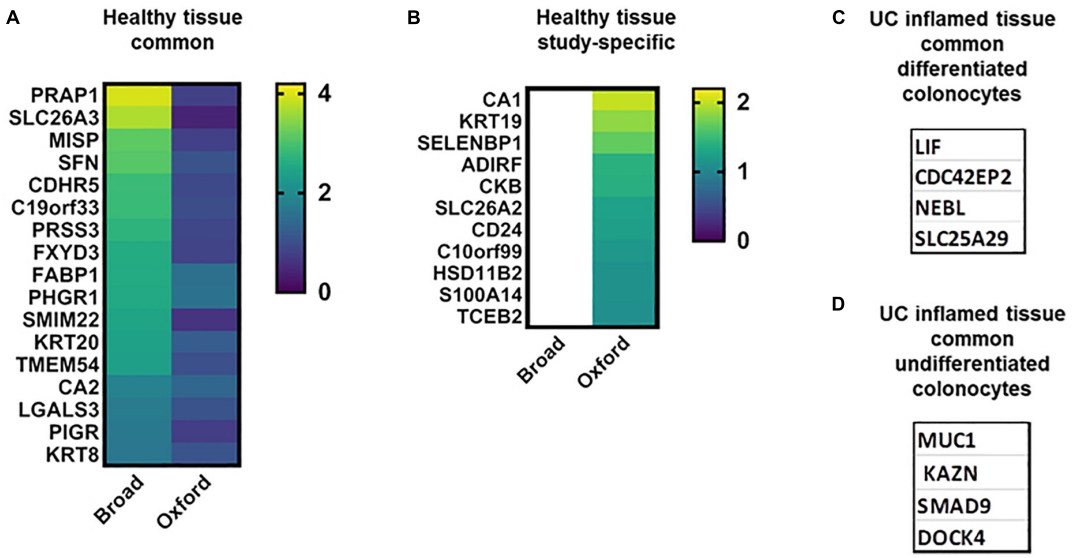

FIGURE 3 | Colonocyte characteristic transcripts in health and UC inflammation. Heat maps of transcripts characteristic for differentiated and undifferentiated colonocytes in healthy tissue [**A,B**; $\log_2$(FC)]; colonocytes – characteristic transcripts in UC in both datasets **(C,D)**.

Undifferentiated Colonocyte Clusters in Health and Ulcerative Colitis Disease

The pool of immature healthy colonocytes is mainly represented by three distinct clusters of Immature Enterocytes 1 and 2, and Enterocyte Progenitors in the Broad study. We searched for specific cluster markers among the substantially elevated protein coding transcripts (log2fc $\geq$ 4.0; **Supplementary Table 2**). DUOX2 and TMEM150B were exclusively elevated in the healthy Immature Enterocytes 1 cluster. Similarly, FOXH1, C14orf180, and HS3ST6 may serve as markers of Immature Enterocytes 2. Surprisingly, no protein transcripts elevated above this threshold were among Enterocyte Progenitors. In addition, there were no transcripts upregulated above the threshold that are common and specific for all three clusters.

In UC-inflamed tissue, according to the Broad study, eight elevated (log2fc $\geq$ 4.0) transcripts were shared among all three clusters (**Supplementary Table 2H**). However, these transcripts were upregulated in numerous other clusters, including differentiated absorptive and secretory lineages. Importantly, the expression pattern of these eight transcripts substantially differs between inflamed UC and healthy tissue. In healthy tissue, they are upregulated in a single or few clusters. However, in UC they are spread across many clusters, further demonstrating that the major UC disease phenotype dissociates the molecular boundaries between different cell types and their stage of differentiation. For example, CCL20 appears as an M-like cell marker in healthy tissue, but is broadly elevated in both secretory and absorptive cells in UC. Similarly, in healthy epithelium, GLDN and DUOX2 are elevated exclusively in Enterocyte Progenitors and Immature Enterocytes 1, respectively, but in UC are elevated in at least seven other secretory and absorptive cell clusters. DUOX2 is the only Immature Enterocytes 1 transcript elevated in both healthy and diseased epithelium. It has been shown that epithelial DUOX2 and DUOXA2 form the predominant enzyme system for H_2O_2 production in human colon and in active UC (58), further supporting these scRNA-seq data.

Elevated MUC1 could be considered as a marker of Immature Enterocytes 1 in active UC (**Figure 3D**), while in healthy tissue MUC1 is expressed throughout all epithelial clusters. SMAD9, KAZN, and DOCK4 (**Figure 3D**) could be considered as markers of Immature Enterocytes 2. Several transcripts highly elevated in the clusters of undifferentiated colonocytes further illustrate the loss of cluster-specificity expression in inflamed UC epithelium compared to healthy tissue. For example, in healthy epithelium, SDR16C5 is elevated in M-like cells, Goblet cells and Tuft cell clusters, MIA is elevated in M-like and Tuft cells; and PRSS22 is elevated in Secretory and Tuft cells; however, all three transcripts are elevated in nearly all the epithelial cell clusters in UC. Importantly, SI, a well-known marker of fully differentiated small intestinal enterocytes was not detected in healthy human colon, but was broadly expressed in UC, including undifferentiated colonocytes. SI has been detected in inflammatory, regenerative, and dysplastic mucosa in Ulcerative Colitis (59). Additionally, OLFM4 is mainly upregulated in Stem and TA1 clusters in healthy epithelium, but in UC is elevated in TA and Absorptive Enterocytes. The expression pattern of these transcripts again shows the loss of cell type specificity in UC.

The transcriptional comparison of immature colonocytes between two studies is challenging and not straightforward due to differences in transcript clustering algorithms. We speculated that Colonocytes and Absorptive Progenitors could be clusters that represent immature colonocytes in Oxford study. Eight transcripts found to be most elevated in all three undifferentiated colonocyte clusters in the Broad study were also significantly elevated in UC Colonocytes in Oxford study. Four transcripts

(TNIP3, S100P, DUOX2, and CXCL1) were also elevated in Absorptive Progenitors. Again, similar to the results of the Broad study, none of the transcripts were cluster-specific and all were upregulated in most clusters except EECs in the Oxford study. Additionally, both datasets suggest that DOCK4, a membrane-associated cytoplasmic protein, which functions as a guanine nucleotide exchange factor involved in regulation of adherence junctions between the cells, may be a reliable marker of undifferentiated colonocytes in UC disease. Although its role in UC disease is not known, DOCK4 was recently identified as a regulator of goblet cell differentiation and MUC2 production in the gut (60).

Several transcripts identified as markers of healthy differentiated colonocytes in the Broad and Oxford studies (VAMP8, OAS1, ISG15, SLC20A1; **Figure 3**) were concordant with data by Li et al. However, the remaining transcripts that were listed as markers of healthy differentiated or undifferentiated colonocytes (**Supplementary Tables 1, 2**) were not reported. CDC42EP2, a potential marker of UC-affected differentiated colonocytes was also reported by Li et al., while MUC1, a potential marker of undifferentiated colonocytes, was elevated in tuft cells in addition to enterocyte progenitors.

COLONIC STEM CELLS

Altered histology and transcriptional profiles in UC epithelia have been observed to persist even after achieving a disease remission (61), suggesting that the colonic stem cell (SC) may be permanently changed during the course of disease (18). Somatic nonsense or gene mutations have been discovered in UC epithelia, including genes involved in IL-17 signaling, such as NFKBIZ, ZC3H12A, and PIGR (62). Inflammation-induced somatic mutations might provide clonal advantage to these stem cells and propagate UC-related pathologies. We next analyzed stem cell profiles in both scRNA-seq data sets to search for potential signatures associated with UC.

The SC cluster from healthy tissue was not described in the Oxford study, possibly because only 38 cells contained the stem cell marker LGR5. The Broad study identified LGR5 as the most SC-enriched and specific transcript (**Table 3**). Among the 18 most elevated SC transcripts ($\log_2$FC $\geq$ 4.0), 16 were SC specific, which might substantially broaden the ability to identify and characterize human colonic SC *via* immunoblotting, immunofluorescence, functional assays and other approaches. OLFM4 is often used as a substitute marker for SC (63) and is elevated ($\log_2$FC 2.4) in both SC and TA1 cells. Interestingly, several other highly expressed SC-specific transcripts are lincRNAs (RP11-219E7.4 and RP11-760H22.2) and RP11-84C10.4 antisense, all of which might be involved in SC-specific regulation of CFTR.

In the Broad study, 65 transcripts were upregulated in SC in active UC relative to healthy controls. Although a majority of these transcripts were also upregulated at least in one other cell cluster, eight transcripts were specific to SC in UC (**Table 3**). Surprisingly, the upregulated transcripts are commonly associated with healthy differentiated cell types such as colonocytes (EZR, IFI27) or goblet cells (IER2, HBEGF, NFKBIA, and FAM3D). The functional significance of these UC-induced SC transformations remains to be determined.

STRING analysis of the 65 upregulated SC transcripts found associations to stress response (GO:0006950) and cell communication (GO:0010646) as the most inclusive gene ontology pathways. These pathways also included a large set of molecules involved in immune response. Multiple HLA and IFI transcripts, either SC-specific or shared between SCs and other epithelial cell clusters in UC, were significantly elevated, indicating a shift in SC function toward antigen presentation in UC. HSPA6 and CCL20 are UC GWAS genes, possibly linking genetic predisposition to UC with changes in the SC transcriptome. In UC, the fate of SC markers LGR5, OLFM4, or AXIN2 is not clear, as these transcripts were not listed among those enriched or downregulated in UC in the Oxford study. According to the Broad study, AXIN2 was significantly downregulated in stem cells in UC. In contrast, OLFM4 was slightly upregulated in stem cells and much more elevated in all TA clusters and in Enterocytes in UC. The lack of overlap between the two studies on SC markers in inflamed colonic tissue indicates that it is too soon to hypothesize on the mechanism(s) contributing to long-lasting changes in SCs in active UC.

The expression of potential stem cell-enriched transcripts (**Table 3**) was not reported in healthy epithelium by Li et al., despite the presence of LGR5 + stem cell clusters in both healthy and UC tissues. In UC, markers elevated in stem cell cluster in either the Oxford or Broad study were discordant with the findings reported by Li et al. (35). EZR, a classical marker of epithelial cells, was found elevated in several epithelial cell clusters, and surprisingly, in fibroblasts. IFI27 was non-specifically elevated in stem cells, while FAM3D and PRAC1 were elevated in stem cells when expression was normalized to the proximal colon transcriptome but not to healthy sigmoid colon. NFKBIA, JUNB, and IER2 were elevated in other epithelial and/or non-epithelial cell clusters, but not in LGR5 + stem cells.

DISCUSSION

RNA-seq of single cells paves the way for discovery of novel epithelial cell clusters in UC. The major differences between the two studies were in the methods and protocols used to isolate cells and the large difference in the number of analyzed cells. In addition, patient selection is also variable and patients are not comparable across studies (**Table 1**). These limitations should not deter researchers from using the findings in these studies, but rather emphasize the need to validate markers by alternative techniques. The Broad and Oxford studies make important contributions to defining the UC epithelia transcriptional landscape, but points of incongruity suggest that expanded profiles from additional subjects are necessary to refine disease-associated hypotheses. Combining scRNA-seq with other -omics into a reference disease atlas would provide a powerful tool to iteratively model, test, and revise mechanistic models in UC experimental settings for drug testing and other clinical studies.

TABLE 3 | Differential gene expression for stem cells.

Health		Ulcerative colitis	
Highly expressed transcripts reported in Oxford Study	**Highly expressed transcripts reported in Broad Study**	**Highly expressed transcripts reported in Oxford Study only**	**Highly expressed transcripts reported in Broad Study only**
None reported	LGR5	EZR	**FN1**
	RP11-219E7.4	NFKBIA	**SI**
	CYP4 × 1	C15orf48	**SPON1**
	OXGR1	JUNB	**B3GNT7**
	SMOC2	IER2	**KCTD12**
	NR0B2	IFI27	**HOXB13**
	SLC39A2	HBEGF	**MT-TV**
	SHISA9	FAM3D	**SLC28A2**
	PTPRO		**PRAC1**
	RP11-84C10.4		
	CAPN6		
	RGMB		
	FMN2		
	CELF5		
	TERT		
	RP11-760H22.2		
	ASCL2		
	C1orf95		

	lnc RNA
	Transcript is downregulated in stem cells in UC
	Transcript is elevated in other healthy cell clusters
	Transcript is elevated in healthy stem cells
Red	Transcript is elevated in other cell types in UC
Blue	Transcript identified in Genome-Wide Association Study (GWAS)

Because parameters vary widely across studies, it is not straightforward to determine whether a particular transcript is specifically changed within a single cluster and thus be a reasonable target for further evaluation in human tissue or organoid models. Our analysis acknowledges this gap and identifies some areas in which non-omics validation is important.

scRNA-seq in the Broad and Oxford studies revealed that transcriptional signatures and cell type boundaries become more nebulous in UC. Multiple transcripts were differentially regulated across many clusters, challenging the classical concept of unique cell cluster markers associated with active UC. The lack of clearly separated transcripts reflects a general loss of cell specialization, replaced by cell phenotypes that do not map to healthy tissue. Evaluating markedly altered transcripts in relation to known interactors (interactome analysis) adds depth to cluster analysis, but these strategies are not useful if cell type clusters lack discrete boundaries in disease.

Study interpretations are limited by the heterogeneity of subjects (including gender, age, ethnicity, site of colon biopsy, stage of UC disease, and prior use of anti-inflammatory therapies). The total number of cells acquired and transcripts sequenced also contribute to variability in sensitivity for detecting rare cell types or transcripts. Validation of findings at the protein level is also imperative to advance understanding of the molecular features conserved in UC epithelia.

AUTHOR CONTRIBUTIONS

JS, JF-A, and OK researched data, contributed to discussion of content and writing, generated figures and tables, and reviewed/edited the manuscript before submission. WH and JH contributed to discussion of content for the article and reviewed/edited the manuscript before submission. JI researched data for the article and reviewed/edited the manuscript before submission. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by NIH grants U01 DK103168, P01 AI125181 to OK, JI, and JF-A, K01 DK113043 to JF-A, and K01 DK106323 to JI, and by University of New Mexico Department of Internal Medicine start-up funds to JI and OK.

ACKNOWLEDGMENTS

We thank Curt H. Hagedorn, Denis M. McCarthy, Henry C. Lin and Mark Donowitz for the critical reading of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.868508/full#supplementary-material

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CASE REPORT
published: 28 April 2022
doi: 10.3389/fmed.2022.895202

Crohn's Disease Complicated by Rare Types of Intestinal Obstruction: Two Case Reports

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Intestinal obstruction is one of the most common complications of Crohn's disease (CD), jeopardizing the quality of life of patients. Numerous factors may contribute to intestinal obstruction in CD. Thus far, the primary reason has been identified as intestinal fibrosis caused by repeated chronic inflammation during the active phase of CD. Herein, we report two rare complicated CD cases and provide a reference for the clinical diagnosis and treatment of similar patients. Case one involves capsule endoscope retention in the small intestine of one CD patient concurrent with intestinal obstruction. Case two is a CD patient with intestinal obstruction caused by a mesangial hernia and ileal stenosis. Individualized and minimally invasive surgical intervention ultimately resulted in the successful management of these two patients. The two cases serve as an excellent guide for diagnosing and treating CD patients who present with similar symptoms.

Keywords: Crohn's disease, intestinal obstruction, capsule endoscopy, internal hernia, minimally invasive surgery

OPEN ACCESS

Edited by:
Antonietta G. Gravina, University of Campania Luigi Vanvitelli, Italy

Reviewed by:
Raffaele Pellegrino, University of Campania Luigi Vanvitelli, Italy
Serkan Karaisli, İzmir Katip Celebi University, Turkey

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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *13 March 2022*
Accepted: *31 March 2022*
Published: *28 April 2022*

Citation:
Xia K, Gao R, Wu X, Ruan Y, Wan J, Wu T, Wang F, Lin Y, Yin L and Chen C (2022) Crohn's Disease Complicated by Rare Types of Intestinal Obstruction: Two Case Reports. Front. Med. 9:895202. doi: 10.3389/fmed.2022.895202

INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory bowel disease that is complicated by genetic susceptibility and environmental triggers (1, 2). It is most frequently diagnosed in individuals between the ages of 20 and 30 (3). At present, the underlying cause of CD is still unknown, and its prevalence is gradually increasing worldwide (4). CD is prone to various complications, with intestinal obstruction being one of the most common. Notably, Lin et al. (5) showed that around 70% of patients inevitably develop fibrosis-associated intestinal stricture 10 years following CD diagnosis, which may contribute to intestinal obstruction. In this study, we described two cases of CD complicated by particular types of intestinal obstruction. The Ethics Committee of Shanghai Tenth People's Hospital approved this case report (Ethical approval number: SHSY-IEC-5.0/22K33), and informed consent was obtained from all patients.

CASE REPORT

Case 1

On January 25, 2021, a 26-year-old man was diagnosed with incomplete intestinal obstruction in another hospital after experiencing vomiting, abdominal pain, and abdominal distension

following a meal. The symptoms improved after fasting, gastrointestinal decompression, anti-infective treatment, and parenteral nutrition support but remained intractable. On March 11 of the same year, the patient experienced severe abdominal pain while undergoing capsule endoscopy in the same hospital, and the capsule endoscope was not excreted after the examination. Enhanced computed tomography (CT) scans of the abdomen revealed small intestinal obstruction, with multiple thickening and strengthening of the small intestinal wall. The obstruction point was located in the small intestine of the pelvic region. According to the images, the first consideration was a CD of the small intestine. As a result, the patient was admitted to the Diagnostic and Treatment Center for Refractory Abdominal Diseases for additional treatment. Physical examination revealed tenderness in the lower abdomen and no other positive signs. The plain abdominal film revealed that the jejunum in the upper left abdomen was dilated, and the intestinal cavity contained a gas-liquid plane. Moreover, metallic shadows could be seen in the overlapping area of the pelvic cavity (**Figure 1A**). The patient was diagnosed as "incomplete small intestinal obstruction, CD (?), small intestinal foreign body (capsule endoscope)." The patient then underwent surgery to remove the thickened intestine and foreign body on March 19. During the operation, laparoscopic exploration revealed that the small intestine presented a segmental lesion intertwined with stenosis and dilation. After exploring the entire small intestine from the Treitz ligament, we discovered that the narrowest point of the intestine was approximately 180 cm from the Treitz ligament, and the stenosis segment of the intestine was approximately 30 cm in length. The distal small intestine was almost normal, while the terminal ileum was narrowed and thickened. Endo-GI was used to resect the intestinal cavity at the beginning of ascending colon, dragging out the narrowed ileum, capsule endoscope incarcerated in the proximal ileum was touched (**Figure 1B**). Then segmental resection of the small intestine with side-to-side stapled anastomosis was performed. Finally, the normal terminal ileum was severed, and the terminal ileostomy was completed. An ileus tube was inserted from the nose to the duodenum. The entire operation lasted 2 h, and the blood loss during the process was about 100 ml. The surgical specimen was inspected, and the multiple polyps of the intestinal wall and capsule endoscope were removed (**Figures 1C,D**). The patient had an uneventful postoperative period and was discharged 8 days after surgery. Pathology analysis revealed that multiple polyps were seen on the mucosal surface of the small intestine with a paving stone-like appearance, the diameters of which were about 0.2–0.6 cm. A longitudinal ulcer was seen on one side of the surgical margin, with a length and width of approximately 4 and 0.9 cm, respectively. Microscopy revealed acute and chronic intestinal wall inflammation with plasma cells, neutrophils, and lymphocytes infiltration. The ulcerative area reached the muscle layer and was alternated with inflammatory polyps. Combined with clinical history, the diagnosis of CD was considered. The patient underwent ileostomy closure surgery after 3 months. The patient is currently in good condition, the disease is well controlled, and no apparent complications have been reported.

Case 2

In 2009, a 36-year-old man with lower abdominal pain and palpable right lower abdominal mass was diagnosed with CD. Since then, he has been followed up regularly in our Gastroenterology Department and treated with medication to slow the progression of the disease. Four days prior to admission, the patient experienced severe abdominal pain and distension following a full meal, which was accompanied by four to five episodes of vomiting. As a result, the patient was admitted to our department for follow-up care. Further examination of the patient's medical history revealed that the patient had not had any excretion or exhaust in the preceding 3 days and had previously defecated two to three times per day. Physical examination revealed tenderness in the middle and lower abdomen, with a palpable mass in the lower abdomen. CT scans of the abdomen showed intestinal tract lesions in the right middle and lower abdomen, causing a proximal small intestinal obstruction (**Figures 2A–C**). Endoscopic examination indicated noticeable mucosal swelling, irregular fissure ulcers, and multiple polypoid hyperplasias in the sigmoid colon (18 cm away from the anus). The intestinal cavity was so narrowed that the endoscope could not pass through it (**Figures 2D–F**). As a result, the patient was diagnosed as " CD (A2 L2 B2 P, active phase), small intestinal obstruction, sigmoid stenosis, severe malnutrition (BMI 16.41 kg/m^2)." After admission, a transnasal ileus tube was inserted under interventional guidance. 1,200, 200, 100 and 10 ml of dark green liquid per day were drained from the tube 4 days before the operation. The patient felt no more abdominal pain after that intervention. Following strict evaluation, the patient underwent subtotal colectomy, ileocecal resection, laparoscopic intestinal adhesiolysis, plication of small intestine, and ileostomy on November 30, 2021.

During the operation, laparoscopic exploration revealed that the proximal small intestine was significantly dilated. Furthermore, a mass wrapped by the omentum majus was seen in the ileocecal junction adhering to the lateral abdominal wall. When an ultrasonic scalpel separated the ileocecal mass, pus was seen flowing from the mass. We then switched to laparotomy because of the unclear anatomical structure and limited vision during the laparoscopic operation. During exploratory laparotomy, contractures were found in the ascending, transverse, descending colon, and mesangium. The proximal small intestine passed through the transverse mesocolon, causing the formation of an internal hernia (**Figure 2G**). Then, a linear cutter stapler severed the descending colon sigmoid junction, and the ileus tube was delivered from the proximal ileum to the terminal ileum. After the ileocecal mass was dragged out, the specimen was severed with a linear cutter stapler at a distance of 10 cm from the ileocecal junction. The terminal ileostomy was then completed. The entire operation lasted 4 h, blood loss during the process was about 600 ml, and the length of the residual small intestine was about 200 cm. The surgical specimen was inspected, as shown in **Figures 2H,I**. The patient had an uneventful postoperative period and was

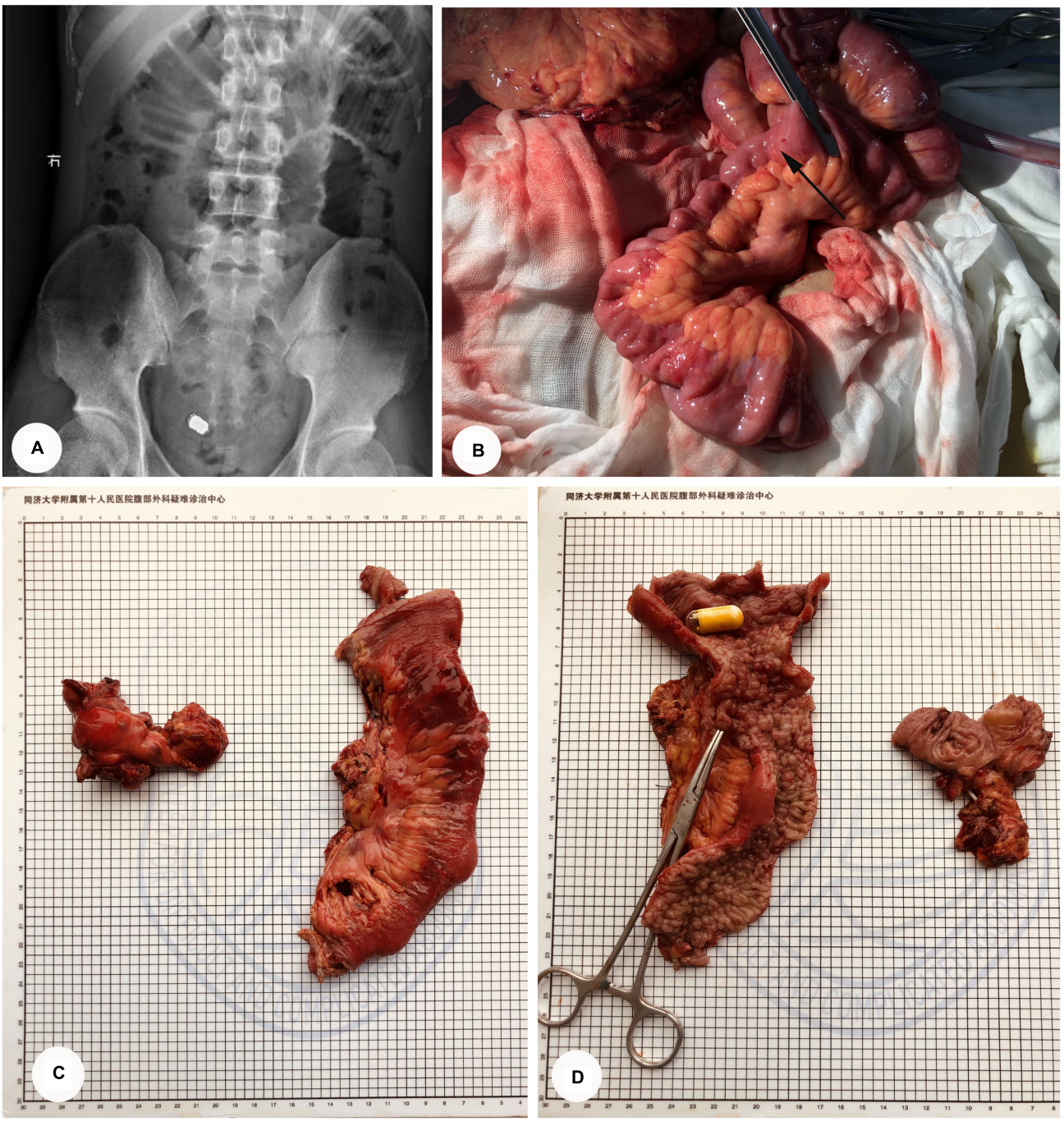

FIGURE 1 | CD complicated by small intestinal obstruction with capsule endoscopic incarceration (Case 1). **(A)** Abdominal plain film: the jejunum in the upper left abdomen was dilated, and the intestinal cavity contained a gas-liquid plane. Metallic shadows could be seen in the overlapping area of the pelvic cavity. **(B)** Intraoperative exploration: the capsule endoscope is incarcerated in the narrowed proximal ileum (Arrow point). **(C,D)** The specimen of surgery: the yellow cylinder is capsule endoscope; vascular clamp showing internal fistula.

discharged 10 days after surgery. Pathology analysis revealed a protrusion lesion in the ileocecal area, measuring 10 × 4 × 4 cm. Under the microscope, chronic active inflammation of colonic mucosa could be seen, with erosion, fissure ulcer and polypoid changes. Lymphoplasmacytic cells were distributed in the colonic mucosa focally. Combining his past clinical history, the diagnosis of CD complicated by intestinal obstruction was given. Since the postoperative follow-up, the patient is in good condition, the disease is under control, and no apparent complications have been reported.

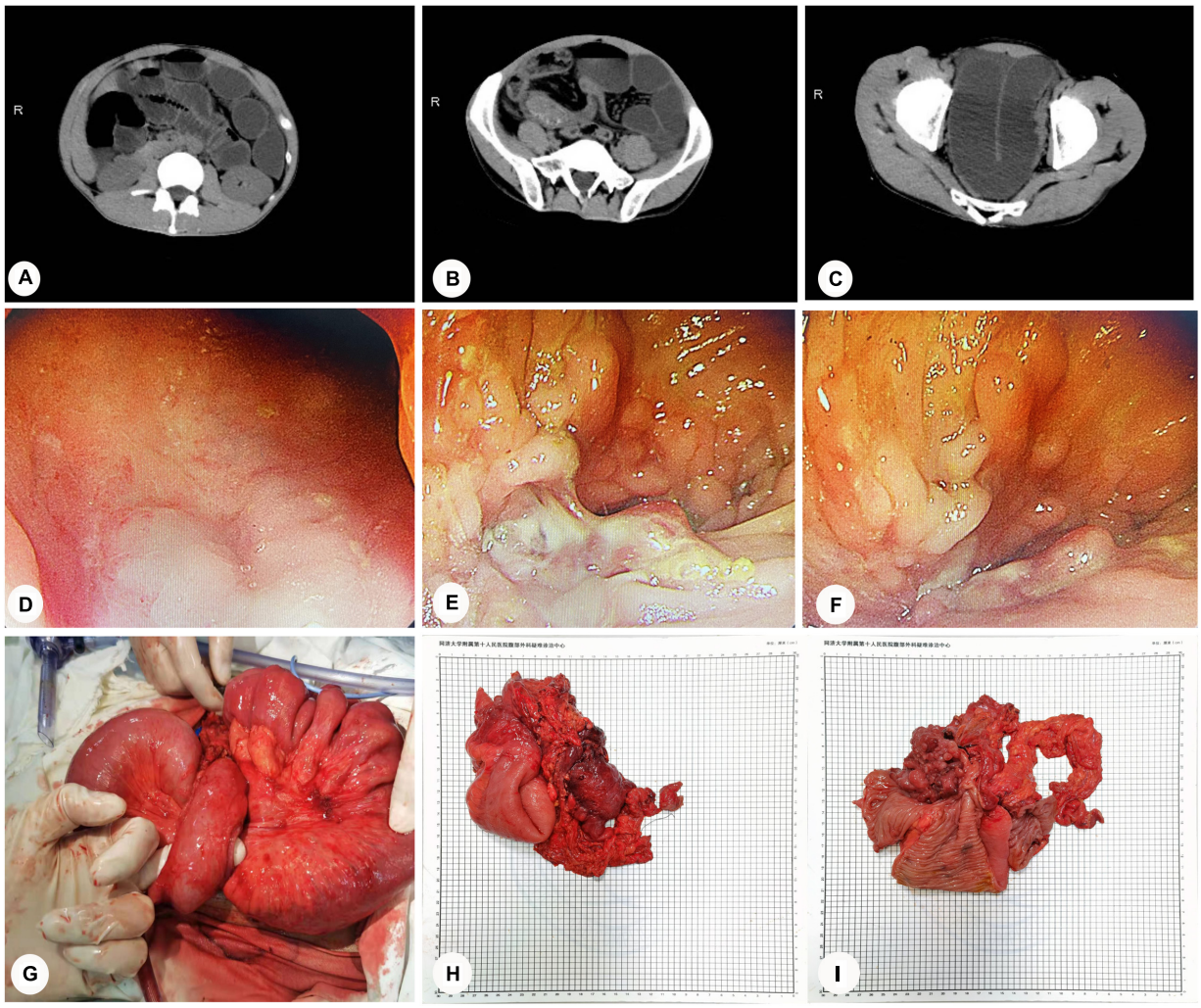

FIGURE 2 | Intestinal obstruction caused by CD combined with internal hernia and ileal stenosis (Case 2). **(A–C)** CT scans of abdomen: intestinal tract lesions in the right middle and lower abdomen, causing a proximal small intestinal obstruction. **(D–F)** Endoscopic examination: noticeable mucosal swelling, irregular fissure ulcers, and multiple polypoid hyperplasias in the sigmoid colon (18 cm away from the anus). The intestinal cavity was so narrowed that the endoscope could not pass through it. **(G)** Intraoperative exploration: the proximal small intestine passed through the transverse mesocolon, causing the formation of the internal hernia. **(H–I)** The specimen of surgery.

DISCUSSION

In CD patients, intestinal obstruction resulting from capsule endoscopy is uncommon. However, according to Guevara-Morales and Castellanos-Juárez (6), the capsule endoscope retention rate in CD patients could reach around 5–6%. Therefore, clinicians should pay closer attention, as this can significantly impact these patients. Capsule endoscopy is a minimally invasive procedure that enables visualization of the entire small intestine mucosa in patients with known or suspected CD (7). The widespread use of capsule endoscopy has significantly compensated for the shortcomings of colonoscopy and enteroscopy. However, intestinal obstruction is recognized as one of the most common complications of CD, which increases the possibility of retention of the capsule endoscope in the gut (8). Therefore, it is necessary to evaluate intestinal cavity patency before swallowing capsule endoscopes. Rozendorn et al. (9) revealed that the ability of magnetic resonance enterography (MRE) to predict capsule endoscope retention in patients with CD is relatively significant. The sensitivity and specificity for the predictions by two radiologists were 92.3, 59% and 100, 52.3%, respectively. Minordi et al. (10) further uncovered that MRE provides a superior soft-tissue contrast resolution and a better visualization of the intestinal wall and its inflammatory and fibrotic characteristics. In addition, computed tomography enterography (CTE) is another well-established diagnostic technique for evaluating the intestinal cavity's patency. Therefore, capsule endoscopy should be used with caution in patients with CD complicated by bowel stenosis.

In case 2, the primary cause of intestinal obstruction is the formation of trans-mesenteric internal hernia. According to the literature, the incidence of internal hernia is about 0.2∼0.9%, accounting for 0.6∼5.8% of all intestinal obstruction cases (11). Internal hernia frequently exhibits no characteristic clinical manifestations prior to the formation of intestinal obstruction, resulting in a low rate of diagnosis preoperatively. Moreover, closed intestinal loops can form quite easily, resulting in intestinal strangulation and necrosis. Patel et al. (12) concluded that when an internal hernia is complicated with acute intestinal obstruction, the mortality rate can reach up to 50%. Therefore, early detection and surgical intervention are critical for optimizing disease outcomes.

Medical treatment is frequently ineffective in CD patients who developed intestinal obstruction, and in both cases, the obstruction was eventually resolved through surgical intervention. Endoscopic intervention or surgical resection is primarily used to treat intestinal stenosis in CD. Endoscopic balloon dilation (EBD) is indicated when the length of strictures is $\leq$ 5 cm, non-angulated, with a sizeable intestinal cavity large enough to allow balloon dilators in the absence of contraindications such as the presence of fistula, abscess, or malignancy (13, 14). In addition, endoscopic stricturotomy (ES) is also an excellent choice when strictures are non-angulated, and the length of the narrowed intestinal cavity is short. Nan et al. (15) demonstrated that the reoperation rate following ES is significantly lower than that following EBD and that ES can result in a higher surgery-free survival rate. However, surgical resection is required when the length of the narrowed intestinal cavity is excessively long or when endoscopic therapy is contraindicated (16). Our department has treated a large number of patients with CD and has accumulated much surgical experience. The narrowed bowel was severed in both cases, and the small intestine cavity was also dilated using an ileus tube. The small intestine must be plicated when the segments of narrowed bowel are long, and the length of the small intestine is insufficient for intestine resection. Not only can plication of the small intestine be used to dilate the intestinal cavity, but it can also be used to prevent the development of adhesive ileus following surgery.

Notably, our department emphasizes minimally invasive surgery, putting the concept of enhanced recovery after surgery (ERAS) into practice. By minimizing pain, hospital stay, and adhesion formation, minimally invasive surgical options have significantly reduced postoperative morbidity (17). Our team recently published a retrospective study confirming the safety and efficacy of laparoscopic minimally invasive surgery as a viable alternative therapeutic option for patients with CD (18).

In conclusion, there are numerous CD cases complicated by intestinal obstruction, and surgery is the mainstay modality to relieve the obstruction. As surgeons, we should thus choose individualized and minimally invasive options that will result in long-term benefits for CD patients.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Shanghai Tenth People's Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data.

AUTHOR CONTRIBUTIONS

KX: disease diagnosis and treatment, collection of cases, and draft manuscript. RG, XW, and YR: disease diagnosis and treatment, collection of cases, and revise manuscript. JW, TW, FW, YL, LY, and CC: disease diagnosis and treatment, and revise manuscript. All authors contributed to the article and approved the submitted version.

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frontiers | Frontiers in Medicine

REVIEW
published: 23 May 2022
doi: 10.3389/fmed.2022.898092

The Impact of Intestinal Ultrasound on the Management of Inflammatory Bowel Disease: From Established Facts Toward New Horizons

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *17 March 2022*
Accepted: *25 April 2022*
Published: *23 May 2022*

Citation:
Nardone OM, Calabrese G, Testa A, Caiazzo A, Fierro G, Rispo A and Castiglione F (2022) The Impact of Intestinal Ultrasound on the Management of Inflammatory Bowel Disease: From Established Facts Toward New Horizons. Front. Med. 9:898092. doi: 10.3389/fmed.2022.898092

Intestinal ultrasound (IUS) plays a crucial role as a non-invasive and accurate tool to diagnose and assess inflammatory bowel disease (IBD). The rationale for using IUS in Crohn's disease (CD), a transmural disease, is widely acknowledged. While the use of IUS in ulcerative colitis (UC), a mucosal disease, is often underestimated, but, recently, it is increasingly expanding. In the context of a treat-to-target approach, the role of IUS is shifting toward a monitoring tool for predicting response to therapy. Hence, adjusting therapeutic strategies based on IUS response could reduce the burden related to endoscopy and speed the decision process with the ultimate goal to alter the natural course of IBD. Assessment of bowel wall thickness (BWT) is the most reliable IUS measure. However, the development of validated and reproducible sonographic scores to measure disease activity and the identification of parameters of therapeutic response remain relevant issues to implement the daily adoption of IUS in clinical practice. Accordingly, this review focuses on the current literature investigating the impact of IUS on CD with emphasis on the concept of transmural healing (TH) and the main related advantages. We further explore new insights on the role of IUS in UC and its clinical implications.

Keywords: transmural healing, transmural remission, Crohn's disease, ulcerative colitis, noninvasive monitoring, intestinal ultrasound

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) belong to inflammatory bowel diseases (IBD). These are chronic, progressive, and disabling diseases whose incidence has dramatically increased during the last 20 years, impacting on the quality of life, social functioning and psychological health (1–3).

Crohn's disease potentially affects any part of the gastrointestinal tract with three different phenotypes (non-stricturing/non-penetrating, stricturing, and penetrating) (4), and it is characterized by a progressive and transmural involvement of the mucosa that may require intestinal resection for patients with complications or those with intractable disease (5).

Ulcerative colitis is usually limited to the mucosal layer and involves the colon starting from the rectum in a continuous manner (6). It has been shown that extensive, severe disease at diagnosis

and early need for corticosteroids are the main factors significantly associated with a higher risk of colectomy ranging from 1 to 5% at 1 year and from 3 to 8% at 5 years after diagnosis (7).

In recent decades, the clinical management of IBD has significantly evolved, moving from a clinical-based strategy toward a concept of "*deep remission*" with the final intent to reduce structural damage, prevent disease progression and improve long-term outcomes for patients.

The Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) represented a turning point in the medical strategy of IBD. In 2015, the International Organization for the Study of Inflammatory Bowel Diseases (IOIBD) first introduced the concept of *treat-to-target strategy and tight monitoring* (8). Since then, the expanding use of immune modulators and the advent of monoclonal antibodies driven against tumour necrosis factor-alpha (TNF-alpha), interleukin-12/23 (IL-12/23), and integrins on the leukocytes surface have made possible to go beyond these conventional treatment goals of the STRIDE toward more ambitious targets. In addition, new advanced non-invasive tools are becoming widely available, providing new opportunities for tight monitoring.

In this scenario, the STRIDE II updated the STRIDE by introducing new additional targets, such as serum and fecal biomarkers, normalization of quality of life, and prevention of disability. Furthermore, transmural healing (TH) in CD and histologic remission in UC have been recognized as adjunct measures with the assumption that deep remission implicates better outcomes (9).

The utility of intestinal ultrasound (IUS) in the assessment of CD is well established (10, 11), while the literature surrounding its use in UC remains questioned. In the context of a "treat-to-target" strategy and close monitoring, IUS plays a crucial role due to its non-invasiveness. In addition, it has been further recognized as a reliable tool for detecting therapeutic responses as it offers notable advantages with respect to endoscopy in terms of cost, safety profile, and lack of bowel preparation (12, 13).

This narrative review aimed to discuss the recent advances of IUS in the management of IBD, starting from the current evidence in CD and focusing on the concept of TH and its clinical implication, to the expanding role in UC.

METHODS

Search Strategy and Selection Criteria

A literature search using the PubMed database from January 2000 to March 2022 was made by two independent reviewers (OMN and GC). The search used the following terms: "transmural healing" or "transmural remission" or "radiological remission" and "Crohn's disease" or "ulcerative colitis" or "inflammatory bowel disease" and "fecal calprotectin" or "non-invasive markers" and "bowel sonography" or "intestinal ultrasound" and "post-operative recurrence."

We screened the title and abstract of 12,798 articles, followed by a full-text analysis of relevant articles. Of these, a total of 41 articles were considered suitable. We selected randomized controlled studies, prospective or retrospective cohort studies, and systematic reviews with meta-analysis and excluded duplications, abstracts and studies in languages other than English. For each study, we collected authors, journal, country, number of enrolled patients, the definition of TH, clinical outcomes, time of follow-up, TH response rate, TH assessment method, IUS accuracy for post-operative recurrence diagnosis, IUS accuracy, and correlation with non-invasive markers.

RESULTS

Cross-Sectional Imaging for Detecting Transmural Healing

Intestinal ultrasound has become an integral part of IBD clinical management in recent years since it accurately determines disease extent, severity, and response to medical therapy (11).

Bowel wall thickness (BWT) of the mucosal and submucosal layers is the most relevant IUS parameter to detect intestinal inflammation. However, additional IUS parameters of transmural activity include increased echogenicity of the submucosal layer, an amplified color Doppler signal indicative of increased vascularity, and proliferation of mesenteric fat (11). Several meta-analyses have investigated the diagnostic accuracy of cross-sectional imaging methods, i.e., magnetic resonance enterography (MRE), CT enterography (CTE), and IUS and they found high accuracy as well as a high agreement between all these tools without any significant differences (14–17). Nevertheless, it is noteworthy that IUS and MRE are radiation-free methods and, thus, a clear advantage is that patients might have less radiation-related risk. However, choosing one examination rather than another one could depend on the operators' availability, expertise, costs and times required to do these procedures.

In a pioneering study in 2013, our group (18) evaluated the diagnostic accuracy of IUS and MRE to diagnose small bowel CD. We showed that MRE outperformed IUS in defining CD extension ($r = 0.71$). The agreement between both the techniques was high in terms of disease location ($k = 0.81$), while MRE had a fair concordance with IUS for strictures ($k = 0.71$) and abscesses ($k = 0.88$), and it showed better detection of entero-enteric fistulas ($k = 0.67$).

Subsequently, in 2017, the same group confirmed a high agreement between IUS and MRE for assessing TH ($k = 0.90$; $p < 0.001$) (19).

Furthermore, Allocca et al. (17), in a single centre study, including 60 patients, analyzed the accuracy of IUS vs. MRE combined with colonoscopy for assessing localization, increased vascular signal at power Doppler, disease activity (ulcers at colonoscopy) and complications (strictures, fistulas and abscesses). Notably, IUS could be a useful tool for detecting ulcers in patients with CD, since it showed a diagnostic accuracy of 91% for localization of disease and 96% for ulcerations. Concerning the complications, they reported diagnostic accuracy of 81% for strictures, 98% for fistulas, and 96% for abscesses.

Similarly, a large randomized controlled trial conducted in eight United Kingdom hospitals (15) compared the accuracy between MRE and IUS for assessing CDs presence, extension and

activity. Notably, they reported significantly higher sensitivity and specificity of MRE for small bowel extent and higher sensitivity for disease presence in respect of IUS. Furthermore, when they evaluated the diagnostic accuracy for colonic disease, they found no significant difference (consistently lower than for small bowel disease) between MRE and IUS, although the latter had greater sensitivity than MRE in newly diagnosed patients (15).

In an attempt to facilitate reliable IUS identification of CD activity, a recent expert consensus through a Delphi method identified four IUS key parameters of inflammation: BWT, bowel wall stratification, hyperemia of the wall assessed by using color Doppler imaging, and inflammatory mesenteric fat. These four variables were included in the final International Bowel Ultrasound Segmental Activity Score (IBUS-SAS) with almost perfect reliability [intra-class correlation coefficient (ICC) 0.97 0.95–0.99, $p < 0.001$]. Significantly, all of them could predict overall disease activity, but BWT and i-fat are required to predict overall disease severity (20).

Transmural Healing—Toward a Standardized Definition

Based on IUS, TH was defined as a BWT less than or equal to 3 mm (18, 19, 21–29). However, some studies used other sonographic features for full and comprehensive characterization. The several definitions of TH are shown in **Table 1**.

For instance, Civitelli et al. (27) added the complete normalization of vascularization assessed by using Doppler US, normal bowel wall stratification, absence of mesenteric fat hypertrophy, nodes enlargement, and disease complications (i.e., strictures, fistulas). Ma et al. (26) used these same features to define TH.

Calabrese et al. (24) defined TH as the normalization of all the IUS parameters, such as BWT less than or equal to 3 mm, Limberg score normalization, normal wall stratification and absence of mesenteric fat hypertrophy, nodes enlargement, and disease complications (i.e., strictures, fistulas).

More recently, Helwig et al. (25) introduced three definitions of TH: simplified TH, extended TH, and complete TH. The first one accounts for BWT and Doppler features normalization; the second one included BWT normalization and the evaluation of at least two parameters among Doppler US normalization, normal wall stratification, and the absence of fibro-fatty proliferation. Finally, the third definition included the normalization of all four parameters.

Clinical Implications of Transmural Healing

Several studies showed that the achievement of TH in CD had been associated with significant improvements in clinical outcomes, hospitalization rates, corticosteroid-free remission, treatment intensification, and need for surgery (**Table 2**).

The relationship between clinical remission (CR) and TH has been investigated in five studies; three studies found a significant correlation between TH achievement and CR (22, 24, 25). In a prospective multi-centre longitudinal study conducted by Ripollés et al. (22), 51 patients with CD who underwent therapy with anti-tumour necrosis factor-alpha (TNF-alpha) drugs were followed at 12 and 52 weeks. A total of 29 (56.9%) patients reported an improvement or normalization of sonographic features at 52 weeks and 28 out of 29 (96.5%) patients showed clinical remission according to the Harvey–Bradshaw Index (HBI) (HBI < 5 for remission) or response (HBI decrease >3). Hence, there was a significant relationship between sonographic score and clinical response at 52 weeks. Indeed, sonographic response after 12 weeks predicted clinical response at 1 year, since patients without sonographic improvement were more likely to have a change or intensification in medication or surgery during the year of follow-up.

In a cohort study conducted by our group (19), 80 patients with CD were prospectively enrolled and followed-up for 2 years to assess the rate of TH after starting anti-TNF-alpha therapy.

Overall, 10 out of 40 patients achieved TH (25%) after 2 years. It is noteworthy that there was a good agreement between mucosal healing and TH assessed by IUS ($k = 0.63$, $p < 0.001$) and by MRE ($k = 0.64$, $p < 0.001$). All the patients who achieved TH were in CR even though the agreement between TH and CR was poor ($k = 0.27$; $P < 0.01$).

Subsequently, the same Italian group prospectively analyzed the clinical implications of achieving TH and compared them with those in patients with only mucosal healing or no healing. Overall, 218 patients completed a 2-year treatment with anti-TNF-alpha. A total of 68 (31.2%) patients reached TH measured with BWT ≤ 3 mm at IUS and mucosal healing, while 60 (27.5%) patients had mucosal healing. Importantly, patients who reached TH had a higher rate of steroid-free clinical remission (95.6%), lower rates of hospitalization (8.8%), and need for surgery (0%) at 1 year compared to those who had mucosal healing (75, 28.3, and 10%, respectively) and no healing (41, 66.6, and 35.5%, respectively) ($P < 0.001$). Furthermore, TH was linked to longer intervals until clinical relapse [hazard ratio (HR) 0.87, $P = 0.01$], hospitalization (HR 0.88, $P = 0.002$), and surgery (HR 0.94, $P = 0.008$) than mucosal healing (21).

Similar evidence comes from a study by Zorzi F et al. wherein patients who achieved transmural remission after anti-TNF-alpha treatment did not require surgery, needed fewer steroids, and showed reduced hospitalization rates at 18 months (28).

Furthermore, in a prospective Spanish study, including 36 patients, TH assessed with IUS in the week before starting anti-TNF-alpha treatment, at 12 weeks and 1 year later, was significantly related to better clinical outcomes, i.e., no need to re-introduce corticosteroids or intensify maintenance therapy and/or need for surgery (23).

Notably in a prospective observational study, Orlando et al. (29) assessed the role of ultrasound elasticity imaging in predicting therapeutic outcomes in 30 patients with CD who underwent IUS and ultrasound elasticity imaging at baseline, 12 weeks, and 52 weeks. A BWT < 3 mm was used to define TH. Bowel wall stiffness was evaluated through the strain ratio between the mesenteric tissue and the bowel wall. Severe ileal fibrosis was defined by a strain ratio ≥ 2. Eight out of 30 patients achieved TH at 14 weeks during the follow-up and one

TABLE 1 | Definitions of transmural healing (TH).

References and number of patients	Definition of transmural healing based on IUS
Castiglione et al. (18), (*n* = 133)	BWT ≤ 3 mm
Ripollés et al. (22), (*n* = 51)	BWT ≤ 3 mm
Civitelli et al. (27), (*n* = 32)	BWT ≤ 3 mm and other IUS features
Castiglione et al. (19), (*n* = 40)	BWT ≤ 3 mm plus absence of hypervascularisation signs
Orlando et al. (29), (*n* = 30)	BWT ≤ 3 mm
Paredes et al. (23), (*n* = 36)	BWT ≤ 3 and 0–1 doppler scale
Castiglione et al. (21), (*n* = 218)	BWT ≤ 3 mm
Zorzi et al. (28), (*n* = 80)	BWT ≤ 3 mm
Ma et al. (26), (*n* = 77)	BWT ≤ 3 mm and normalization of stratification, no hypervascularisation, resolution of mesenteric inflammatory fat, and no complications (IUS)
Calabrese et al. (24), (*n* = 188)	BWT ≤ 3 mm ileum/4 mm for colon, normal Limberg score and no complications
Helwig et al. (25), (*n* = 137)	BWT and Color-Doppler normalization, no loss of stratification and no fibro fatty proliferation

BWT, bowel wall thickness; IUS, intestinal ultrasound.

TABLE 2 | Relationship between transmural healing and clinical outcomes in patients with Crohn's disease (CD).

References	Country	Clinical outcomes (*p*-value for correlation with TH)	Time of follow-up	TH response rate	TH assessment method
Castiglione et al. (18), (*n* = 133)	Italy	CR: CDAI < 150 (poor agreement)	2 yo	25%	IUS
Ripollés et al. (22), (*n* = 51)	Spain	CR: HBI < 5 ($p = 0.001$)	12 w–1 yo	29.5%	IUS
Castiglione et al. (19), (*n* = 40)	Italy	CR: CDAI < 150 (poor agreement)	2 yo	25%	IUS/MRE
Orlando et al. (29), (*n* = 30)	Italy	Surgery ($p = 0.003$)	14 w – 1 yo	30%	IUS
Paredes et al. (23), (*n* = 36)	Spain	Steroid use; hospital admission; surgery (global $p = 0.001$)	12 w	42.4%	IUS
Castiglione et al. (21), (*n* = 218)	Italy	Corticoid free remission ($p < 0.01$); longer time to clinical relapse ($p = 0.01$); surgery ($p < 0.001$); hospital admission ($p < 0.001$); need for drug escalation ($p < 0.001$); switch/swap therapy ($p < 0.01$); Clinical outcomes in patients on biologics withdrawal ($p < 0.001$)	12 w	31.2%	IUS
Zorzi et al. (28), (*n* = 80)	Italy	Need of hospitalization ($p = 0.005$); need for steroids $p = 0.007$; surgery ($p = 0.004$)	12–24 mo	32%	SICUS
Ma et al. (26), (*n* = 77)	China	Corticoid free remission ($p < 0.001$); drug escalation ($p = 0.003$); hospital admission ($p = 0.005$); surgery ($p = 0.03$)	12–42 mo	32%	IUS
Calabrese et al. (24), (*n* = 188)	Italy	HBI < 5 ($p = 0.002$)	12 mo	31.2%	IUS
Helwig et al. (25), (*n* = 137)	Germany	HBI < 5 ($p = 0.001$)	12 w	43%	IUS

TH, transmural healing; CR, clinical remission; CDAI, Crohn's Disease Activity Index; HBI, Harvey-Bradshaw Index; yo, years; m, months; w, weeks; IUS, intestinal ultrasound; SICUS, small intestine contrast ultrasonography; MRE, magnetic resonance enterography.

patient achieved TH at 52 weeks. Regarding clinical outcome, the frequency of surgery was significantly higher in patients with a strain ratio ≥2 groups than in <2 groups ($p = 0.003$).

A prospective, longitudinal cohort study (26) conducted in a single tertiary hospital in China investigated the impact of TH assessed with IUS on long-term positive outcomes compared to mucosal healing. Both the mucosal healing and TH were associated with better long-term outcomes based on the univariate analysis, while in the multi-variate analysis, TH was an independent predictor of steroid-free CR [odds ratio (OR), 52.6; $p < 0.001$], drug escalation (OR, 0.1; $p = 0.002$), and hospitalization (OR, 0.05; $p = 0.005$).

More recently, Helwig et al. (25), in a *post hoc* analysis, including 351 patients with IBD belonging to multi-centre studies such as the TRUST and the TRUST&UC, confirmed the positive association between TH and clinical outcomes. Indeed, patients with CD who achieved TH were more likely to reach clinical remission at week 12 [odds ratio (OR), 3.33 (1.09–10.2); $p = 0.044$].

With the aim of measuring therapeutic response, Allocca et al. (30) have proposed an ultrasound score [bowel ultrasound score (BUS)] to best predict the endoscopic activity of CD. It included the following parameters: BWT; bowel wall pattern; bowel wall flow, vascular signals at color Doppler; stricture,

fistula, abscess, enlarged mesenteric lymph nodes (short axis >5 mm) and mesenteric hypertrophy. These features have been evaluated for each intestinal segment affected by the disease. The worst segment was selected and used for the BUS calculation. A score of <3.52 was considered predictive of disease remission.

Clinical Application of Intestinal Ultrasound for Detecting Post-operative Crohn's Disease Recurrence

The application of IUS in post-surgical CD recurrence has become an essential part of disease monitoring, despite colonoscopy being still considered the gold standard for detecting post-operative recurrence (POR) (31, 32). Early therapeutic intervention is paramount to prevent disease recurrence, as recently suggested (33). However, patients not easily accept and are often reluctant to undergo colonoscopy only a few months after surgery. In this context, IUS could be a surrogate tool to guide early therapeutic strategies and, thereby, the right timing to perform colonoscopy. Several studies have investigated the correlation of IUS and colonoscopy during the first year after CD surgery. A prospective study conducted by our group (34) showed that IUS and small intestine contrast ultrasound (SICUS) had a sensitivity of 77 and 82%, respectively, and they both had a specificity of 94% for POR detection. Subsequently, in a study cohort of 72 patients with CD, Calabrese et al. (35) found a good correlation between BWT (BWT > 3 mm considered predictive of disease recurrence) assessed by SICUS and the Rutgeerts score (RS) (RS ≥ i2 indicating endoscopic recurrence) at colonoscopy ($P < 0.0001$; $r = 0.67$). The same group in 2013 (36) confirmed a good correlation between SICUS and CTE in detecting BWT ($k = 0.79$) and disease extent ($k = 0.89$; $p < 0.0001$), supporting the use of SICUS in routine clinical practice.

In a 5-year experience conducted on 40 post-operative patients with CD, Onali et al. (37, 38) reported the usefulness of IUS for 1-year POR assessment; even though, SICUS was not predictive of clinical recurrence at 4 and 5 years. Subsequently, a meta-analysis (39), including 536 patients, observed a good pooled sensitivity and specificity of IUS for POR detection (sensitivity = 0.94; specificity = 0.84). Of note, a BWT ≥ 5.5 mm was predictive of severe endoscopic POR (Rutgeerts score > i3), so it could be reasonable to manage therapeutic escalation and decisions based on these IUS findings. However, it is noteworthy that colonoscopy cannot be avoided even in patients with a low risk of POR with BWT < 5.5 mm.

More recently, a retrospective study (40), including 201 post-operative patients with CD followed for a median of 7.6 years, showed that IUS recurrence (defined as either anastomotic BWT > 4 mm or new abdominal complication) predicted surgical recurrence, i.e., new major abdominal surgery or symptoms not controlled by medical treatments, while endoscopic recurrence was not predictive of late clinical recurrence (after 36 months) and, thus lacks prognostic value.

Carmona et al. (41) conducted a retrospective observational study on 31 patients with CD who previously underwent ileocecal resection. They found a sensitivity and specificity of 100% and 86.6% for BWT of 3.4 mm with the area under the receiver operating characteristic (AUROC) curve of 92.9%. Based on these findings, IUS represents a feasible and reliable alternative to colonoscopy, especially in early stage of surgery.

Further non-invasive methods such as faecal calprotectin (FC) have been shown to be accurate for the assessment of POR. In a prospective cohort study, Lopes et al. (42) reported a good correlation between increased FC and POR based on modified Rutgeerts score (MRS – MRS > i2b defining POR). Of note, the AUROC for FC cut-off value of 100 μg/g was 0.831 ($p < 0.05$).

In a systematic review by Tham et al. (43), FC 150 μg/g was considered the best cut-off value for predicting POR (defined as a RS > i2) with a pooled sensitivity and specificity of 70% and 69%.

However, large prospective studies are needed to determine how IUS can be collocated in the algorithm of POR and explore the magnitude of benefit of combined IUS and FC for detecting POR and establishing an optimal FC threshold.

Clinical Application of Intestinal Ultrasound Combined to Non-invasive Markers

In recent years, we have seen the advent of a new era of non-invasive monitoring of IBD.

The use of biomarkers, such as C-reactive protein (CRP) and FC, is more and more expanding in clinical practice. CRP is the most widely used inflammatory marker and it has been shown a strong correlation with CD activity, while less correlation with UC.

A possible explanation could be that in UC, the inflammation is limited to the mucosa unless very severe, whereas, in CD, it is extended to all the bowel wall layers (44). However, CRP normalization does not predict a complete clinical and endoscopic remission (45–47).

The correlation between CRP levels and TH was investigated in 2013 by our group (18) in a longitudinal observational study, including patients with CD. There was a good concordance between TH and CRP levels ($k = 0.79$; $p = 0.02$). Subsequently, these results were confirmed in 2017 (19) ($k = 0.77$; $p = 0.02$).

Despite practical concerns about variability, lack of standardized cut-off for disease activity and patients' reluctance to collect stool samples serially, FC is now routinely used in clinical practice for monitoring disease activity and response to medication (45, 48).

The trial "Effect of Tight Control Management on Crohn's Disease (CALM)" has paved the way for tight monitoring, including FC measurement (49). When compared with patients in the standard of care group, the treat-to-target group achieved better outcomes, such as mucosal healing, deep remission, and less need for steroids.

Weinstein-Nakar first explored the diagnostic accuracy of FC for predicting deep remission assessed by MRE in a pediatric population. A cohort of 243 patients showed that FC had the AUROC 0.93–0.94 for predicting deep healing, defined as mucosal healing [Simple Endoscopic Score-CD (SES-CD) 0–2] plus TH on MRE. Deep healing resulted in lower FC levels [median 10 μg/g, interquartile range (IQR) 10–190] than endoscopic healing or TH alone, with optimal cut-offs of

300 μg/g and 100 μg/g for mucosal healing and deep healing, respectively (50).

In a retrospective study, 268 Korean patients diagnosed with CD and treated with anti-TNF-alpha agents underwent colonoscopy, radiological assessment and FC measurement in 3 months. They found that an FC cut-point level of 81.1 mg/kg predicted deep healing, defined as the combination of endoscopic and radiological healing, with a sensitivity of 0.623 and a specificity of 0.817 (AUROC, 0.767; 95% CI, 0.702–0.832). The fecal calprotectin AUROC increased to 0.805 when serum albumin and CRP were added to the evaluation (95% CI, 0.752–0.858) (51).

However, there are limited data about IUS and FC. Calabrese et al. (24) explored the correlation between TH and combined CRP and FC. They found that a significant ($p < 0.002$) proportion of patients achieving TH had clinical remission and normalization of CRP and FC assessed by using qualitative measurement at 3, 6, and 12 months compared to the baseline.

In the *post hoc* analysis of the TRUST study (25), patients with CD who achieved TH at week 12 reported improved CRP and FC levels, even though this was not significant compared with patients without TH, defined as no IUS improvements.

With regards to UC, Maaser et al. (52), in the prospective, observational TRUST&UC study conducted at 42 German IBD-specialized centres, found that an FC normalization (intended as a value >250 μg/g becoming <250 μg/g at week 12) positively correlates to a BWT normalization in sigmoid ($p = 0.023$) and descending colon ($p = 0.029$).

Furthermore, in a single Italian study, Allocca et al. (53) investigated the diagnostic accuracy of IUS and/or FC compared with colonoscopy (CS) in assessing endoscopic activity. The sensitivity and specificity of BWT > 3 mm or FC > 101 μg/g was 100 and 53%, respectively, for endoscopic disease activity, while when taken into account together, they resulted in a sensitivity of 84 and specificity of 93%.

Regardless, more studies are awaited to integrate IUS, CRP, and FC into the definition of deep healing.

Intestinal Ultrasound and Ulcerative Colitis: Ready for Use?

The application of IUS in UC is less well established since endoscopic evaluation and histology have been considered the critical stakeholders for assessing disease extent and severity.

However, in the setting of tight monitoring, IUS has become the focus of current intense study (54), even in UC, due to the need for non-invasive, easily available tools.

Growing evidence suggests that its use is reliable, objective and well accepted because it could reduce the need for colonoscopy with related risks, potential complications, sedation, and bowel preparation.

Currently, the role of IUS in UC could be to evaluate disease activity and disease extension.

Assessment of BWT is the most reliable IUS measure. A recent expert panel identified several key IUS features complementary to the bowel wall thickness, such as parietal blood flow, Doppler signal, wall layer stratification, and fatty wrapping. They further assessed the reliability of IUS parameters among expert sonographers, and it is noteworthy that inter-observer agreement was almost perfect for BWT [intra-class correlation coefficient (ICC): 0.96] and substantial for color Doppler signs ($\kappa = 0.63$) (55).

Several studies found also differences between different colonic segments and the other age groups of patients (56). Maaser et al. (52), in the TRUST&UC study, identified a threshold of 4 mm for the sigmoid colon and 3 mm for descending, transverse, and ascending colons for defining active disease. In a prospective multi-centric study, Kinoshita et al. (54) described four grades of severity according to the presence of the following features: the first one consisted of normal wall colonic thickness; the second one consisted of thickened mucosa and sub-mucosa without hypoechoic change of the sub-mucosa; the third one consisted of bowel wall thickness with loss of stratification; the last one consisted of bowel wall thickness with loss of stratification and irregular mucosa.

Regarding UC extension, the overall accuracy of IUS in terms of BWT has been acceptable compared to endoscopy. In addition, when BWT was combined to color Doppler assessment, the accuracy increased, and it has been reported around 95% across all the bowel segments measured.

A recent systematic review highlights a significant heterogeneity between all the IUS measures for assessing disease activity and identified that increased BWT and detection of increased blood flow by color Doppler were the most often applied criteria for disease activity and distribution (56).

In a prospective observational study, including 53 patients with UC, Allocca et al. (53) reported that BWT > 3 mm, hypoechogenicity, a signal on power Doppler, and lymphadenopathy correlated with endoscopic disease activity. Based on them, a score consisting of BWT > 3 mm plus vascularity or flow within the colonic wall or BWT > 4.43 mm alone without vascular signal was built. This score had a sensitivity of 0.71 and a specificity of 1.00 in assessing disease activity and inter-observer agreement was excellent ($\kappa = 0.86$) so it has been recently validated under the name 'Milan ultrasound criteria (MUC)' (57) in a cohort of 98 patients with UC. Importantly, there was a strong correlation between MUC and the Mayo Endoscopic Score (MES) at baseline ($p = 0.653$; $p < 0.001$). In addition, a baseline MUC > 6.2 was predictive of a negative disease course (HR: 3.87, 95% CI: 2.25–6.64, $p < 0.001$), while patients with MUC < 6.2 had a significantly lower cumulative probability of treatment escalation, need of corticosteroids, hospitalization, and colectomy (58).

Similarly, Bots et al. (59) developed and internally validated, in 60 patients with UC, a new index for grading disease activity, using endoscopy with MES as the reference standard. Based on this index, a BWT > 2.1 mm discriminated between remission (MES 0) and mild (MES 1) endoscopic activity, while a cut-off of 3.2 mm distinguished between mild and moderate endoscopic activity (MES 0–1 vs. MES 2–3) and, finally, a BWT > 3.9 mm correlated with severe endoscopic activity. The other parameters included in this score were an enhanced color Doppler signal and a lack of haustrations, both predicting disease activity. In addition, fat wrapping was predictive of severe disease.

The TRUST&UC study (52) has shown that BWT correlates with disease clinical activity scores measured using the Simple Clinical Colitis Activity Index (SCCAI) at the beginning of a flare and after 12 weeks of therapy. High BWT (> 4 mm in the sigmoid colon and >3 mm in the other segments) and the Doppler signal were observed at the time of diagnosis and over 12 weeks (at 2, 6, and 12 weeks). Loss of haustration, loss of wall stratification, ascites, lymphadenopathy, and mesenteric fat proliferation were further evaluated during follow-up. It is noteworthy that the percentage of patients with high BWT changed significantly over time from baseline to 12 weeks after starting therapy and BWT at week 2 predicted the response to medical treatment followed by the SCCAI.

Kinoshita et al. (54) performed the first prospective multicentric study to compare US with colonoscopy for assessing UC. They found a significant overall correlation between US and colonoscopy in all the colonic segments (k = 0.55; $p < 0.001$) and the concordance for each colonic segment was moderate, whereas, for the rectum, it was poor.

The poor diagnostic accuracy of IUS for detecting inflammation in the rectum in patients with UC was further confirmed by a recent meta-analysis (60). Good sensitivity and specificity were reported for detecting active disease (when BWT > 3 mm) in the right and transverse colons. However, this accuracy decreased toward the rectum due to the rectum's deep position in the pelvis and to the small intestine gas distension.

Hence, IUS is not considered a reliable tool for assessing rectal disease. Sagami et al. (48) used the IUS combined with transperineal ultrasound (TPUS) and FC for active patients with UC who required colonoscopy as the gold standard to evaluate the rectum. BWT < 4 mm in TPUS was a significant independent predictor for rectal endoscopic and histologic healing ($p < 0.05$) and, notably, the predictability was better than FC. Thus, the authors proposed TPUS in combination with IUS to assess the whole colon (61).

FUTURE DIRECTIONS

Current evidence suggests that IUS is shifting from a tool to diagnose IBD and simply discriminate active vs. inactive disease to a more complex, valid, and reliable instrument for closely monitoring patients with IBD and predicting therapeutic response.

Recent evidence supports that adjusting therapeutic approaches based on IUS parameters appear to be a reasonable strategy in CD. Indeed, assessing IUS findings after starting therapy could be crucial to establish the early response to treatment and speed up making clinical decisions.

The use of IUS in UC is still challenging, given that endoscopy and, more recently, histology remain the reference standard for assessing UC activity. However, based on the latest findings, it is time to include IUS in routine clinical management.

Developing IUS scores to determine disease activity and response to treatment is the next crucial step toward standardized IBD monitoring and expanding IUS adoption.

We strongly believe that in the next future, the full acknowledgment of the role of IUS in IBD could allow us to replace invasive assessment of endoscopic response/remission and thus reduce the psychological burden of colonoscopies on the patients.

AUTHOR CONTRIBUTIONS

OMN and GC contributed to the conception, design, drafting, and revision of the manuscript. AT, AC, and GF contributed to the manuscript draft. AR critically reviewed the manuscript. FC contributed to the conception and critically reviewed the manuscript for important intellectual content and provided overall supervision. All authors have contributed to the article and approved the submitted version of the manuscript.

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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.

frontiers | Frontiers in Medicine

MINI REVIEW
published: 23 May 2022
doi: 10.3389/fmed.2022.865131

Overview of Three Proliferation Pathways (Wnt, Notch, and Hippo) in Intestine and Immune System and Their Role in Inflammatory Bowel Diseases (IBDs)

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Reviewed by:
Harpreet Kaur,
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 29 January 2022
Accepted: 14 April 2022
Published: 23 May 2022

Citation:
Khoramjoo SM, Kazemifard N, Baradaran Ghavami S, Farmani M, Shahrokh S, Asadzadeh Aghdaei H, Sherkat G and Zali MR (2022) Overview of Three Proliferation Pathways (Wnt, Notch, and Hippo) in Intestine and Immune System and Their Role in Inflammatory Bowel Diseases (IBDs). Front. Med. 9:865131. doi: 10.3389/fmed.2022.865131

Inflammatory bowel disease (IBD) is a disorder, which involves the gastrointestinal (GI) tract consisting Crohn's disease (CD) and ulcerative colitis (UC). The etiology of this disease is not yet clear and, hence, there are numerous medications and treatments for patients with IBD, although a definite and permanent treatment is still missing. Therefore, finding novel therapeutic approaches are vital for curing patients with IBD. In the GI tract, there are various lineages of cells with different roles that their existence is necessary for the barrier function of intestinal epithelial cells (IECs). Therefore, signaling pathways, which manage the hemostasis of cell lineages in intestine, such as Wnt, Notch, and Hippo, could have crucial roles in regulation of barrier function in the intestine. Additionally, these signaling pathways function as a governor of cell growth, tissue homeostasis, and organ size. In patients with IBD, recent studies have revealed that these signaling pathways are dysregulated that it could result in depletion or excess of a cell lineage in the intestine. Moreover, dysregulation of these signaling pathways in different cell lineages of the immune system could lead to dysregulation of the immune system's responses in IBD. In this article, we summarized the components and signaling of Wnt, Notch, and Hippo pathways and their role in the intestine and immune system. Furthermore, we reviewed latest scientific literature on the crosstalk among these three signaling pathways in IBD. An overview of these three signaling pathways and their interactions in IBD could provide a novel insight for prospective study directions into finding efficient medications or treatments.

Keywords: inflammatory bowel disease, Wnt signaling, Notch signaling, Hippo signaling, immune system

INTRODUCTION

Inflammatory bowel disease (IBD) is a regressive inflammatory condition, which occurs in the gastrointestinal tract (1). Patients with IBD fall into two clinical types: ulcerative colitis (UC) and Crohn's disease (CD). In patients with UC, part of involvement is limited to the colon and it can spread from the rectum to the cecum. In this type of IBD, these parts show large mucosal ulceration. On the other hand, in patients with CD, the parts, which are affected the most, are the ileum and the colon, but other parts of the gastrointestinal (GI) tract could be influenced patchily (2). In spite of the vast studies to find a causative factor for etiology of IBD, it is still known as a multifactorial disorder (3). Different factors, such as internal triggers (genetic susceptibility and immunoregulatory impairments), environmental factors (diet and chemicals), and microbial exposure, are considered to cause IBD (2, 4, 5). Moreover, recent studies have shown that the dysbiosis of gut microbiota profoundly contributes to the development of IBD (2, 6–8).

Various cell lineages arise by differentiation and proliferation of intestinal stem cells (ISCs), which are controlled by multiple signaling pathways, including Hippo, Notch, and Wnt (9–13). These signaling pathways accelerate undifferentiated columnar cells, named crypt base columnar cells, to regenerate into absorptive and secretory cell types in the GI tract (14). Some studies have illustrated that in patients who suffer from IBD, especially those with ulcerative colitis (UC), overexpression of Notch and inhibition of Wnt lead to a lack of Paneth cells that exist in crypts (15). Similarly, other studies have demonstrated that an imbalance in components of Hippo signaling pathway in the intestine of patients with IBD resulted in excess of ISCs and shortage of secretory cells, such as goblet cells and Paneth cells (16). Therefore, dysregulation in pathways that play a role in proliferation and differentiation may explain the defective mucus secretion and wound healing, which could ultimately induce the failure of intestinal barrier in patients with IBD (15). Furthermore, recent studies on Wnt, Notch, and Hippo showed that these signaling pathways play a regulatory role in the function and generation of various immune cells' types that in IBD, it could be dysregulated (17–19). However, numerous studies have been conducted on understanding the function and regulation of the proliferation pathways in the gut epithelium and their specific role in IBDs is still unknown (20).

In IBD, the process of wound healing and mucus secretion is dysregulated that could lead to impaired barrier function of the GI tract and ultimately leaky gut. Herein, we briefly explain the barrier functionality of intestinal epithelial cells (IECs) and the process of wound healing in IBD. In addition, it is illustrated that some proliferation pathways, including Wnt, Notch, and Hippo, could have critical impacts on these processes and the immune system. We also summarize these three signaling pathways and their role in the intestine and immune system. Finally, we concisely discuss the interactions of these signaling pathways in IBD.

BARRIER FUNCTION OF INTESTINAL EPITHELIAL CELLS

The intestinal epithelial cells (IECs) establish a barrier, which is selectively permeable and sets apart luminal content from beneath tissues (21, 22). Basically, IECs function as a barrier, which prevents unacceptable solutes, microorganisms, viruses, and luminal antigens from passing the epithelium and entering the lamina propria (22, 23). Multiple components that participate in the intestinal barrier consist of the epithelial cells with tight junctions, adherens junctions, and luminal secretions, such as mucus or unstirred layers, on the apical side of the epithelium (22).

PROCESS OF WOUND AND MUCOSAL HEALING IN INFLAMMATORY BOWEL DISEASE

The process of wound healing starts when a part of the intestinal epithelium gets injured. Intestinal wound healing depends on the accurate balance between migration, proliferation, and differentiation of the epithelial cells, which are nearby the wounded area (24). First, epithelial cells surround the wounded area, which loses their columnar polarity. Then, they proliferate to surge the pool of cells for resurfacing the wound. Finally, to maintain the mucosal barrier function, maturation and differentiation of epithelial cells are vital (25). Dysfunction of these three steps during the wound healing's process in patients with IBD results in the broken differentiation and proliferation of different cell lineages in gut, such as goblet cells or Paneth cells, that lead to flawed mucosal secretion and leaky gut (26).

IMPORTANCE OF PROLIFERATION PATHWAYS IN INTESTINE

Many cell lineages are vital for maintenance of intestinal epithelial barrier integrity, which arise by differentiation and proliferation of intestinal stem cells (ISCs) (27). Crypt-based stem cells, which are near the base of the crypts, need to actively proliferate to maintain continuous renewal of different cell lineages (28). As these cells move up from crypt to villus, proliferation ends gradually and differentiation into one of the four primary cell types occurs (i.e., enterocytes, goblet, Paneth, and enteroendocrine cells) (29, 30). Multiple signaling pathways, such as Hippo, Notch, and Wnt, are responsible for regulating the proliferation and the differentiation in intestinal epithelial cells (9–13, 31). Finally, an imbalance among these types of pathways in epithelium could lead to colorectal cancer and IBD (27).

WNT PATHWAY

The Wnt signaling pathways are a group of signaling pathways, which commence with proteins that transmit signals into a cell by cell surface receptors (32, 33). Therefore, this pathway is activated by the cell–cell communications and it has been conserved

throughout the biological evolution (27). Wnt pathway is divided into β-catenin dependent (canonical) and β-catenin independent (noncanonical) types (34). This signaling pathway gets activated when Wnt proteins contact with Frizzled (Frz) receptor on the cells' surface (18, 33). Thereafter, Wnt proteins run a complex signaling cascade that plays an important role in regulating cell proliferation and differentiation by regulating the β-catenin, which is an important mediator (34–36). When the Wnt pathway is silenced, β-catenin can be phosphorylated by the ubiquitin-proteasome system [including glycogen synthase kinase 3 (GSK3), casein kinase Iα (CKIα), axin, and adenomatosis polyposis coli (APC)] and then transcription complexes prohibit gene transcriptional activity (37, 38). In opposition, when the Wnt signaling pathway has been activated, β-catenin degradation is banned, which is leading to its aggregation (38, 39). As a result, transcription complexes are changed by accumulated β-catenin, which activates targeted expression of genes related to cell proliferation and migration, EphB2/B3, Cylind-1, and c-Myc (40, 41).

ROLE OF WNT PATHWAY IN INTESTINE

Wnt signaling pathway plays a vital role in the intestinal epithelium, specifically in regulating the stem cells' behavior, proliferation, differentiation, and migration (42). This pathway is one of the many signaling pathways for the maintenance of stem cells (43). Recent studies have shown that deletion of β-catenin's encoding gene (CTNNB1) results in disruption of secretory cells' differentiation (44). Wnt pathway is able to direct the beginning development of secretory cells lineage and the endpoint of differentiation of Paneth cells for sustaining homeostasis (45). Reduced Wnt pathway, particularly diminished expression in its transcription factor 4 (TCF-4), could mediate Paneth cells differentiation flaws that it induces specific deficiency of Paneth cell defensins, which is a principal factor in IBD pathogenesis (15, 46). In addition, evidence found in TCF-4 knockout mice illustrated that the reduced level of defensins in gut permits bacteria to invade the epithelium and resulting in colitis (42, 47). On the other hand, excessive Wnt pathway accumulates β-catenin in the cytoplasm, then they are translocated to the nuclear, and finally induces overexpression of Wnt target genes, which lead to colon cancer (48). Furthermore, recent studies on IBD-associated colorectal cancer (CRC) revealed that negative regulators of Wnt, such as AXIN2 and RFN43, are downregulated in 31 tissue sample of patients with IBD-CRC (49).

ROLE OF WNT PATHWAY IN IMMUNE SYSTEM

One of the important roles of Wnt pathway is in multiple layers of immune regulation. Presence or absence of Wnt proteins could have impact on different immune cells, such as dendritic cells, macrophages, CD8+ T cells, and CD4+ T cells (18, 50). Based on recent studies on Wnt proteins and dendritic cells (DCs), it is demonstrated that Wnt proteins may be involved in promoting DCs into a tolerogenic state (51). Manicassamy et al. showed that reduced expression of β-catenin in DCs enhances inflammatory responses in the mice model of inflammatory bowel disease. Therefore, in DCs, β-catenin signaling causes a tolerogenic state and prevents them from inflammatory responses (52). Recent studies on Wnt pathway and macrophages showed that in macrophages, Wnt ligands have crucial roles in repairing injured tissues, since macrophages' role in tissue repairing and wound healing is well known (53, 54); however, in some studies, it is demonstrated that macrophage-derived Wnt5a maintains immune functions and stimulates the secretion of proinflammatory cytokines (55). Some previous studies showed that proteins of Wnt signaling play a regulatory role in the function of CD8+ T-cell effector and generating memory T-cell pool (18, 56). Functional regulation of β-catenin-mediated CD8+ T-cell immune responses remains unclear (57–60). The role of Wnt pathway in CD4+ T-cells is not yet clear and it needs more accurate investigations for various Wnt ligands; however, some studies suggested that the overexpression of β-catenin in regulatory T (Treg) cells enhanced Treg function in IBD (61, 62). The canonical Wnt signaling proteins are able to induce their roles in T-cell differentiation and effector function in various inflammatory diseases, such as IBD, cancer, as well as in autoimmunity and viral infections (18). Wnt signaling is important in inflammatory and fibrotic diseases and it is in harmony with the roles of Wnt proteins in repairing injured tissue. Recent studies' outcomes demonstrated that Wnt signaling plays vital roles in lymphomyelopoiesis and immune responses (56). Moreover, Wnt pathway and inflammatory signaling pathways, such as nuclear factor-kappa B (NF-κB), Janus kinase-signal transducer and activator of transcription 3 (JAK-STAT3), and mitogen-activated protein kinase (MAPK), affect each other, which regulate inflammatory factors' secretion during the pathogenesis of colitis (18, 63).

NOTCH PATHWAY

The Notch signaling pathway is conserved during the evolution that is presented in most animals (27). It regulates the differentiation and development of cells, tissues, and organs by interactions among nearby cells (27). The pathway consists of receptors, ligands, transformation complexes, and several regulatory molecules (64). The Notch transmembrane receptor plays a critical role in the signaling pathway that regulates the fate and development of a wide range of metazoan cells through local cell interactions (65–67). There are at least four different Notch receptors (Notch 1–4) in mammals that the Notch-1 is dominant in the intestine (66, 68). As a result of binding ligands, slight structural conformations in the membrane around the binding site activate matrix metalloproteinases (MMPs) and γ-secretase (69). With the assistance of activated γ-secretase and a disintegrin and metalloproteinase (ADAM)-family MMPs, Notch intracellular domain (NICD), which is the activated form of Notch receptors, is generated (64, 66, 70). Thereafter, NICD enters the nuclear and by the help of activator transcription

complexes, it regulates the *HES* genes to determine the fate of the cell (64, 71–74).

ROLE OF NOTCH PATHWAY IN INTESTINE

In the intestine, Notch is necessary for the survival of ISCs. Notch is also responsible for determining ISCs differentiation into secretory or absorptive lineages (63). High Notch signaling leads to absorptive differentiation, whereas low Notch signaling induces differentiation of secretory cells (75). Abnormal activity of the Notch pathway in IBD induces increased expression of the HES1 transcription factor in human colon cell lines, which thereafter inhibits differentiation of secretory cell lineages and weakens the mucus barrier, which is linked to chronic colitis (76). Accordingly, it is demonstrated that the Notch signaling pathway has a crucial role in maintaining goblet cells in the lesion of patients with UC that is so important in mucosal and wound healing in IBD. Zheng et al. illustrated that abnormality in expression of Notch intracellular domain (NICD) in ulcers induces reduction in the quantity of goblet cells in patients with UC (77). NICDs imposed expression leads to a decrease in phenotypic genes for goblet cells located in human IECs (77). In addition, several signaling pathways and cytokines cascade with Notch pathway mediate epithelial regeneration, such as interleukin-22 (IL-22) and tumor necrosis factor-α (TNF-α) (27, 78). Accordingly, Kuno et al. found that messenger RNA (mRNA) expression of OFLM4, intestinal stem cell marker, is upregulated by TNF-α and Notch pathway in patients with IBD (63). Moreover, it is reported that Notch signaling pathway contributes to the maintenance of tight junctions and adherens junction proteins in mice. Ahmed et al. showed that during the infection with *Citrobacter rodentium* and absence of Notch pathway, the function of tight junctions and adherens junctions impaired, which could result in increased permeability of epithelial cells and more exposure of luminal contents with immune system and inflammation (79). Notch dysregulation has also been demonstrated in colon cancer (80).

ROLE OF NOTCH PATHWAY IN IMMUNE SYSTEM

Notch signaling is important pervasively all over the immune system, since it has lineage and context-dependent impacts on a broad range of cells. In the immune system, Notch1 ligands, especially Jagged1, are present in regulatory T-cells (Tregs) (17). The activation of Notch1 in dendritic cells (Notch1 intracellular domain) induces the interaction of signaling elements and components that result in overexpression and the transport of pSmad3, which is known to facilitate the effector function of Tregs (17, 81). Notch signaling is also involved in the improvement of inflammatory conditions. Some studies revealed that Notch signaling pathway improves an inflammatory cascade in macrophages in inflammation and blocking Notch reduces the production of proinflammatory cytokines, such as interleukin-1β (IL-1β) (82, 83).

HIPPO PATHWAY

The Hippo pathway is a pathway, which is remained conserved throughout the evolution and it controls the size and homeostasis of an organ by regulating cell proliferation, survival, apoptosis, and stemness (84, 85). Specifically, intercellular contacts and membrane adhesion complexes modulate the transduction of a signal by the fundamental constituents of this pathway, which are highly conserved in mammals (86). These components contain the mammalian sterile 20-like kinases, MST1 and MST2, with their regulatory protein WW45 (SAV1) and the large tumor suppressor 1 and 2 kinases (LATS1 and LATS2) with their regulatory protein MOBKL1A/B (MOB1) (87). When the MST1/2 kinases get activated and LATS kinases get phosphorylated, it leads to negative regulation of cell proliferation (88). Concisely, phosphorylation of LATS kinases results in a process of phosphorylation of the transcriptional coactivators Yes-associated protein (YAP) at Ser127 and PDZ-binding motif (TAZ) at Ser89, so it makes binding sites for 14-3-3 proteins that accumulate YAP/TAZ in the cytoplasm (88). Once this inhibitory phosphorylation does not work, these transcription factors will be able to enter to the nucleus and contact with other transcriptional factors that enhance cell proliferation (89, 90). In cells that are in apoptotic phase because of exposure to severe DNA damage stress, YAP activates the transcription of proapoptotic genes through binding to the p73 transcription factor that is a p53-like tumor suppressor. This process is moderated by phosphorylation of YAP at the Tyr357 position through c-Abl protein, which provides a higher affinity of YAP compared to p73 (89, 91).

ROLE OF HIPPO PATHWAY IN INTESTINE

It is confirmed that YAP/TAZ enhances regeneration of tissues in the mammalian intestine (92). Accordingly, Yui et al. demonstrated that YAP/TAZ is associated with the expression of Sca1, which is a cell surface protein representing a marker for the repairing epithelium (93). YAP/TAZ has two different roles in the renewal of the intestinal epithelium: one is ISCs' proliferation that happens through collaboration of YAP/TAZ with transcription factor TEADs (94) and the other role is encouraging goblet cells differentiation by cooperation with transcription factor klf4 (95, 96). The activity of MST1/2 gets higher, as cells move from the crypts toward the lumen, so MST1/2 has decreased activity in the crypts (97). Contrarily, YAP is plentiful in the nucleus of the cells, which are located in lower crypts; however, this molecule is also found in cytoplasm of upper cells in the villi (97). In general, the expression of YAP in the nucleus of cells diminishes as cells move from the crypts to the villi; in contrary, expression of YAP in cytoplasm surges (16). Deletion of MST1/2 in mouse intestinal epithelium cells induces an improved amount of nuclear YAP; as a result, it increases proliferation of undifferentiated ISCs and lack of secretory cells both in the small and large intestines (16). In another study, it is demonstrated that in IECs, conditional knockout of MST1/2 results in disorganized villus structures, increased undifferentiated cells, and dysplastic epithelia (98). It is also reported that in mouse gut, deficiency

of SAV1 induces enlargement of crypt structures (92). YAP modulates the regeneration of mucus both in the patients with IBD and the DSS-induced colitis mouse model (99). Moreover, previous studies conducted by Ou et al. illustrated that YAP/TAZ expression is linked with promotion of fibrosis in patients with CD by activating intestinal fibroblasts (100). To sum up, YAP in the nucleus has a positive role in the regeneration of intestinal epithelium in IBD and may provide a novel therapeutic target for IBD.

ROLE OF HIPPO PATHWAY IN IMMUNE SYSTEM

Recent studies have shown that the Hippo pathway plays an important role in the modulation of immune system. MST1/2 has a pivotal role in mediating, migration, adherence, and survival of T cells by its downstream effectors, such as LATS1/2, NDR1/2, and YAP (101). MST1/2 promotes the function of regulatory T-cell (Treg) through modulating Foxp3 acetylation (16, 102). It is shown the deficiency of MST1/2 leads to impairment of Foxp3 expression and Treg cell development and its function in mice (16, 102). It is also illustrated that the deficiency of MST1/2 might induce the lack of naïve T cells, which could lead to autoimmune demonstrations or resulting in recurrent bacterial or viral infections (103, 104). Additionally, in a study by Geng et al., it has been found that the TAZ is able to determine the fate of a T cell to become a proinflammatory T-helper (Th) 17 cell or an immunosuppressive Treg cell (105). Particularly, lack of TAZ improves the differentiation of Treg cells; however, activation of TAZ enhances Th17 cell differentiation (105). On the other hand, YAP in macrophages was shown to deteriorate the IBD, since it negatively affects M2 polarization of macrophages, which is induced by IL-4/IL-13 and promotes the activation of M1 macrophages that is caused by lipopolysaccharide (LPS) or interferon-γ (IFN-γ) (19).

CROSSTALK BETWEEN WNT, NOTCH, AND HIPPO SIGNALING PATHWAYS IN INFLAMMATORY BOWEL DISEASE

There is an interplay among the proliferation signaling pathways, including Hippo, Wnt, and Notch, in intestinal regeneration (**Figure 1**) and imbalance among these pathways results in different problems, which are associated with different diseases, including IBD.

Once the Hippo pathway gets activated, it negatively affects the Wnt signaling pathway by cytoplasmic and phosphorylated YAP/TAZ; however, deactivation of the Hippo pathway has a positive effect on the expression of Wnt target genes by nuclear and dephosphorylated YAP (20). Additionally, it is reported that β-catenin activates and upregulates YAP and TAZ (106, 107). Imajo et al. illustrated that the YAP/TAZ regulates Wnt signaling that relies on the state of phosphorylation and cellular localization of YAP/TAZ proteins (108). Cytoplasmic YAP/TAZ downregulates the Wnt signaling through the regulation of nuclear translocation and activation of β-catenin (108, 109). This is in contrast to nuclear YAP, which stabilizes β-catenin and resulting in the improved expression of Wnt target genes (110, 111). It is shown that YAP and β-catenin grow in nucleus within regeneration following inflammation. Once the nuclear YAP is overexpressed, it enhances Wnt/β-catenin signaling and significantly leads to the improvement of the IECs' healing ability, thereby demonstrating that nuclear YAP improves the IECs' proliferation by the activation of Wnt/β-catenin signaling pathways (112). In addition, within intestinal regeneration following tissue damage, cytoplasmic YAP restricts Wnt signals, interrupts the ISCs, and decreases the stem cells' growth, which induce abnormal migration of Paneth cells and reduction of ISCs (97).

In previous studies, it is reported that the Hippo pathway is able to regulate Notch signaling. In intestine, conditional knockout of MST1/2 leads to increased amount of NICD in nucleus (16). Reduction of MST1/2 results in activation of Notch signaling through decreasing of phosphorylation and increasing the abundance of nuclear accumulation of YAP (16). Intrinsically, the YAP molecules, which are located in nucleus, facilitate Notch signaling (98). Moreover, it is shown that administration of gamma-secretase inhibitors (GSIs), which restrict YAP-activating Notch, induce colitis (98, 113). In general, the Hippo pathway is able to downregulate Notch signaling *via* phosphorylation and suppression of the YAP.

Wnt and Notch signaling pathways are so intertwined that it has been suggested that they established an integrated signaling termed as "Wntch" (114). An accurate balance between Wnt and Notch is required for the homeostasis of intestine, as their dysregulation may result in inflammation, colitis, and tumorigenesis. It is demonstrated that activation of Notch pathway upregulates the expression of β-catenin (115). On the other hand, Kay et al., by utilizing chemical reaction network theory (CRNT), found that Wnt-mediated actions on Hes1 promoter are able to change dynamic transition of Notch signaling pathway from multistability to monostability, highlighting the role of β-catenin in modulating Notch signaling pathway (116).

DISCUSSION

In this short article, we aimed to overlook on three proliferation pathways playing important roles in intestine and immune system. We also reviewed some of their impacts on pathogenesis of IBD. In the process of wound and mucosal healing in a healthy condition, adjacent cells to the lesion start to proliferate and migrate to retrieve the columnar polarity of the epithelial cells (24). This process is faulty in the intestine of patients with IBD, particularly those who have UC. Multiple factors contribute in this flaw, but it is shown that one of the most important factors is the dysregulation of proliferation pathways, such as Hippo, Wnt, and Notch, and also an imbalance among them (27). Moreover, dysregulation of these pathways leads to an imbalance of cell lineages in intestine. Importantly, this dysregulation results in depletion of goblet cells and Paneth cells, which leads to impaired secretion of mucus and defensins and invasion of various bacteria to epithelial cells (26). Accordingly, this results in massive responses of the immune system and inflammation that cause tissue damage and ulcers, which are the clinical symptoms

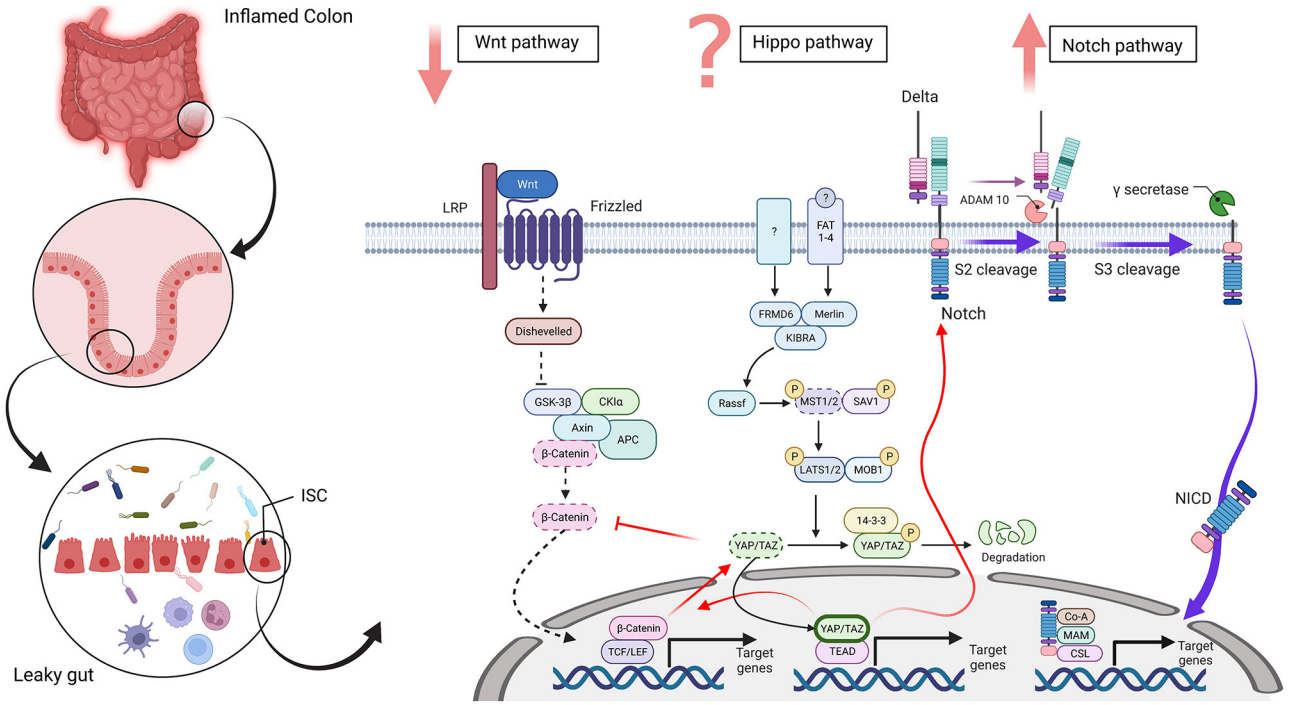

FIGURE 1 | Wnt, Hippo, and Notch signaling in an intestinal stem cell [inflammatory bowel disease (IBD) condition]. The disrupted intestinal epithelial barrier promotes a leaky gut in inflammatory conditions, such as IBD. In this state, differentiation-related pathways, such as Wnt, Hippo, and Notch, are dysregulated in intestinal stem cells. The Wnt signaling pathway plays a vital role in the intestinal epithelium and downregulation of β-catenin disrupts the differentiation of secretory cells in IBD. The Hippo pathway regulates cell proliferation, survival, apoptosis, and stemness. An improved amount of nuclear YAP/TAZ occurred in IBD and increased the proliferation of undifferentiated intestinal stem cells (ISCs) and a lack of secretory cells. In contrast, cytoplasmic YAP/TAZ overexpression is seen in inflamed tissue. Also, MST1/2 is downregulated and disorganized villus structures and increased undifferentiated cells and dysplastic epithelia. However, it is controversial that Hippo pathway is upregulated or downregulated in IBD. The Notch pathway regulates the differentiation and development of cells, tissues, and organs by interactions among nearby cells. Upregulation of the Notch pathway in IBD inhibits differentiation of secretory cell lineages and abnormality in the expression of NICD in ulcers reduces the quantity of goblet cells in patients with ulcerative colitis (UC). Red arrows show crosstalk between these three pathways and dotted lines display downregulated pathways and molecules. Abbreviations: low-density-lipoprotein-related protein (LRP), adenomatosis polyposis coli (APC), glycogen synthase kinase 3 beta (GSK3β), casein kinase 1α (CK1α), T-cell factor (TCF), lymphoid enhancer factor (LEF), FERM domain-containing protein 6 (FRMD6), mammalian Sterile 20-related 1 and 2 kinases (MST1 and MST2), Salvador 1 (SAV1), Large tumor suppressor 1 and 2 kinases (LATS1 and LATS2), Mps One Binder Kinase Activator-Like 1A (MOB1), Yes-associated protein 1 (YAP), PDZ-binding motif (TAZ), TEA domain family member (TEAD), Fat-related atypical cadherins 1-4 (FAT 1-4), A Disintegrin and metalloproteinase domain-containing protein 10 (ADAM 10), Notch intracellular domain (NICD), meprin A-5 protein (MAM) and CBF1, Suppressor of Hairless, Lag-1 (CSL). The figure has drawn by BioRender (www.Biorender.com).

of IBD (117). These signaling pathways also play important roles in various immune cells, including dendritic cells, macrophages, and T cells. These signaling pathways could impact immune cells to differentiate to a particular type that it could attenuate or strengthen immune responses (117). Moreover, there is a crosstalk between these signaling pathways and inflammatory signaling pathways, such as NF-κB, JAK-STAT3, and MAPK, that could influence an immune cell to produce proinflammatory or anti-inflammatory cytokines (104). Dysregulation in these pathways in immune cells is reported to be important in immune responses to inflammation in patients with IBD (117).

FUTURE PERSPECTIVE

This study needs more investigation in different aspects. First, a precise analysis of the relationship among these three pathways needs to be more investigated. By doing so, we will be able to understand the impact of different molecules of signaling pathways on each other. In addition, current findings demonstrated the role of these pathways in several immune cells yet not all of them. More experiments could be done in finding their role in other immune cells. Finally, these proliferation pathways can be a potential target for medications. More studies are required to develop efficient drugs for triggering epithelial cells to regulate these pathways. The results of prospective studies can dwindle the morbidity and mortality linked to IBD, hence reduce the worldwide incidence of this disease.

AUTHOR CONTRIBUTIONS

SMK: literature search and writing. SBG, MF, SS, HA, GS, and MZ: literature search. NK: drawing of figure. All authors contributed to the article and approved the submitted version.

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frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 08 June 2022
doi: 10.3389/fmed.2022.881027

Impact of BNT162b2 mRNA Vaccination on the Development of Short and Long-Term Vaccine-Related Adverse Events in Inflammatory Bowel Disease: A Multi-Center Prospective Study

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OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *22 February 2022*
Accepted: *03 May 2022*
Published: *08 June 2022*

Citation:
Shehab M, Alrashed F, Abdullah I, Alfadhli A, Ali H, Abu-Farha M, Channanath AM, Abubaker JA and Al-Mulla F (2022) Impact of BNT162b2 mRNA Vaccination on the Development of Short and Long-Term Vaccine-Related Adverse Events in Inflammatory Bowel Disease: A Multi-Center Prospective Study. Front. Med. 9:881027. doi: 10.3389/fmed.2022.881027

Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination has been effective in protecting against severe COVID-19 infections and related mortality. It is recommended for all individuals including patients with inflammatory bowel disease (IBD). However, safety data are lacking in this group of patients. Therefore, we aim to evaluate the short- and long-term vaccine related adverse events (AEs) in patients with IBD.

Methods: This is a prospective, observational cohort study investigating short- and long-term AEs related to the BNT162b2 vaccine in patients with IBD (study group) after the first and second dose compared to healthy participants (control group). Patients were recruited at the time of attendance to the clinic or infusion rooms. Short term (<3 weeks) localized and systemic AEs were assessed *via* questionnaire. Follow-up phone-based survey was made to collect data on long term (up to 24 weeks) AEs.

Results: A total of 408 patients answered the questionnaires, 204 patients in each group, the study and control group. No serious adverse events were reported in either the study or the control group after the first or the second dose. Participants in the control group reported more frequent pain at the injection site than those in the study group after the first dose [58 (57%) vs. 38 (37%) respectively, $P = 0.005$]. After the second dose, tiredness was reported more frequently in the control group [49 (48%)] compared to the study group [25 (24%) ($P < 0.001$)]. At 20–24 weeks post vaccination, 386 out of 408 (94.6%) patients were willing to participate in the follow-up phone based questionnaire [196 (96.1%) in the study group vs. 190 (93.1%) in the control group]. In both groups, none of the patients reported local, systemic, or severe adverse events (0 out of 386) at week 20–24 post second dose.

Conclusion: The BNT162b2 vaccine is safe in patients with IBD. No severe or long-term adverse events were reported in our study. The frequency of local and systemic adverse events after the second dose was generally higher among healthy participants compared to patients with IBD. Further studies including a larger cohort with a longer follow-up duration are needed to assess for possible rare adverse events.

Keywords: IBD, COVID-19, vaccine, safety, symptoms

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China in December 2019 (1). The disease has been known to cause a significant morbidity and mortality among many of those infected. The outbreak, which was later declared a pandemic, had global health and socioeconomic consequences (2). This has led to an international effort for vaccine development and the introduction of the first vaccine, the BNT162b2 mRNA (Pfizer/BioNTech) vaccine in December 2020 followed by ChAdOx1 nCoV-19, mRNA-1273 (Moderna) and other vaccines, which later were authorized under the emergency use authorization (3, 4).

Thereafter, clinical trials and real-world data have shown efficacy and safety of these vaccines in reducing COVID-19 infection severity and decreasing both hospitalization and mortality in patients with COVID-19. Nevertheless, patients with inflammatory bowel disease (IBD) were largely excluded from these trials (5–7).

Despite the fact that many of the patients with IBD are on immune-modifying medications, those patients were not found to be at higher risk of developing COVID-19 infection. However, being on corticosteroids was found to be a risk factor for developing more severe infection (8). Vaccination against the SARS-CoV-2 virus in patients with IBD is highly recommended by most international gastrointestinal societies (9, 10).

A study showed that the overall prevalence of COVID-19 vaccination among patients with IBD on biologic therapies was lower than that of the general population (11). Furthermore, many studies focused on the efficacy of COVID-19 vaccination in patients with IBD receiving biologic therapies, while the safety of vaccination was not extensively explored (12, 13). However, evidence regarding the vaccine safety in patients with IBD is slowly emerging, with the majority of studies investigating only the short-term adverse events following vaccination in patients with IBD (14, 15). Therefore, it is imperative to assess the long-term safety of SARS-CoV-2 vaccine in patients with IBD. This study aims to evaluate the short- and long-term adverse events following vaccination with BNT162b2 mRNA vaccine among patients with IBD.

MATERIALS AND METHODS

We performed a prospective multi-center cohort study at two tertiary care centers (Muabark Alkabeer Hospital and Dasman Center) to assess short and long-term adverse events related to COVID-19 mRNA vaccine, BNT162b2 (Pfizer/BioNTech) in patients with IBD (study group) compared to healthy participants (control group).

This study was performed and reported in accordance with Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (16). This study was reviewed and approved by the Ethical Review Board of Mubarak Alkabeer Hospital and Dasman Center "Protocol # RA HM-2021-008" as per the updated guidelines of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and of the US Federal Policy for the Protection of Human Subjects. The study was also approved by the regional health authority (reference: 3799, protocol number 1729/2021). Subsequently, patient informed written consent was obtained before inclusion in the study.

Localized and systemic adverse events to the BNT162b2 vaccine were assessed *via* paper questionnaires at the time of attendance at the gastroenterology infusion rooms and outpatient clinics from 1 August 2021 to 15 September 2021, and patients were followed up to 10 February 2022, using a phone based questionnaire. Outcomes were stratified by first and second doses.

Study group patients were eligible to be included if they: (1) had confirmed diagnosis of inflammatory bowel disease (IBD) before the start of the study, (2) had received one or two doses of COVID-19 vaccination with BNT162b2 (Pfizer-BioNTech), (3) were at least 18 years of age or older. Patients were excluded if they received any vaccine other than the BNT162b2 or if they tested positive for SARS-CoV-2 previously or had symptoms of COVID-19 since the start of the pandemic up to the time of vaccination. Patients were also excluded if they have one of the following within 8 weeks of vaccination: stool fecal calprotectin levels >250 ug/g, C-Reactive Protein (CRP) levels >10 mg/L, active symptoms of IBD or endoscopic active disease (refer below), use of corticosteroids or active extraintestinal manifestation of IBD (e.g., inflammatory uveitis, arthritis, skin rashes, etc).

Patients with Harvey Bradshaw Index (HBI) >4 and partial clinical Mayo score >1 are considered to have active symptoms of IBD. In addition, patients who had colonoscopies with an endoscopic Mayo score >1 for ulcerative colitis or Simple Endoscopic Score for Crohn's Disease (SES-CD) >4 are considered to have active endoscopic. In addition, patients who had severe allergic reactions to a previous vaccine in their life or were unwilling to participate in the study were excluded.

On the other hand, healthy participants (control) group were individuals who volunteered to participate in the study at Dasman Center with no previous history of chronic

medical illnesses such as diabetes, hypertension, cardiovascular disease, autoimmune diseases, osteoarthritis, chronic obstructive pulmonary disease, renal disease, asthma, hyperlipidemia, or history of stroke and bleeding disorder. In addition, basic laboratory tests were performed (full blood count, renal function tests, liver function tests, lipid profile, HbA1c, ESR, and CRP) to objectively screen for underlying diseases.

The baseline questionnaire assessed the type of immunization, date of immunization(s), patient demographics and IBD characteristics, and data regarding IBD medication use around the time of vaccination. Participants were asked to report short-term localized and systemic adverse events defined as adverse events occurring within 14 days after receiving the BNT162b2 vaccine dose 1 and 21 days of receiving vaccine dose 2. The follow-up phone-based survey collected data on long term (20–24 weeks from the first dose) adverse events of BNT162b2 vaccination. Both groups were tested with SARS-CoV-2 PCR within 72 h before each vaccine dose. Positive subjects were excluded from the study. Patients were also monitored 24 weeks post vaccination. Any subjects who reported symptoms of COVID-19 or tested positive for it were also excluded.

Vaccine adverse events were classified as the injection site (localized) or systemic reactions. Adverse localized reactions included pain, redness, itching, swelling, or tenderness at the injection site. Systemic adverse reactions included fever, chills, fatigue, headache, joint pain, muscle aches, nausea, allergic reaction, rash, or other. Severe adverse events were defined as incidence of pulmonary embolism, acute myocardial infarction, immune thrombocytopenia, and disseminated intravascular coagulation per the Food and Drug Administration (FDA) definition of potential adverse events of interest (17).

Diagnosis of inflammatory bowel disease (IBD) was made according to the international classification of diseases (ICD-10 version: 2016). Patients were considered to have IBD when they had ICD-10 K50, K50.1, K50.8, K50.9 corresponding to Crohn's disease (CD) and ICD-10 K51, K51.0, K51.2, K51.3, K51.5, K51.8, K51.9 corresponds to ulcerative colitis (UC) (18).

Statistical Analysis

Analysis was conducted using R (19). We performed descriptive statistics to present the demographic characteristics of patients included in this study. The McNemar test was used to determine whether the proportion of participants who had any symptoms (yes or no) after the first dose of the vaccine differed after the second dose. Pearson's Chi-squared test was used to compare the proportions of symptoms of the control group vs. the study group. Participants in both groups were matched for *Age* and *Gender*. The technique attempts to choose matches that collectively optimize an overall criterion. The criterion used is the sum of the absolute pair distances in the matched sample.

RESULTS

Baseline Characteristics

Between 1 August 2021 and 15 September 2021, a total of 204 patients diagnosed with inflammatory bowel disease (IBD) answered the questionnaire. Of these, 119 (58%) were males. The median age of the patients included was 34.6 years (IQR 25–41). A total of 140 (68.7%) patients and 64 (31.3%) patients had Crohn's disease and ulcerative colitis, respectively. Most patients were receiving biologic therapy [82(40%)], followed by immunomodulators [75 (37.0%)], whereas 47 (23.0%) patients were on 5-aminosalicylates.

Before receiving the first dose, the mean CRP was 7 mg/L in the study group and 5 mg/L in the control group. Mean stool fecal calprotectin was 85 mcg/g in the study group.

In the study group, half of the patients ($n = 102$) received one dose of the Pfizer-BioNTech vaccine, while the other half ($n = 102$) received two doses of the vaccine. Asthma (8%), arthritis (5%), and diabetes (3.9%) were the most common comorbidities in the study group. Demographics are shown in **Table 1**. No serious adverse events were reported in either the study or the control group after the first or the second dose.

Symptoms After the First Dose in the Study Group vs. Control Group

Adverse events after the first vaccine dose are shown in **Table 2**. The most common local adverse event was pain at the injection site reported in 37% of the study group. In general, after receiving the first dose, local adverse events were reported more frequently by patients in the control group. Specifically, subjects in the control group reported more frequent pain at the injection site than those in the study group [58 (57%) vs. 38 (37%), respectively, $P = 0.005$]. Redness and swelling at the injection site were also more common in the control group compared to the study group, [17 (17%) vs. 3 (2.9%), $P < 0.001$, and 18 (18%) vs. 4 (3.9%), $P = 0.002$], respectively.

Similarly, after receiving the first dose, more subjects reported systemic reactions in the control group compared to the study group. Specifically, tiredness was reported by 35 (34%) of the subjects in the control group as opposed to 19 (19%) subjects in the study group ($P = 0.011$), muscle pain in 24 (24%) subjects in the control group and 11 (11%) subjects in the study group ($P = 0.016$), chills in 14 (14%) subjects in the control group compared to 4 (3.9%) subjects in the study group ($P = 0.014$), and nausea in 9 (8.8%) subjects from the control group compared to 1 (1.0%) subjects in the study group ($P = 0.009$). There was no significant difference in the occurrence of headaches, fever, or joint pain between the control group and the study group after the first dose of the vaccine.

Symptoms After the Second Dose in the Study Group vs. Control Group

The frequency of local adverse events after the second dose was also generally higher among subjects in the control group than those in the study group (**Table 3**). Pain at the injection site was reported by 50 (49%) subjects in the control group and 32 (31%) subjects in the study group ($P = 0.01$). Additionally, 15 (15%) subjects in the control group reported redness at the injection site compared to 4 (3.9%) subjects in the study group ($P = 0.008$), while 29 (28%) subjects had swelling at the injection site in the control group compared to 3 (2.9%) subjects in the study group ($P < 0.001$).

TABLE 1 | Baseline characteristics of patients with inflammatory bowel disease (IBD).

Characteristic	*N* = 204
Age (years)[a]	34.6 (25, 41)
Gender	
Female	85 (42%)
Male	119 (58%)
Nationality	
Kuwaiti	180 (88%)
Non-Kuwaiti	24 (12%)
Disease extent, *n* (%)	
Ulcerative colitis (UC)	64 (31.3%)
E1: ulcerative proctitis	12 (18.7%)
E2: left sided colitis	20 (31.2%)
E3: extensive colitis	32 (50.0%)
Crohn's disease (CD)	140 (68.7%)
L1: ileal	62 (44.2%)
L2: colonic	24 (17.1%)
L3: ileocolonic	48 (34.4%)
L4: upper gastrointestinal	6 (4.3%)
B1: inflammatory	68 (48.8%)
B2: structuring	33 (23.3%)
B3: penetrating	39 (27.7%)
Past medical history	
Diabetes	8 (3.9%)
Hypertension	5 (2.0%)
Heart disease	5 (2.0%)
Arthritis	11 (5.0%)
COPD	2 (1.0%)
Kidney	5 (2.0%)
Asthma	16 (8.0%)
Hyperlipidemia	7 (3.0%)
IBD medications	
5-aminosalicylates	47 (23.0%)
Immunomodulators	75 (37.0%)
Biologics and small molecules	82 (40.0%)

[a]*Median (IQR) or frequency (%).*

TABLE 2 | Comparison of symptoms after the first dose in patients with IBD (study) group vs. healthy participants (control) group.

Characteristic	Study group, *N* = 102[a]	Control group, *N* = 102[a]	*P*[b]
Age_in years	33 (26, 41)	34 (28, 43)	
Gender			
Female	48 (47%)	52 (51%)	
Male	54 (53%)	50 (49%)	
Injection site			
Pain	38 (37%)	58 (57%)	0.005
Redness	3 (2.9%)	17 (17%)	<0.001
Swelling	4 (3.9%)	18 (18%)	0.002
Systemic AEs			
Tiredness	19 (19%)	35 (34%)	0.011
Headache	15 (15%)	16 (16%)	0.8
Muscle pain	11 (11%)	24 (24%)	0.016
Chills	4 (3.9%)	14 (14%)	0.014
Fever	13 (13%)	14 (14%)	0.8
Nausea	1 (1.0%)	9 (8.8%)	0.009
Joint pain	8 (7.8%)	11 (11%)	0.5

[a]*Median (IQR) or frequency (%).*
[b]*Wilcoxon rank sum test; Pearson's Chi-squared test.*

When comparing systemic reactions among subjects in the control group as opposed to the study group, tiredness was reported by 49 (48%) subjects vs. 25 (24%) subjects ($P < 0.001$), headaches in 35 (34%) subjects vs. 21 (20%) ($P = 0.001$), muscle pain in 34 (33%) vs. 16 (15%) subjects ($P < 0.001$), chills in 19 (19%) vs. 9 (8.8%) subjects ($P = 0.042$), nausea in 14 (14%) subjects vs. 2 (2.0%) subjects ($P = 0.002$), and joint pain in 20 (20%) vs. 4 (3.9%) subjects ($P < 0.001$) respectively. Conversely, no significant difference was found in the frequency of fever between the control group and the study group [26 (25%) vs. 20 (20%), $P = 0.3$] (**Table 3**).

Other than nausea, none of the participants reported gastrointestinal (GI) related symptoms such as diarrhea or abdominal pain after the first or second dose. Furthermore, none of the patients reported any severe adverse events after the first or second dose. Additionally, no significant differences in any adverse reaction frequency were seen based on sex or age.

Symptoms After the First Dose vs. the Second Dose in the Study Group

The frequency and type of adverse reactions after the first dose of the vaccine were compared with adverse reactions after the second dose of the vaccine among patients with IBD (study group). There was no significant difference in the frequency of symptoms reported after the first and second dose among subjects in the study group. The most common local reaction was pain at the injection site reported by 38 (37%) patients after the first dose and 32 (31%) patients after the second dose ($P = 0.3$). Redness was reported in 3 (2.9%) subjects after the first dose and 4 (3.9%) after the second dose ($P > 0.9$) and swelling 4 (3.9%) subjects after the first dose and in 3 (2.9%) after the second dose ($P > 0.9$).

Systemic reactions were reported as follows: tiredness in 19 (19%) after dose 1 vs. 25 (24%) after dose 2, $P > 0.9$, headache in 15 (15%) after dose 1 vs. 21 (20%) after dose 2, $P > 0.9$, muscle pain in 11 11% after dose 1 vs. 16 (15%) after dose 2, $P > 0.9$, chills in 4 (3.9%) after dose 1 vs. 9 (8.8%) after dose 2, $P = 0.2$, fever in 13 (13%) after dose 1 vs. 20 (20%) after dose 2, $P = 0.2$, nausea in 1 (1%) after dose 1 vs. 2 (2%) after dose 2, $P > 0.9$, and joint pain in 8 (7.8%) after dose 1 vs. 4 (3.9%) after dose 2, $P = 0.3$ (**Table 4**).

Long-Term Adverse Events

At 20–24 weeks post vaccination, 386 out of 408 patients were willing to participate in the follow-up phone-based questionnaire. In the study group, 196 (96.1%) patients and 190

TABLE 3 | Comparison of symptoms after the second dose in patients with IBD (study) group vs. healthy participants (control) group.

Characteristic	Study group, $N = 102$[a]	Control group, $N = 102$[a]	P[b]
Age_in years	34 (25, 41)	33 (25, 45)	
Gender			
Female	43 (42%)	49 (48%)	
Male	59 (58%)	53 (52%)	
Injection site			
Pain	32 (31%)	50 (49%)	0.010
Redness	4 (3.9%)	15 (15%)	0.008
Swelling	3 (2.9%)	29 (28%)	<0.001
Systemic AEs			
Tiredness	25 (24%)	49 (48%)	<0.001
Headache	21 (20%)	35 (34%)	0.001
Muscle pain	16 (15%)	34 (33%)	<0.001
Chills	9 (8.8%)	19 (19%)	0.042
Fever	20 (20%)	26 (25%)	0.3
Nausea	2 (2.0%)	14 (14%)	0.002
Joint pain	4 (3.9%)	20 (20%)	<0.001

[a]*Median (IQR) or frequency (%).*
[b]*Wilcoxon rank sum test; Pearson's Chi-squared test.*

TABLE 4 | Symptoms after the first dose and second dose in patients with IBD (study group).

Characteristic	First dose, $N = 102$[a]	Second dose, $N = 102$[a]	P[b]
Injection site			
Pain	38 (37%)	32 (31%)	0.3
Redness	3 (2.9%)	4 (3.9%)	>0.9
Swelling	4 (3.9%)	3 (2.9%)	>0.9
Systemic AEs			
Tiredness	19 (19%)	25 (24%)	>0.9
Headache	15 (15%)	21 (20%)	>0.9
Muscle pain	11 (11%)	16 (15%)	>0.9
Chills	4 (3.9%)	9 (8.8%)	0.2
Fever	13 (13%)	20 (20%)	0.2
Nausea	1 (1.0%)	2 (2.0%)	>0.9
Joint pain	8 (7.8%)	4 (3.9%)	0.3

[a]*n (%).*
[b]*McNemar's Chi-squared test with continuity correction; McNemar's Chi-squared test.*

(93.1%) in the control group answered the questionnaire. 21 (10.7%) out of 196 patients in the study group, and 19 (10.0%) out of 190 in the control group reported having a breakthrough SARS-CoV-2 infection confirmed by PCR test after the second dose of vaccination. None of the patients who tested positive were hospitalized.

In both groups, none of the patients reported local, systemic, or severe adverse events (0 out of 386). In addition, short-term local and systemic adverse events have been resolved in the control and study groups.

DISCUSSION

We performed a survey-based study to explore the onset of adverse events related to the BNT162b2 vaccine in patients with inflammatory bowel disease (IBD) compared with healthy participants. In our study, none of our patients had severe vaccine-related adverse events, as they are very rare (20). We found that the most common adverse events after the first and the second doses were tiredness and headache, followed by local pain at the injection site. Nausea was reported in both study and control group, however, none of the groups reported other gastrointestinal (GI) related symptoms such as diarrhea or abdominal pain.

Weaver et al. (21) explored vaccine related adverse events among patients with IBD and the effect of vaccination on IBD disease course. Similar to our study, they found that severe localized and systemic vaccine-related adverse events were rare in patients with IBD. Injection site tenderness (68%) and fatigue (46% dose 1, 68% dose 2) were the most commonly reported localized and systemic adverse events after vaccination.

Interestingly, we also found that local and systemic adverse events were more common in healthy participants compared to patients with IBD. Given that the majority of our IBD cohort are on biologics or immunomodulators, it is possible that these medications blunt the immune response to vaccination. Botwin et al. (14) evaluated post-mRNA vaccination adverse events in 246 vaccinated adults with IBD participating in a longitudinal vaccine registry. Similar to our finding, the study found that adverse events were less common in individuals receiving biologic therapy. The authors concluded that patients with IBD can be reassured that the risk of adverse events is likely not increased, and may be reduced while receiving concomitant biologic therapy.

None of the participants in our study experienced severe short- or long-term adverse events. One systematic review and meta-analysis evaluated SARSCoV-2 vaccination in patients with IBD. The study did not find any severe adverse events or vaccine-related mortality in patients with IBD and the majority of patients reported mild adverse events after vaccination, including fatigue, headache, dizziness, and gastrointestinal symptoms (22).

Another study (23) observed a higher rate of diarrhea and abdominal pain in vaccinated patients with IBD compared to the general population. They also found that age and disease remission was inversely correlated with the onset of GI symptoms. To our knowledge, no evidence has emerged of IBD flare-ups caused by COVID-19 vaccination. Furthermore, in a population-based study, the effect of SARS-CoV-2 vaccination on IBD course was evaluated for a period of 4 weeks in patients with IBD. The study reported no clinical and laboratory exacerbation compared with the pre-vaccination baseline and no increase in corticosteroid prescription 1 month after vaccination in a large retrospective cohort compared with a matched unvaccinated cohort (24).

Another study (15) explored immediate (within 1 day) adverse events after mRNA SARS-CoV-2 vaccines in patients with IBD in the United States. Similar to our study, the authors found that the incidence of adverse events including acute myocardial

infarction, anaphylaxis, facial nerve palsy, and coagulopathy in patients with IBD after COVID-19 vaccination was small and similar to a matched cohort of patients without IBD. Immediate adverse events after vaccination were rare in both cohorts.

In our study, none of our patients reported long-term adverse events 20–24 weeks after vaccination. One study (20) involved more than 1.5 million BNT162b2 vaccinated persons from an integrated healthcare organization, followed over a period of 42 days. The study reported an excess risk of lymphadenopathy (78.4 events per 1,00,000 persons), herpes zoster infection (15.8 events), appendicitis (5.0 events), and myocarditis (2.7 events) in the vaccinated cohort. However, the author concluded that their results indicate that SARS-CoV-2 infection is itself a very strong risk factor for myocarditis, and it also substantially increases the risk of multiple other serious adverse events.

Taken together, these emerging data provide reassurance that COVID-19 vaccination does not cause severe adverse events in patients with IBD and support recent consensus recommendations to vaccinate all patients with IBD. British Society of Gastroenterology (BSG) (10), the International Organization for the Study of Inflammatory Bowel Diseases (IOIBD) (25), and the Canadian Association of Gastroenterology (9) recommend that all patients with IBD should receive SARS-CoV-2 vaccination regardless of whether patients were in remission or not. In addition, the CORALE-IBD study group is assessing and conducting studies at Cedars-Sinai to understand the effects of vaccination against COVID-19 in people with IBD, and their recent publications reassured patients with IBD and provider communities that symptoms after the second and third dose of mRNA vaccine are generally mild and well tolerated. It also showed that responses after mRNA vaccination in adults with IBD receiving various medication regimens are robust (26, 27).

Our study has several strengths. It provides real world data for the public about adverse effects and vaccine safety in a subpopulation that was not studied in the initial clinical trials. In addition, the comparison to healthy participants and the rigorous matching allowed for precise estimation of the rate of adverse events in patients with IBD. Finally, the long-term follow-up period helps detect any possible late events that may occur several weeks after vaccination.

Our study also has some limitations. Given the observational nature of this study, it is possible that some hidden confounding variables were still not properly addressed. In addition, this study was performed prospectively in the context of an ongoing pandemic, therefore, the association between any breakthrough infection and any given adverse events cannot be ruled out. In addition, COVID antibody testing was not performed for all patients which could help identify those patients with previous silent infection. However, in our follow-up phone-based survey we asked patients about any previous or current SARS-CoV-2 infection. Despite these limitations, our study provides highly anticipated data regarding the short- and long-term adverse events of the SARS-CoV-2 vaccination in patients with IBD.

CONCLUSION

The BNT162b2 vaccine is safe in patients with IBD. No severe or long-term adverse events were reported in our study. The frequency of local and systemic adverse events after the second dose was generally higher among healthy participants compared to patients with IBD. Further studies including a larger cohort with a longer follow-up duration are needed to assess for possible rare adverse events.

DATA AVAILABILITY STATEMENT

The data presented in this study are available upon request from the corresponding author. The data are not publicly available due to local legal and ethical regulations.

ETHICS STATEMENT

This study was reviewed and approved by the Ethical Review Board of Mubarak Alkabeer Hospital and Dasman Center Protocol # RA HM-2021-008 as per the updated guidelines of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and of the US Federal Policy for the Protection of Human Subjects. The study was also approved by the Regional Health Authority (Reference: 3799, Protocol Number 1729/2021). Subsequently, the patient informed written consent was obtained before inclusion in the study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MS: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and submission of the manuscript. FA, MS, and IA: acquisition of data and drafting of the manuscript. AC: statistical analysis and interpretation of data. HA: data collection and supervision. JA and FA: critical revision of the manuscript for important intellectual content and study supervision. All the authors contributed to the article and approved the submitted version.

FUNDING

This study was funded by Kuwait Foundation for the Advancement of Sciences (KFAS) Grant (RA HM-2021-008).

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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.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 13 June 2022
doi: 10.3389/fmed.2022.910623

Impact of Inflammatory Bowel Disease (IBD) and IBD Medications on Risk of Hyperlipidemia and *in vitro* Hepatic Lipogenic-Related Gene Expression: A Population-Based Cohort Study

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Patients with inflammatory bowel disease (IBD) present a higher risk of developing cardiovascular diseases (CVDs) due to chronic inflammation, which plays an essential role in atherogenesis. Hyperlipidemia is another risk factor for CVDs; however, the association between IBD, IBD medications, and hyperlipidemia remains controversial. We conducted a nationwide, population-based, retrospective, cohort study to examine the effect of IBD and IBD medications on the risk of developing hyperlipidemia. The effects of IBD medications on the expression of lipogenesis-related hepatic genes were also evaluated. We obtained data from the Longitudinal Health Insurance Database of Taiwan from patients with new-onset IBD and a comparison cohort of patients without IBD. A Cox proportional hazards regression model was used to analyze the difference in the risk of developing hyperlipidemia between the two cohorts. We also examined the influence of IBD medications on the expression of lipogenesis-related hepatic genes. After adjusting for comorbidities and confounding factors, the case group ($N = 14,524$) had a higher risk for hyperlipidemia than the control group ($N = 14,524$) [adjusted hazards ratio (aHR), 2.18]. Patients with IBD that did not receive IBD medications exhibited a significantly higher risk of hyperlipidemia (aHR, 2.20). In those treated with IBD medications, the risk of developing hyperlipidemia was significantly lowered than those without such medications

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Edited by:
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Reviewed by:
Kevin Sheng-Kai Ma,
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Specialty section:
This article was submitted to Family Medicine and Primary Care, a section of the journal Frontiers in Medicine

Received: 01 April 2022
Accepted: 16 May 2022
Published: 13 June 2022

Citation:
Tien N, Wu T-Y, Lin C-L, Wu C-J, Hsu C-Y, Fang Y-J and Lim Y-P (2022) Impact of Inflammatory Bowel Disease (IBD) and IBD Medications on Risk of Hyperlipidemia and in vitro Hepatic Lipogenic-Related Gene Expression: A Population-Based Cohort Study. Front. Med. 9:910623. doi: 10.3389/fmed.2022.910623

(all aHR $\leq$ 0.45). Gene expression analysis indicated that IBD medications downregulated the expression of lipogenesis-related genes. Screening blood lipids in IBD patients is needed to explore the specific role and impact of IBD medications in the development of CVD.

Keywords: inflammatory bowel disease (IBD), IBD medications, hyperlipidemia, Longitudinal Health Insurance Database (LHID), lipogenesis

INTRODUCTION

Inflammatory bowel diseases (IBDs) have no cure and are characterized by chronic, recurrent exacerbation and intestinal inflammation, resulting in altered gut functions (1). IBDs consist of Crohn's disease (CD) and ulcerative colitis (UC). The fundamental causes of these autoimmune diseases include the interplay between genetic and environmental factors, excluding pathogenic infections (2). IBD diagnoses depend on multiple factors, including clinical, endoscopic, radiological, and histological features, but not infectious etiology (1). In Taiwan, crude CD and UC incidences increased from 0.17 to 0.47 and 0.54 to 0.95 new cases per 100,000 persons, respectively, between 2001 and 2015. Moreover, CD and UC prevalence increased from 0.6 to 3.9 and 2.1 to 12.8 cases per 100,000 persons, respectively, within the same time frame. The male-to-female ratio in the study samples were 2.19 for CD and 1.62 for UC (3–5). Meanwhile, IBD prevalence in the USA is estimated to be 1.1–3 million adults (6). In the Western world, IBD prevalence is approximately 50–200 and 120–200 cases per 100,000 persons for CD and UC, respectively (7). J. Cosnes, C. Gower–Rousseau, P. Seksik, and A. Cortot, "Epidemiology and natural history of inflammatory bowel diseases," *Gastroenterology*, vol. 140, no. 6, pp. 1785–1794.e4, 2011. View at: *Publisher Site*. UC, as a chronic and recurrent intestinal disease, is mainly an autoimmune disease caused by genetic–environmental interactions, rather than colonic colitis caused by general bacterial and viral infections.IBD cases increase annually, and drugs and changes to the environment can improve patient quality of life. Medications remain the main therapeutic strategy for IBD and to relieve inflammation. In addition, surgery can be introduced if medications are unsuccessful or result in serious adverse reactions (1).

IBDs coincide with clotting abnormalities and vascular-related comorbidities, such as deep vein thrombosis, portal vein thrombosis, and ischemic vascular diseases (8). It has been reported that patients with IBD have a venous thromboembolism (VTE) risk 1.7–5.9 times greater than the general population. This has been found to affect 0.55–6.15% of patients with IBD, and the overall prevalence of VTE in IBD subjects was estimated as 1–8% (9). Moreover, VTE-associated mortality is twice as high in patients with IBD than in the general population (10). Further, a meta-analysis demonstrated that IBD is associated with an 18% higher risk of CVD (11), and the risk is higher for females than males [adjusted odds ratio (aOR), 1.28] (8). Additionally, the risk of mesenteric ischemia is increased 3.4-fold, and that of VTE is increased 1.4-fold (12).

The relationship between IBD and hyperlipidemia risk should be evaluated. Moreover, further research is warranted to devise therapeutic modalities to prevent hyperlipidemia and consequently decrease the CVD risk for IBD patients. Preventive or therapeutic strategies can also be developed to identify the pathogenic causes of these complications (9, 11, 13–19). Hyperlipidemia, a well-established CVD risk factor (20), is defined as abnormal lipid levels with total cholesterol (TC) $\geq$ 200 mg/dL, triglycerides (TGs) $\geq$ 150 mg/dL, high-density lipoprotein cholesterol (HDL-C) $\leq$ 40 mg/dL, and low density lipoprotein cholesterol (LDL-C) $\geq$ 130 mg/dL. Hyperlipidemia can originate from genetics, diet, lifestyle, metabolic disorders, and other diseases (21). Additionally, the degree of hyperlipidemia correlates with CVD severity and can predict prognosis. For example, patients with IBD often have changing blood lipid profiles, similar to those reported in CVD, frequently caused by IBD medications. Thus, risk factor modulation is needed to reduce blood lipid levels and CVD risk (8).

In contrast to lipid levels in hyperlipidemia, patients with IBD have low levels of TC and HDL-C and high levels of LDL-C and TGs (22). The exact mechanism underlying these altered levels is unknown; however, active inflammation and changes to lipid, apolipoprotein, and lipoprotein profiles via altered lipid *de novo* synthesis and degradation might play a role (22–24). Despite these lipid levels, patients with IBD have an increased risk of CVD (25), resulting in a "lipid paradox". However, studies showing these results are limited by small sample sizes. Moreover, the relationship between IBD therapeutics and reduced CVD risk remains controversial (26). Thus, we conducted a long-term, retrospective study with a large cohort to evaluate patients with IBD and the effects of their medications.

Abbreviations: ACLY, ATP citrate lyase; ACP, acid phosphatase; AMI, acute myocardial infarction; aOR, adjusted odds ratio; CAD, coronary artery disease; CD, Crohn's disease; CI, confidence interval; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CVD, cardiovascular disease; DMSO, dimethyl sulfoxide; ESR, erythrocyte sedimentation rate; FAS, fatty acid synthase; HDL-C, high density lipoprotein cholesterol; HR, hazard ratio; IBDs, inflammatory bowel diseases; ICD-9-CM, International Classification of Diseases, Clinical Modification; IFN-γ, interferon γ; IL-6, interleukin 6; LDL-C, low density lipoprotein cholesterol; LHID, Longitudinal Health Insurance Database; LXRs, Liver X receptors; NAFLD, non-alcoholic fatty liver disease; NHIRD, National Health Insurance Research Database; NHRI, National Health Research Institutes; NO, nitric oxide; NOS, nitric oxide synthase; PNPP, para-nitrophenylphosphate; qRT-PCR, quantitative real-time polymerase chain reaction; RR, relative risk; SCD, stearoyl CoA desaturase; SD, standard deviation; SE, standard error; SREBP-1c, sterol regulatory element binding protein 1c; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TGs, triglycerides; TNF, tumor necrosis factor; UC, ulcerative colitis; VTE, venous thromboembolism.

The mechanisms underlying the increased risk of CVD in patients with IBD are under investigation; however, systemic inflammation might play a role. Different drugs are selectively and broadly used to inhibit inflammation in IBD, controlling the active disease and inhibiting remission. Pharmacological treatment for IBD can be divided into four main classes, (1) aminosalicylates (5-aminosalicylic acid derivatives), (2) corticosteroids, (3) immunosuppressants, and (4) monoclonal antibodies (26). 5-Aminosalicylates are the most widely used and are ideal for IBD with mild to moderate symptoms mainly by blocking prostaglandin and leukotriene production (27). Corticosteroids are also used for reducing inflammation, however, they frequently have side effects dependent on the dose and treatment duration (28). Immunosuppressive and immunoregulatory agents could include the suppression of a specific subgroup of T cells, achieving a therapeutic response after a prolonged period (28). Therefore, these drugs are only useful for long-term control, rather than acute disease. Biologics are groups of monoclonal antibodies for patients with a reduced response to IBD drugs with small molecules. Several anti-tumor necrosis factor (TNF) therapies could inhibit TNF production by macrophages through altered regulatory peptide expression with IBD, which might lead to monocyte apoptosis (28). In addition, biologics can block leukocyte migration by blocking integrin adhesion molecules. Nevertheless, when prolonged treatment results in complications or adverse events, surgery can also be performed.

The liver is responsible for most processes involved in lipid homeostasis, including lipogenesis and blood lipid balancing. Therefore, any changes in hepatic lipid metabolism might affect the balance and homeostasis of blood lipid levels and could result in the development of non-alcoholic fatty liver disease (NAFLD) (29, 30). Liver X receptor alpha (LXRα), a nuclear receptor activated by ligands and act as transcription factor, is highly and specifically expressed in liver, responsible for lipid metabolism and *de novo* synthesis and excretion of cholesterol (31). LXRα activation results in the development of steatosis, which is mediated by the hepatic lipogenic pathway, primarily through sterol regulatory element binding protein 1 (SREBP-1c) (32). In addition, hepatic expression of LXRα, SREBP-1c, and their target genes, was found to be significantly upregulated in liver biopsies from NAFLD patients (33). Ultrasonographic monitoring revealed hepatic steatosis in approximately 50% of patients with associated with hypertriglyceridemia (29). Hyperlipidemia is commonly associated with NAFLD and is an independent risk factor of atherogenic dyslipidemia based on various clinical studies (33). Thus, the most important role of LXRα is the maintenance of lipid homeostasis as it regulates the balance of lipid-metabolism genes.

Further studies are warranted to determine how treatments for IBD affect hyperlipidemia risk. Thus, we performed a nationwide, population-based, cohort study to evaluate the risk of hyperlipidemia in patients with IBD from the 2000 to 2012 National Health Insurance Research Database (NHIRD) compared to that in the general population in Taiwan. We also examined the impact of IBD medications on the risk of hyperlipidemia and the expression of lipogenesis-related genes in differentiated HepaRG cells. We show that patients with IBD are more likely to have hyperlipidemia than those without IBD.

MATERIALS AND METHODS

Data Source

We obtained data from the NHIRD. The database covers >99% of the population of 23 million in Taiwan and was constructed using comprehensive inpatient and outpatient health care information, including demographic data, diagnostic codes, and prescription details. The dataset from the NHIRD is a subset of the Longitudinal Health Insurance Database, which comprises data of one million randomly sampled beneficiaries enrolled in the NHI program. The International Classification of Diseases, Clinical Modification (ICD-9-CM, procedure code 555 and 556) was used as the disease diagnostic tool. This study was approved by the Central Regional Research Ethics Committee of China Medical University, Taichung, Taiwan (CMUH-104-REC2-115-R5).

Study Population

In this population-based cohort study, we established an IBD cohort and a non-IBD cohort of patients enrolled in the database from January 1, 2000 to December 31, 2012 to compare their risk of hyperlipidemia. The index date of the case group (IBD cohort) was defined as the date of the first diagnosis of IBD and that of the control group (as the non-IBD cohort) was a random date during the study period. We excluded patients who had a history of hyperlipidemia before the index date. According to age (5-year intervals), gender, and the index year, the cohorts were frequency matched at a 1:1 ratio. The end date of the follow-up period was the onset of hyperlipidemia, death, or the end of study period (December 31, 2013), whichever came first. The primary outcome of the study was an individual event of hyperlipidemia (ICD-9-CM code 272) is defined as increased serum fasting levels of TC (≥200 mg/dL), LDL-C (≥130 mg/dL), or TG (≥150 mg/dL), and elevations of fasting TC concentration, which may or may not be associated with the elevated TG concentration, and decreased of HDL-C. To avoid subjects being mistakenly diagnosed or mistakenly coded as hyperlipidemia cases, we therefore defined patients with at least two claims for outpatient care and/or one hospitalization visit to ensure the validity of diagnosis. We selected potential confounders based on the previous research for multivariable analysis, including age, sex, and comorbidities of type 2 diabetes mellitus (T2DM) (ICD-9-CM 250), obesity (ICD-9-CM 278), coronary artery disease (CAD) (ICD-9-CM 410–414), hypertension (ICD-9-CM 401–405), and chronic kidney disease (CKD) (ICD-9-CM 585, 586). Further analysis was performed to investigate the effect of IBD treatment available in Taiwan on the risk of hyperlipidemia compared to non-IBD controls, IBD without medical treatment, and IBD without surgical treatment.

Chemicals and Cell Culture

All chemicals were purchased from Sigma-Aldrich (St. Louis, Missouri, USA) and were of the highest-purity grade available. Chemicals were dissolved in dimethyl sulfoxide (DMSO)

at appropriate concentrations before use. Human hepatoma HepaRG™ cells were purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Frozen cells were thawed and maintained in Williams' E medium (Sigma-Aldrich) supplemented with 10% Fetal Clone™ II serum (Hyclone™, GE Healthcare, Chicago, Illinois, USA), 1 × L-glutamine, 5 μg/mL human insulin, and 50 μM hydrocortisone hemisuccinate without antibiotics for 2 weeks. Next, the medium was replaced with the same medium plus 2% DMSO for two additional weeks to induce differentiated hepatocyte-like properties. Cells were cultured in a humidified atmosphere of 5% CO_2 at 37°C. Cell viability was assessed using *p*-nitrophenylphosphate in an acid phosphatase assay (ACP), as previously reported (34).

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

To evaluate the effects of IBD medications (corticosteroids, immunomodulators, and aminosalicylates) on hepatic lipogenesis-related gene expression, mRNA levels were measured. Total RNA was extracted from differentiated HepaRG cells under various treatment conditions using a Direct-zol™ RNA MiniPrep kit (ZYMO Research, Irvine, CA, USA) according to the manufacturer's protocol. The quantity and purity of RNA were confirmed by calculating the ratio of the absorbance at 260 nm to the absorbance at 280 nm. Total RNA (1 μg) was subjected to synthesis of first-strand cDNA using a MultiScribe™ reverse transcriptase kit (ThermoFisher Scientific). Expression of *SREBP-1c*, *SCD*, *FAS*, adenosine 5′-triphosphate citrate lyase (*ACLY*), *ACC*, *LXRα*, and *β-actin* was analyzed by qRT-PCR using Luminaris Color HiGreen qPCR master mix (ThermoFisher Scientific) in the StepOnePlus™ Real-Time PCR System following the standard procedure. Each pair of specific primers used for RT-PCR analysis is listed in **Table 1**. The amount of target cDNA in each sample was calculated by determining a fractional PCR threshold cycle number (Ct value). The relative mRNA levels were normalized to those of *β-actin*, and the target cDNA expression was calculated as follows: $2^{-(\mathrm{Cttargetgene}-\mathrm{Ct}\beta\text{-}actin)}$. Data are presented as fold-change compared to the control group.

Statistical Analysis

We evaluated the frequency and percentage of each categorical variable and the mean and standard deviation (SD) of each continuous variable. A chi-squared test was used to examine the differences of the demographic categorical variables between the IBD and non-IBD cohorts. Student's *t*-test was used to measure the association of continuous demographic variables between the two cohorts. To address the concern of constant proportionality, we examined the proportional hazard model assumption using a test of scaled Schoenfeld residuals. Results showed that there was no significant relationship between Schoenfeld residuals for IBD and follow-up time (p-value = 0.68) in the model evaluating the hyperlipidemia risk. Stratified Cox models were used to estimate the risk of hyperlipidemia by sex, age, and comorbidity between the two cohorts. The aHR was obtained for age, sex, and comorbidities of T2DM, obesity, CAD, hypertension, and CKD disease through multivariable analysis. The Kaplan–Meier method was applied to estimate cumulative incidence curves of hyperlipidemia in both cohorts, with significance based on the log-rank test. Analyses were performed in SAS software, version 9.4, and survival curves were drawn using R software.

For *in vitro* studies, data obtained from separate measurements were reported as the mean ± standard error (SE). The *P*-value for each experimental comparison was determined using analysis of variance, followed by the least significant difference test for multiple comparisons. All *P*-values were determined relative to the control group, as indicated in the figures. All statistical analyses were performed using SPSS for Windows, version 20.0 (IBM SPSS, Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics: Demographic and Association Findings

Table 2 shows the baseline characteristics of the study population from 2000 to 2013. After propensity score matching, and calculating the real-world database power as 0.9996, this study included 14,524 patients with and without IBD, respectively. Among the types of IBD, 1,362 (9.38%) and 13,162 (90.6%) patients had UC and CD, respectively. The mean ages for the IBD and non-IBD cohorts were 44.3 (SD = 16.4) and 43.9 (SD = 16.7) years, respectively. No significant differences were observed between the two cohorts in sex, age, or comorbidity of CKD. Patients with IBD were at higher risk of T2DM, obesity, CAD, and hypertension. Regarding treatment for patients with IBD, 68.6%, 0.97%, 3.60%, 0.03%, and 1.46% of patients received corticosteroids, immunomodulators, aminosalicylates, monoclonal antibodies, and surgical treatment, respectively. As shown in **Figure 1**, the Kaplan–Meier plots revealed that the cumulative incidence curve of hyperlipidemia showed a significantly higher risk in patients with IBD than those without.

Table 3 displays the stratified analyses of the risk of hyperlipidemia by sex, age, and comorbidity between the two cohorts. Compared with the control cohort, the IBD cohort faced a 2.18-fold higher risk of hyperlipidemia (95% CI, 2.03–2.34). Compared with that in the control cohort, UC resulted in a 2.60-fold increased hyperlipidemia risk (95% CI, 2.06–3.28), and the CD group was associated with a 2.15-fold increased hyperlipidemia risk (95% CI, 1.99–2.32). Moreover, females had a 2.10-fold higher risk of hyperlipidemia in the IBD cohort after controlling for other factors (95% CI, 1.90–2.32), while the risk for males in the IBD cohort was 2.25-fold (95% CI, 2.03–2.49). Among patients aged ≤ 49 years, the IBD cohort had a 2.00-fold higher risk of hyperlipidemia than the non-IBD cohort (95% CI, 1.81–2.21). Similarly, those aged 50–64 years had a 2.10-fold higher risk of hyperlipidemia in the IBD cohort (95% CI, 1.86–2.37). Additionally, among those aged ≥65 years, the IBD cohort had a 2.78-fold higher risk of hyperlipidemia (95% CI, 2.34–3.31). Moreover, among patients without comorbidities, the IBD cohort had a 2.06-fold higher risk of hyperlipidemia compared to the non-IBD cohort. Meanwhile, patients with comorbidities in the

TABLE 1 | Sequences of PCR primers.

Gene	Species	Forward primer (5′-3′)	Reverse primer (5′-3′)
SREBP-1c	human	CGC TCC TCC ATC AAT GAC AA	TGC AGA AAG CGA ATG TAG TCG AT
SCD	human	CCG ACG TGG CTT TTT CTT CT	GCG TAC TCC CCT TCT CTT TGA C
FAS	human	ACA TCA TCG CTG GTG GTC TG	GGA GCG AGA AGT CAA CAC GA
ACLY	human	GTG TGG ACG TGG GTG ATG TG	TTG ATG TCC TCA GGA TTC AGT TTC
ACC	human	CTC TTG ACC CTG GCT GTG TAC TAG	TGA GTG CCG TGC TCT GGA T
LXRα	human	CGA TC GAG GTG ATG CTT CTG	GGC AAA GTC TTC CCG GTT AT
β-actin	human	CCT GGC ACC CAG CAC AAT	GCC GAT CCA CAC GGA GTA CT

TABLE 2 | Demographic characteristics, comorbidities, and medications in patient with and without inflammatory bowel disease (IBD).

Variable	IBD		*p*-value
	No *N* = 14,524 *N* (%)	Yes *N* = 14,524 *N* (%)	
Type of IBD			
Ulcerative colitis (UC)		1,362 (9.38)	
Crohn's disease (CD)		13,162 (90.6)	
Sex			0.99
Female	7,820 (53.8)	7,820 (53.8)	
Male	6,704 (46.2)	6,704 (46.2)	
Age, mean (SD)	43.9 (16.7)	44.3 (16.4)	0.08
Stratify age			0.99
≤49	9,765 (67.2)	9,765 (67.2)	
50–64	2,798 (19.3)	2,798 (19.3)	
65+	1,961 (13.5)	1,961 (13.5)	
Comorbidity			
Type 2 diabetes mellitus (T2DM)	394 (2.71)	453 (3.12)	0.04
Obesity	101 (0.70)	132 (0.91)	0.04
Coronary artery disease (CAD)	1,055 (7.26)	1,479 (10.2)	< 0.001
Hypertension	2,341 (16.1)	2,808 (19.3)	< 0.001
Chronic kidney disease (CKD)	120 (0.83)	143 (0.98)	0.15
Medications			
Corticosteroids		9,959 (68.6)	
Immunomodulators		141 (0.97)	
Aminosalicylates		523 (3.60)	
Monoclonal antibody and small molecule		5 (0.03)	
Surgical treatment		212 (1.46)	

[#]*Chi-Square Test.*
Two sample T-test.

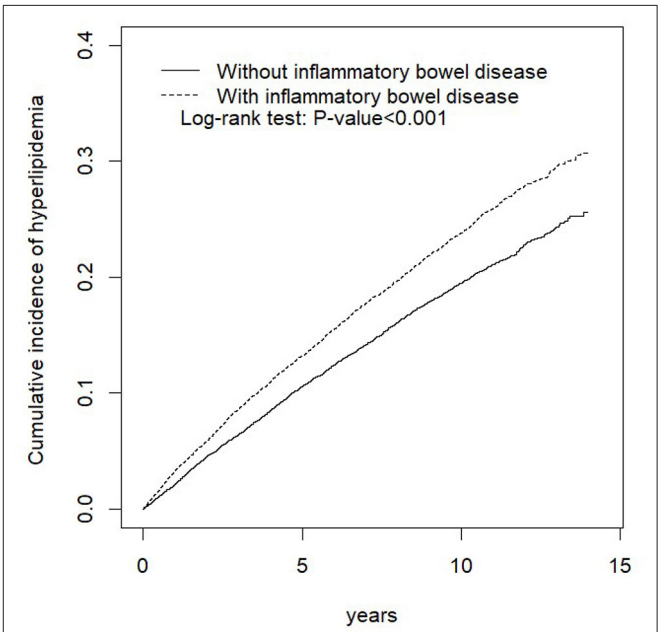

FIGURE 1 | Cumulative incidence of hyperlipidemia compared between the cohort with and without inflammatory bowel disease (IBD) using the Kaplan–Meier method.

IBD cohort had a 2.25-fold higher risk of hyperlipidemia than those in the non-IBD cohort.

Table 4 presents the incidence, as well as the crude and aHR of hyperlipidemia among IBD patients with and without treatment compared to those without IBD. Compared to the control cohort, the IBD cohort without treatment had a 2.20-fold higher risk of hyperlipidemia (95% CI, 2.05–2.36). Stratification of each type of IBD treatment suggested that immunomodulators, aminosalicylates, and corticosteroids could significantly decrease the risk of hyperlipidemia (aHR, 0.29; 95% CI, 0.18–0.49, aHR, 0.43; 95% CI, 0.34–0.55, aHR, 0.45; 95% CI, 0.42–0.49, respectively) compared to that of patients with IBD without treatment. Further stratification according to surgical treatment for IBD showed that surgical treatment could reduce the risk of hyperlipidemia (aHR, 0.32; 95% CI, 0.20–0.51).

Effects of IBD Medications on Lipogenesis-Related Gene Expression

We assessed the influence of corticosteroids (budesonide, prednisolone, methylprednisolone, and hydrocortisone), immunomodulators (azathioprine, methotrexate, cyclosporine, tacrolimus, and 6-mercaptopurine), and aminosalicylates (sulfasalazine, balsalazide, and 5-aminosalicylate) on hepatic gene expression. The concentrations were adjusted according to the maximum plasma or serum drug

TABLE 3 | Comparison of incidence (CI) and hazard ratio (HR) of hyperlipidemia stratified by sex, age, and comorbidities between with and without inflammatory bowel disease (IBD).

	IBD							
	No			Yes				
Variable	**Event**	**PY**	**Rate#**	**Event**	**PY**	**Rate#**	**Crude HR (95% CI)**	**Adjusted HR† (95% CI)**
All	2,345	107,920	21.7	2,927	106,110	27.6	1.27 (1.20, 1.34)***	2.18 (2.03, 2.34)***
Type of IBD								
UC	252	10,484	24.0	310	9,904	31.3	1.30 (1.10, 1.54)***	2.60 (2.06, 3.28)***
CD	2,093	97,436	21.5	2,617	9,6213	27.2	1.27 (1.20 1.34)***	2.15 (1.99, 2.32)***
Sex								
Female	1,301	59,279	22.0	1,540	58,317	26.4	1.20 (1.12, 1.29)***	2.10 (1.90, 2.32)***
Male	1,044	48,641	21.5	1,387	47,793	29.0	1.35 (1.25, 1.47)***	2.25 (2.03, 2.49)***
Stratify age								
≤49	1,128	77,553	14.5	1,520	76,695	19.8	1.36 (1.26, 1.47)***	2.00 (1.81, 2.21)***
50–64	822	18,830	43.7	952	18,262	52.1	1.19 (1.09, 1.31)***	2.10 (1.86, 2.37)***
65+	395	11,537	34.2	455	11,153	40.8	1.19 (1.04, 1.36)*	2.78 (2.34, 3.31)***
Comorbidity‡								
No	1,429	91,362	15.6	1,742	85,918	20.3	1.30 (1.21, 1.39)***	2.06 (1.88, 2.26)***
Yes	916	16,558	55.3	1,185	20,191	58.7	1.06 (0.97, 1.16)	2.25 (2.01, 2.51)***

#incidence rate, per 1,000 person-years; Crude HR, crude hazard ratio.
† Multivariable analysis including age, sex, and comorbidities of T2DM, obesity, CAD, hypertension, and CKD.
‡ Patients with any one of the comorbidities T2DM, obesity, CAD, hypertension, and CKD were classified as the comorbidity group.
**p < 0.05.*
****p < 0.001.*

TABLE 4 | Incidence, crude, and adjusted hazard ratio (aHR) of hyperlipidemia compared among inflammatory bowel disease (IBD) patients with and without IBD treatment compared to non-IBD controls.

Variables	***N***	**Event**	**PY**	**Rate#**	**Crude HR (95% CI)**	**Adjusted HR† (95% CI)**	**Adjusted HR† (95% CI)**
Non-IBD controls	14,524	2,345	107,920	21.7	1 (Reference)	1 (Reference)	
IBD without anti-IBD treatment	4,425	1,115	24,689	45.2	2.07 (1.92, 2.22)***	2.20 (2.05, 2.36)***	1 (Reference)
IBD with anti-IBD treatment							
Immunomodulators	138	15	1,171	12.8	0.59 (0.36, 0.98)*	0.65 (0.39, 1.08)	0.29 (0.18, 0.49)***
Aminosalicylates	478	68	2,937	23.2	1.06 (0.83, 1.35)	0.95 (0.74, 1.21)	0.43 (0.34, 0.55)***
Monoclonal antibody and small molecule	5	0	26	23.2	-	-	-
Corticosteroids	9,478	1,729	77,287	22.4	1.03 (0.97, 1.10)	0.99 (0.93, 1.05)	0.45 (0.42,0.49)***
IBD without surgical treatment	14,312	2,910	104,631	27.8	1.28 (1.21, 1.35)***	2.20 (2.05, 2.36)***	1 (Reference)
Surgical treatment	212	17	1,479	11.5	0.53 (0.33,0.85)**	0.69 (0.43, 1.12)	0.32 (0.20, 0.51)***

#Incidence rate, per 1,000 person-years; Crude HR, crude hazard ratio.
† Multivariable analysis including age, sex, and comorbidities of T2DM, obesity, CAD, hypertension, and CKD.
**p < 0.05.*
*** p < 0.01.*
**** p < 0.001.*

concentrations as they exhibit hepatotoxicity (35–47). We tested the toxicity of the drugs in a cell viability assay using human hepatoma HepaRG cells. The concentrations of corticosteroids were 0.01, 1.13, 0.72, and 1.42 μM for budesonide, prednisolone, methylprednisolone, and hydrocortisone, respectively; those of immunomodulators were 0.15, 1.77, 1.91, 0.03, and 0.77 μM for azathioprine, methotrexate, cyclosporine, tacrolimus, and 6-mercaptopurine, respectively; and those of aminosalicylates were 61.5, 1.40, and 60.1 μM for sulfasalazine, balsalazide, and 5-aminosalicylate, respectively.

The cells were treated with IBD medications for 48 h, and the ACP assay was used to assess the cell viability. Our results indicated that the cell viability from each drug remained >95% compared with the control group (**Figure 2**). Moreover, the expression of lipogenesis-related hepatic genes, such as *SREBP-1c, SCD, FAS, ACLY, ACC,* and *LXRα* were assessed using RT-PCR in differentiated HepaRG cells treated with IBD medications

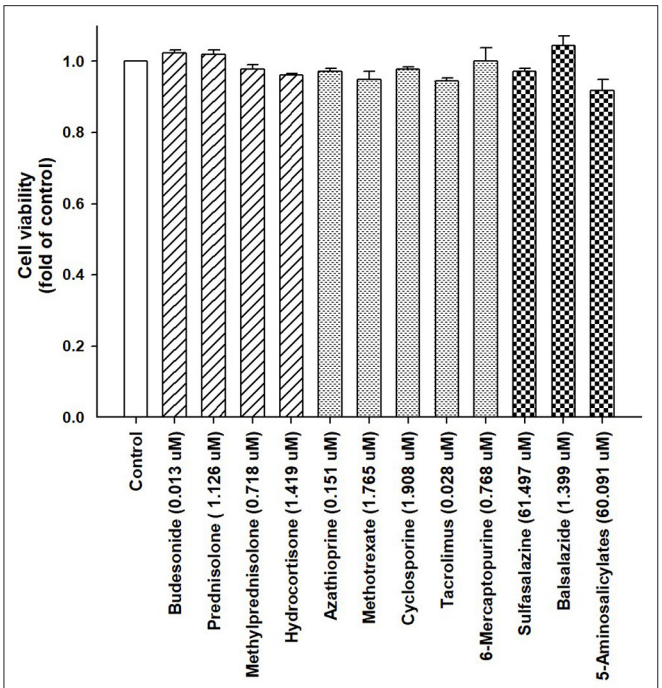

FIGURE 2 | Viability of HepaRG cells following exposure to IBD medications. HepaRG cells were exposed to corticosteroids (budesonide, prednisolone, methylprednisolone, and hydrocortisone), immunomodulators (azathioprine, methotrexate, cyclosporine, tacrolimus, and 6-mercaptopurine), and aminosalicylates (sulfasalazine, balsalazide, and 5-aminosalicylate) for 48 h. Cell viability was monitored by cellular acid phosphatase activity using *p*-nitrophenylphosphate as a substrate. The data are shown as the mean ± SE (error bars) ($n = 4$).

for 48 h. The total RNA was extracted, and gene expression was analyzed. *SREBP-1c*, a transcription factor that regulates lipid homeostasis by modulating the expression of a series of target lipogenic genes (33), is mainly distributed in the liver and participates in hepatic fatty acid synthesis by upregulating the expression of downstream genes. T0901317, a synthetic *LXRα* agonist, significantly induced the expression of these target genes. However, the expression levels of *SREBP-1c, SCD, FAS, ACLY, ACC,* and *LXRα* were significantly lower in most treatment groups than the untreated groups (**Figures 3A–F**). IBD medications reduce the risk of hyperlipidemia and partially reduce the expression of hepatic genes involved in lipogenesis, resulting in improved blood lipid profiles.

DISCUSSION

To the best of our knowledge, this was the first study to report the correlation of IBD medications and risk of hyperlipidemia in patients with IBD, particularly in Asian populations. This study assessed 23 million people living in Taiwan using the NHIRD (47). The results show that the risk of hyperlipidemia was 2.18 times higher in patients with IBD than the general population after adjusting for confounding factors and comorbidities ($P < 0.001$). Moreover, this exclusive, nationwide population-based cohort study presented a comprehensive and complete assessment of an Asian population and discovered a significant association between IBD, IBD medications, and hyperlipidemia. We also found that without IBD therapies (medications and surgical treatment), the risk of hyperlipidemia was higher in patients with IBD than in the control cohort (aHR, 2.20; 95% CI, 2.05–2.36, $P < 0.001$). Furthermore, with IBD therapies (medications and surgical treatment), the risk of hyperlipidemia decreased in patients with IBD compared to those without IBD (all aHR ≤ 0.99, not significant). In addition, among patients with IBD, the risk of hyperlipidemia significantly decreased in those that received IBD treatment compared to those that did not (all aHR ≤ 0.45, $P < 0.001$). Finally, IBD medications in hepatocyte-derived HepaRG cells significantly reduced the expression of lipogenesis-related genes, such as *SREBP-1c, SCD, FAS, ACLY, ACC,* and *LXRα*, contributing to improved blood lipid profiles. These findings provide valuable insights for clinical physicians regarding the benefits of IBD therapies for improving blood lipid profiles and CVD prognosis, as well as reducing the risk of CVD through blood lipid control. Our study clarifies the impact of IBD medications on the development of hyperlipidemia, and the decreased risk of hyperlipidemia in patients receiving treatment may be due to drug-induced downregulation of lipogenic genes, especially the master lipogenic transcription factors, *SREBP-1c* and *LXRα*.

Our analysis of 29,048 people (14,524 with IBD and 14,524 without) with a mean follow-up period >7 years showed an overall significant increase in risk of hyperlipidemia compared with the general population (aHR, 2.18). Moreover, males, patients >65 years of age, and those with comorbidities were at higher risk than those in the general population matched by age, sex, and comorbidities of T2DM, obesity, CAD, hypertension, and CKD. As such, these groups may require early and aggressive intervention for CVD risk management. Furthermore, a significant association was observed between hyperlipidemia and IBD; however, patients with IBD that received IBD therapy had a decreased risk of hyperlipidemia compared to those that did not receive therapy.

Several large meta-analyses and cohort studies have reported increased rates of CVD in patients with IBD. However, hyperlipidemia was less likely to be assessed among such patients, which could result in the silent progression of atherosclerosis and vascular defects. Moreover, acute myocardial infarction (AMI) was found to be higher in patients with IBD than in those without (OR, 12.05; 95% CI, 11.16–13.01), as was the rate of VTE (12, 18). A prospective study of 24 patients with IBD who had undergone surgical treatment, exhibited increased levels of HDL-C, TC, and LDL-C in patients in remission compared to those with recurrent active IBD (48). In another retrospective study, TC and LDL-C levels were significantly lower in patients with IBD after restorative proctocolectomy than in control subjects (49). These findings may be due to malabsorption from accelerated transit and the exclusion of the terminal ileum caused by the covering ileostomy (49). TC and HDL-C levels were significantly lower, TG levels were significantly higher in patients with IBD compared to controls were also reported (50). Ripollés Piquer

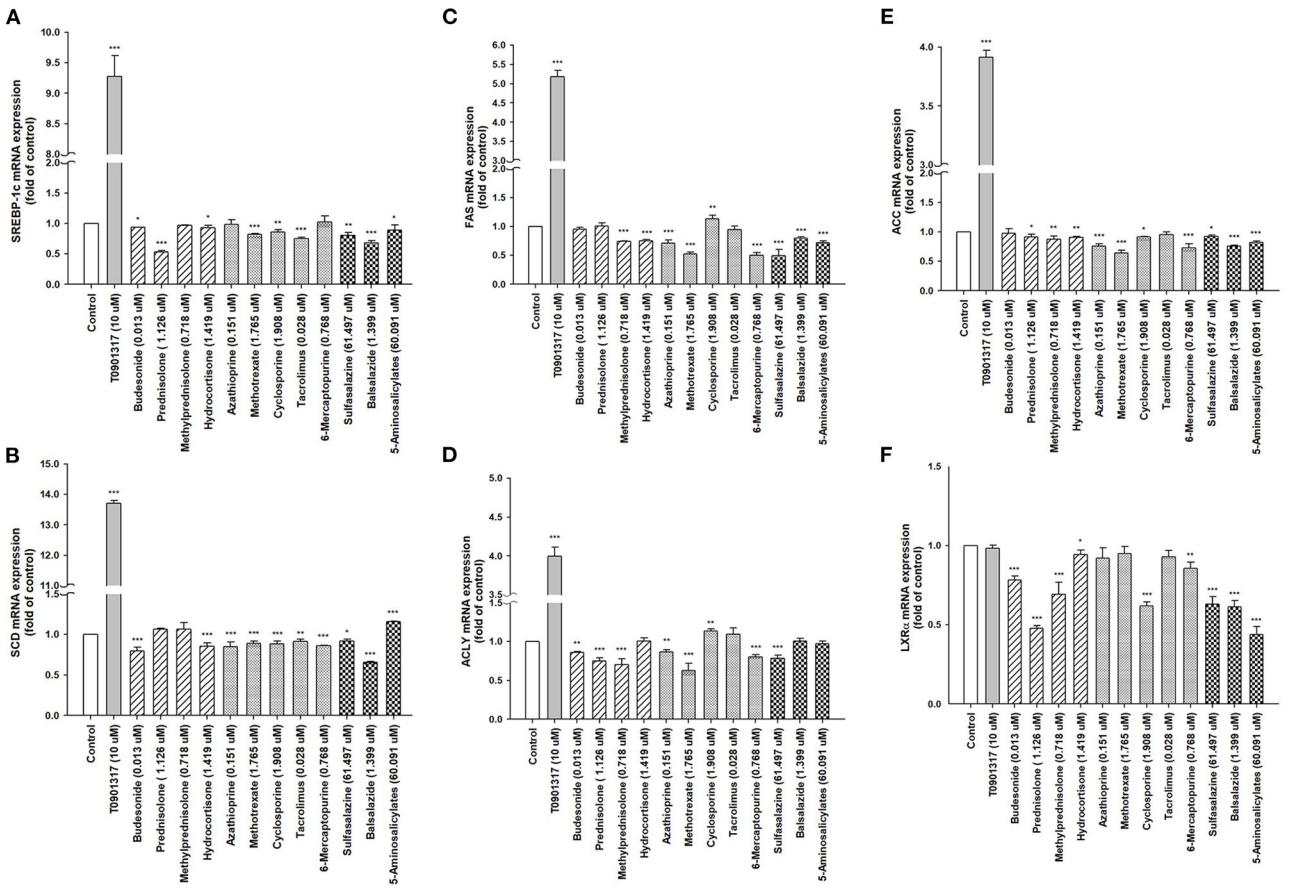

FIGURE 3 | Expression of hepatic lipid metabolism-related genes following treatment with T0901317 and IBD medications. Differentiated HepaRG cells were treated for 48 h with T0901317 (10 μM), corticosteroids (budesonide, prednisolone, methylprednisolone, and hydrocortisone), immunomodulators (azathioprine, methotrexate, cyclosporine, tacrolimus, and 6-mercaptopurine), and aminosalicylates (sulfasalazine, balsalazide, and 5-aminosalicylate). Following treatment, mRNA was extracted and the expression levels of **(A)** *SREBP-1c*; **(B)** *SCD*; **(C)** *FAS*; **(D)** *ACLY;* **(E)** *ACC*; and **(F)** *LXRα* were analyzed by qRT-PCR. Values were normalized to the expression of *β-actin*, with the levels of DMSO-treated cells set at 1. Results are expressed as the mean ± SE ($n = 3$). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with cells treated with DMSO.

et al. suggested that the levels they observed corresponded with those of atherogenesis and may contribute to the development of CVD (24). Nevertheless, more large-scale and epidemiological studies are warranted to evaluate lipid alterations in patients with IBD more in depth.

Lipid profile alterations in patients with IBD have been presented in previous studies. First, Becker and colleagues (51) showed an association of decreased TC levels and increased TG levels with intestinal resection in patients with CD in a study of 22 children with CD and 10 healthy controls. Moreover, a US study (22) concluded that patients with IBD have lower TC and HDL-C levels and higher LDL-C and TG levels compared to controls from the NHANES 2005–2006 database. Another report showed that patients with IBD have lower TC and LDL-C levels compared to control subjects due to systemic inflammation from reduced lipoprotein and hepatic lipase activity and decreased cholesterol absorption (52). The conflicting results of these studies may be partially explained by the differences in study population, methodology, data interpretation, duration of sampling, and baseline characteristics of the study samples (21, 22, 50). While our findings are not consistent with all studies, we performed an in-depth analysis of different treatment modalities over a prolonger follow-up period. Moreover, our study was based on a nationwide population database rather than a single institution.

No study has previously reported the correlation of IBD therapies with hyperlipidemia. We found that IBD treatments can prevent patients from developing hyperlipidemia. Thus, while IBD can neither be prevented nor cured, hyperlipidemia can be partially prevented by appropriate interventions such as diet control, regular exercise, and reduced body weight. In view of hyperlipidemia-related CVD, physicians who participate in the long-term care of patients with IBD should consider aggressive modification of risk factors associated with hyperlipidemia. Finally, we conclude that the association of IBD and hyperlipidemia remains to be explored due to conflicting results of each study.

The association between IBD and the increased risk of CVD has been previously reported in a systemic review. The review observed 6,478 coronary events in 123,907 patients with IBD and found a 19% higher risk of CVD in those with IBD (53). Additionally, patients with IBD exhibited a higher risk of carotid atherosclerotic plaque, warranting evaluation of carotid intima-media thickness through ultrasonography for this high-risk subpopulation. Moreover, Kristensen et al. (13) found that compared with controls, patients with IBD presented an increased risk of stroke [relative risk (RR), 1.15; 95% CI, 1.05–1.27] and hospitalization due to heart failure (RR, 1.37; 95% CI, 1.26–1.49) (13). Since, hyperlipidemia is a major risk factor for stroke, which is a leading cause of death (54, 55), may play a role in this situation. In addition, a case-control study reported an increased risk of stroke in patients with IBD (aOR, 2.93; 95% CI, 1.44–5.98) especially in those younger than 50 years of age (56). Thus, the periodic and continuous monitoring of CVD risk in this population is necessary. However, no specific guidelines exist for patients with IBD regarding the prevention and management of CVD (9, 53). Although the current American Heart Association/American College of Cardiology guidelines on blood cholesterol management showed that chronic inflammatory disorders increase the risk of CVD, patients with IBD have not been specifically assessed (57).

Our findings show that patients with IBD were likely to have more comorbidities. For example, several pathophysiological factors are involved in the development of hyperlipidemia in these patients and may increase the risk of CVD. However, lipid profile monitoring in IBD patients is not routinely performed in Taiwan. Additionally, awareness of hyperlipidemia is lower than that of diabetes and hypertension; therefore, hyperlipidemia is diagnosed only when physical complications, such as CVD accompanied by chest pain (58). Thus, hyperlipidemia is rarely managed in patients with IBD. We suggest that clinicians perform blood lipid profiling for patients with IBD due to their increased risk of hyperlipidemia to provide suitable treatment and complete medical care.

Although a previous report showed that body mass index and blood lipid levels are often low in patients with IBD due to poor absorption of bile during active inflammation and after surgery (59). Our data showed that obesity is pronounced in these patients, which may further increase the risk of hyperlipidemia. Another report revealed that the inflammation-based increase of TGs is caused by increased hepatic lipoprotein production and decreased lipoprotein clearance. TGs are mediated by apolipoprotein C-III, which is a main risk factor for atherosclerogenesis (60). Several studies have indicated that chronic inflammation is associated with altered blood lipid profiles and CVD and generates inflammatory cytokines such as *TNF*α, reduces *NOS* expression, decreases NO bioavailability, impairs endothelium vasodilation, and increases cell adhesion molecules and stimulating leukocyte migration, therefore promoting atherosclerosis (61). Inflammatory cytokines, such as *TNF*, *IL-6,* and *IFN-*γ may downregulate lipolytic enzyme activity (8); however, the pathophysiological activities in IBD are more complex because of the chronic inflammation, malnutrition, and lipid malabsorption due to intestinal damage or resection (25).

Previous study found that low TC and LDL-C levels were correlated with systemic inflammation and CRP levels in IBD (52). Meanwhile, one study that observed a significant association of low TC and high TGs levels with disease severity failed to present any association with increased levels of inflammatory biomarkers (CRP, ESR, and albumin) (21). They reported increased risk of CVD in patients with IBD along with lower TC levels and chronic inflammation reflects a complicated and poorly understood pathogenic mechanism, described as a "lipid paradox" or dyslipidemia. A better understanding of dyslipidemia in IBD will help prevent and manage atherosclerosis and CVD.

We found that, each group of patients that received IBD medications showed a significant decrease in risk of hyperlipidemia (all aHR $<$ 0.46) after adjusting for age, sex, and comorbidities with T2DM, obesity, CAD, hypertension, and CKD. Whether IBD medications play an important role in reducing the risk of hyperlipidemia and CVD had not been previously elucidated. Moreover, although it has been reported that corticosteroids may increase the risk of atherosclerosis and acute coronary syndrome resulting from hypertension, hyperlipidemia, and thromboembolism (11, 26), they overall reduce the risk of CVD through inhibition of inflammatory response. In the current study, we also found that corticosteroids may inhibit hepatic lipogenic gene expression. Thus, the contribution of corticosteroids to the reduced risk of CVD remains controversial. Nevertheless, methotrexate has been reported to rarely induce steatohepatitis associated with acute coronary syndrome and thromboembolic events (62, 63). We found that among the immunomodulators, methotrexate and 6-mercaptopurine inhibited the expression of lipogenic genes. Moreover, cyclosporine was associated with increased risk of acute coronary syndrome and heart failure (64). In addition, 5-aminosalicylates, a standard first-line treatment for IBD, may be used to prevent CVD and VTE in patients with IBD (14, 65, 66) due to their anti-inflammatory effects and ability to inactivate platelets. Moreover, we found that most 5-aminosalicylates were shown to inhibit lipogenic gene expression.

Increased carotid intimal thickness, wall stiffness, and endothelial dysfunction observed in patients with IBD may be due to the increase in circulating inflammatory cytokines and CRP (67). IBD medications are beneficial for their dual effects of decreasing the expression of lipogenic genes and treating IBD symptoms; however, the net effects of IBD medications on the lipid profile in terms of CVD risk remain uncertain. Thus, the metabolic profiles of patients with IBD should be considered when making therapeutic decisions. Nevertheless, prospective studies are needed to evaluate the dual effects of IBD medications in the general IBD population.

We performed a longitudinal population-based cohort study with cases and controls matched for sex and age to explore the possible correlation of hyperlipidemia and IBD in Taiwan. We also evaluated the effect of IBD medications on the risk of developing hyperlipidemia. Nevertheless, our study had several limitations. First, the database we used may have misclassified IBD and hyperlipidemia, limiting the reliability and validity. However, in Taiwan, universal health insurance is distributed, and as a result, a peer review system is enacted by specialists

to reduce the possibilities of false positives. Second, several potential risk factors of hyperlipidemia, such as smoking habits, alcohol intake, body mass index, lifestyle and dietary habits, and family history of hyperlipidemia were not included in this study as they are not provided in the NHIRD. In addition, the risk of hyperlipidemia according to the severity of IBD could not be estimated, as the database had no information regarding the severity of IBD, such as Harvey-Bradshaw Index and Mayo score. Third, this claim database was initially created for charging purposes, and as some information was anonymized, we could not contact the patients directly to get individual data from them. Fourth, several clinical laboratory data were not included in this database, and as such, we could not assess the degree of hyperlipidemia in patients with IBD. For example, serum TC, TGs, HDL-C, and LDL-C. Cases of hyperlipidemia were defined indirectly according to the physicians' records based on lipid levels under IBD medication therapy. In this study, both IBD and hyperlipidemia were accurately analyzed and coded (ICD-9-CM codes) by specialists according to the standard symptomatic criteria by considering the normal side effects, signs, research facility information, and imaging findings. Additionally, this study reduced the confounding effect of medications by adjusting for comorbidities. However, more information should be obtained from other databases to conduct a comprehensive prospective study or randomized controlled trial to further investigate the relationships of IBD, IBD medications, and hyperlipidemia. Most crucially, evidence derived from a retrospective cohort study is typically lower in statistical quality because of numerous sources of inherent bias, including the classification bias. However, the NHI program has a high coverage rate, and medical reimbursement specialists and peer reviewers scrutinized all insurance claims, ensuring that the diagnoses and coding of diseases were highly reliable in the NHIRD. The classification bias was also supposed to be non-differential, and this should not invalidate our result. Primary human hepatocytes (PHHs) are a well defined *in vitro* hepatic model to predict drug responses with respect to metabolism and toxicity, based on the proper maintenance of metabolism, transport, and biological signaling pathways. However, the significant variability among individuals and high cost of PHHs has led to the development of alternative cell line models. Thus, HepaRG cells have emerged as a promising alternative to PHHs as an *in vitro* model, as this cell model, upon reaching phenotypic maturity, can grow to confluence and differentiate over 4 weeks (from progenitor cells) into cocultures of hepatocyte-like and cholangiocyte-like cells (68). However, it must be kept in mind that cell lines do not behave identically to primary cells and do not necessarily completely replace and reflect the effects and outcomes of primary cells.

This study had several strengths. First, we used a nationwide, population-based cohort of patients with anonymized data to minimize selection bias. Additionally, we evaluated the effect of IBD and IBD medications on the risk of hyperlipidemia over a prolonged follow-up period. Moreover, by adjusting for age and sex in a 1:1 ratio, we accounted for confounders that may affect the occurrence of hyperlipidemia. Finally, although detection bias may have occurred if patients had more hospital visits than the control population by increasing the possibility of detecting hyperlipidemia, the risk of hyperlipidemia was still increased 2.2-fold in patients with $\geq$2 hospital visits per year. Overall, this is the first study to investigate the IBD-associated risk for developing hyperlipidemia and evaluate the effects of IBD medications on lipogenic gene expression.

CONCLUSION

In conclusion, patients with IBD have a significantly increased risk of developing hyperlipidemia compared to non-IBD controls. However, under all IBD medications and surgical treatment, patients with IBD experienced a reduced risk of hyperlipidemia compared with non-treated IBD patients. We further confirmed that the effects of IBD medications on the decreased expression of lipogenic-related genes contributed to the beneficial effects on the blood lipid profiles. Therefore, regular monitoring of blood lipid levels should be considered for the early detection of hyperlipidemia in patients with IBD, especially in elderly patients, to decrease the risk of CVD. Large, population-based studies or randomized clinical trials are warranted to confirm our observation that IBD and IBD medications play an important role in the development of hyperlipidemia. In addition, early detection, monitoring, aggressive, and comprehensive treatment for metabolic disturbances, and alleviation of acquired risk factors are highly recommended. Further investigations into the possible biological and pathological mechanisms underlying the relationship between hyperlipidemia and the use of IBD medications are essential.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The NHIRD encrypts patient personal information to protect privacy and provides researchers with anonymous identification numbers associated with relevant claims information, including sex, date of birth, medical services received, and prescriptions. Therefore, patient consent is not required to access the NHIRD. This study was approved to fulfill the condition for exemption by the Institutional Review Board (IRB) of China Medical University (CMUH-104-REC2-115-R5). The IRB also specifically waived the consent requirement.

AUTHOR CONTRIBUTIONS

NT, T-YW, Y-JF, and Y-PL: conceptualization, methodology, investigation, supervision, and funding acquisition. C-LL, C-JW, and C-YH: software and formal analysis. NT and Y-PL: validation. C-LL, C-YH, and Y-PL: resources and data curation. C-YH and Y-PL: project administration. All authors: writing—original draft preparation, writing—review and editing, and

visualization. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Ministry of Science and Technology, Taiwan, R.O.C. (MOST110-2320-B-039-016-MY3), China Medical University, Taichung, Taiwan (CMU110-MF-29), China Medical University Hospital, Taichung, Taiwan (DMR-111-105; DMR-111-228), partially supported by the Taiwan Ministry of Health and Welfare Clinical Trial Center (MOHW110-TDU-B-212-124004), Taichung Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (TTCRD111-31), Show Chwan Memorial Hospital, Changhua, Taiwan (SRD-110027).

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ORIGINAL RESEARCH
published: 04 July 2022
doi: 10.3389/fmed.2022.933996

Immunogenicity of BNT162b2 Vaccine Booster Dose in Patients With Inflammatory Bowel Disease Receiving Infliximab Combination Therapy: A Prospective Observational Study

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Introduction: Few data exist regarding the immunogenicity of the third dose of BNT162b2 relative to the second dose in patients with inflammatory bowel disease (IBD) on different immunosuppressive therapies. We investigated the immunogenicity of BNT162b2 vaccine booster dose in patients with IBD on infliximab combination therapy.

Method: This is a prospective single-center observational study conducted from January 1, 2022 to February 28, 2022. Patients were recruited at the time of attendance at the infusion center. Eligibility criteria included patients with a confirmed diagnosis of IBD who are receiving infliximab with azathioprine or 6-mercaptopurine. Patients who received two doses of BNT162b2 vaccine (second dose group) were compared to patients who had received three doses of BNT162b2 vaccine [third dose (booster) group]. Patients were excluded if they were infected or had symptoms of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) previously since the start of the pandemic or received other vaccines than the BNT162b2. Our primary outcome was the concentrations of SARS-CoV-2 antibodies Immunoglobulin G (IgG) and neutralizing antibodies 40–45 weeks from the first dose of BNT162b2 vaccine in patients with IBD receiving infliximab combination therapy. Medians with interquartile range (IQR) were calculated.

Results: In total, 162 patients with IBD and receiving infliximab combination therapy were recruited, and the number of patients in both the second dose group and third dose (booster) group was 81. Mean age was 35 years old in both groups. Median (IQR) SARS-CoV-2 IgG levels were significantly lower after the second dose [125 BAU/ml (43, 192)] compared to patients who received the third booster dose [207 BAU/ml (181, 234)]

OPEN ACCESS

Edited by:
Antonietta G. Gravina, University of Campania Luigi Vanvitelli, Italy

Reviewed by:
Jin-Wen Song, Fifth Medical Center of the PLA General Hospital, China
Polona Žigon, University Medical Centre Ljubljana, Slovenia

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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *01 May 2022*
Accepted: *07 June 2022*
Published: *04 July 2022*

Citation:
Shehab M, Alrashed F, Alfadhli A, Alsayegh A, Aldallal U, Alsayegh M, Cherian P, Alkhair I, Thanaraj TA, Channanath A, Dashti AA, Albanaw A, Ali H, Abu-Farha M, Abubaker J and Al-Mulla F (2022) Immunogenicity of BNT162b2 Vaccine Booster Dose in Patients With Inflammatory Bowel Disease Receiving Infliximab Combination Therapy: A Prospective Observational Study. Front. Med. 9:933996. doi: 10.3389/fmed.2022.933996

($P = 0.003$). Neutralizing antibody levels were also lower after the second dose [80% (21, 95)] compared to patients who received the third booster dose [96% (93, 99)] ($P \leq 0.001$). The percentage of patients who achieved positive SARS-CoV-2 IgG levels in the third (booster) dose group was 96.3%, whereas it was 86.4% in the second dose group. The percentage of participants who received the third (booster) dose and achieved a positive SARS-CoV-2-neutralizing antibody level was 100%, whereas it was 88.9% in the participants who received the second dose only.

Conclusion: Most patients with IBD on infliximab combination therapy had positive SARS-CoV-2 IgG and neutralizing antibody concentrations 40–45 weeks post BNT162b2 vaccination. However, SARS-CoV-2 IgG and neutralizing antibody concentrations were lower in patients who received two doses only compared to patients who received a third dose. A longer follow-up study is needed to evaluate decay in antibodies over time.

Keywords: IBD, vaccine, infliximab, COVID-19, immunogenicity, booster

INTRODUCTION

Coronavirus was first identified to have infected people in the mid-1960s. Since then, multiple subgroupings have emerged. Recent and most notable of which have been severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) originating from Wuhan, China (1, 2). SARS-CoV-2 is particularly dangerous in part due to its heterogeneous symptoms resulting in anything between diarrhea to severe respiratory distress and its high infectious rate. The transmission of the virus occurs *via* the presence of angiotensin-converting enzyme 2 (ACE2) receptors, present on the epithelial type II cells in the lungs and the brush border of gut enterocytes. Here, the virus can then utilize these receptors to access the host's tissue resulting in infection (3). As a result of its rapid spread, SARS-CoV-2, as of May 23, 2022, has infected 525,467,084 confirmed cases, of which 6,285,171 resulted in deaths (4). Despite new variants like Delta and Omicron continuing to cause a global surge in cases, the advent of vaccines, such as boosters, brings confidence that the spread will be curbed.

Global efforts led to the development of highly effective COVID-19 vaccines, with early findings reporting 95% efficacy of mRNA vaccine BNT162b2 against COVID-19 (5, 6). Since then; however, variants sprouted and immunity dwindled. This resulted in the reported efficacy of BNT162b2 against Omicron dropping to 34–37% after 15 weeks of the second dose. Thankfully, the arrival of boosters for BNT162b2 primary course recipients helped push COVID-19 vaccine efficacy back over 70%, making it especially important for the immunocompromised patients such as those with inflammatory bowel disease (IBD) (7).

Inflammatory bowel disease is a chronic immune-mediated inflammatory process often distinguished between two subtypes, Crohn's disease (CD) and Ulcerative Colitis (UC), impacting millions of patients. IBD is often treated using immunosuppressive drugs like corticosteroids, tumor necrosis factor (TNF) inhibitors, thiopurines, and Janus kinase inhibitors, raising concerns over the risk of patient complications from infectious sources. This is exceptionally significant today, as recent evidence suggests that corticosteroid and 5-aminosalicylic acid (5-ASA) use is associated with more severe COVID-19 outcomes such as hospitalization and death (8–11). This can be explained due to a blockage of intracellular signals needed for the host to fight pathogens with patients on immunosuppressive drugs (12, 13). Furthermore, early findings in patients receiving biological treatments such as infliximab combination therapy demonstrated lower SARS-CoV-2 IgG, IgA, and neutralizing antibody levels after BNT162b2 vaccination compared with healthy participants (14). In addition, a previous study demonstrated that the immunogenicity of COVID-19 vaccines varies according to the immunosuppressive drug exposure and is attenuated in recipients of infliximab, infliximab plus thiopurines, and tofacitinib (15). All things considered, most patients displayed significant immunogenicity after two doses of the vaccines, illustrating the potential greater benefits of a third dose (16). Thus, this study aims to assess the immunogenicity of the second dose compared to the third (booster) dose of BNT162b2 vaccine in patients with IBD receiving infliximab with azathioprine or 6-mercaptopurine (infliximab combination).

MATERIALS AND METHODS

A prospective single-center observational study was conducted at a tertiary care IBD center, Mubarak Al-Kabeer Hospital. Patients were recruited at the time of attendance at the infusion center from January 1, 2022, to February 28, 2022. Patients who received two doses of BNT162b2 vaccine (second dose group) were compared to patients who had received three doses of BNT162b2 vaccine [third dose (booster) group]. This study was performed and reported in accordance with Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (17). The international classification of diseases (ICD-10 version: 2016) was used to diagnose IBD. Patients were considered to

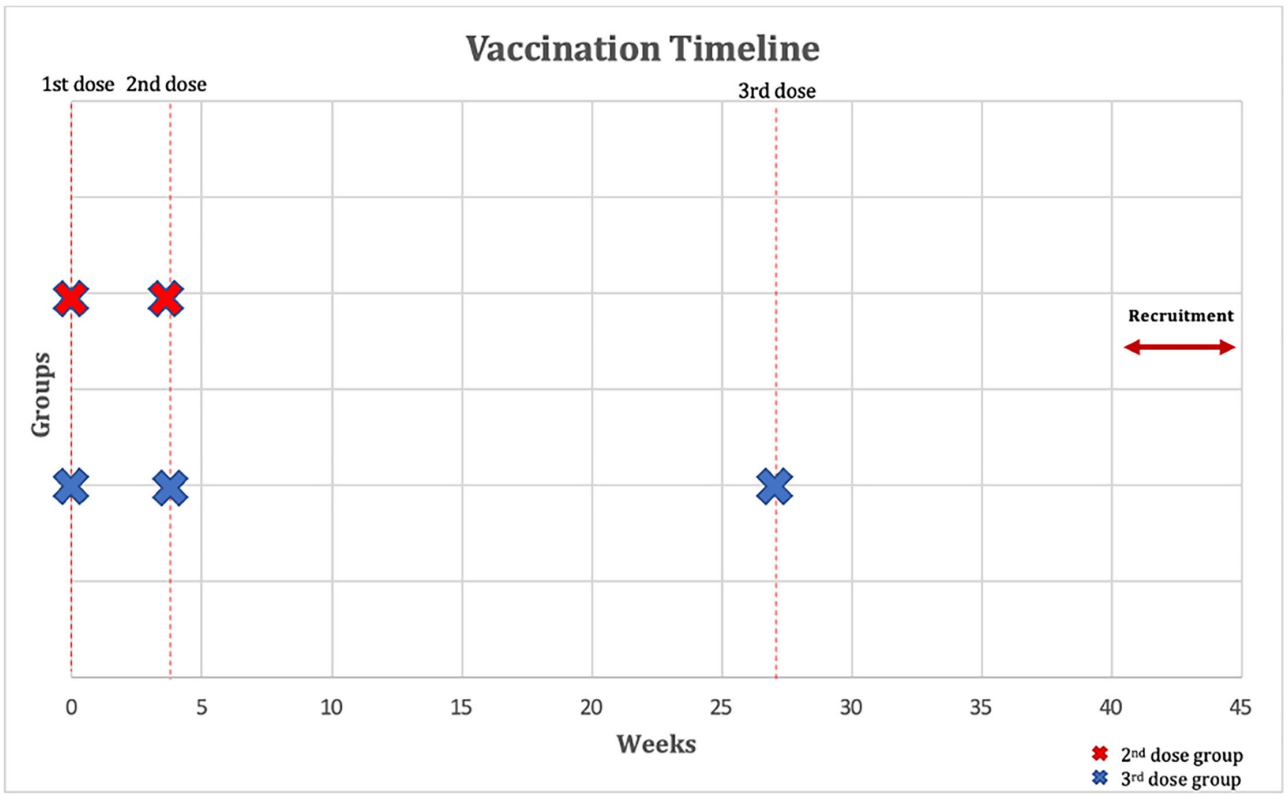

FIGURE 1 | Outline of vaccination timeline and time when a serological response was assessed. The figure shows that participants have received two-dose of COVID-19 vaccination with BNT162b2 vaccine 3 weeks apart and that the third (booster) dose group have received a third dose with BNT162b2 vaccine 24 weeks after the second dose. Immunoglobulin G (IgG) and neutralizing antibodies were measured 40–45 weeks after their first dose of BNT162b2.

have IBD when they had ICD-10 K50, K50.1, K50.8, K50.9 corresponding to CD and ICD-10 K51, k51.0, k51.2, k51.3, k51.5, k51.8, k51.9 corresponding to UC (18).

Patients were eligible to be included if they: (1) had a confirmed diagnosis of IBD before the start of the study; (2) were receiving infliximab with azathioprine or 6-mercaptopurine for at least 6 weeks or more for the induction of remission or with at least one dose of drug received for the maintenance of remission in the previous 8 weeks before the first dose of vaccination; (3) Second dose group: have received two-dose of COVID-19 vaccination with BNT162b2 vaccine, 3 weeks apart. Third dose (booster) group: have received two-dose of COVID-19 vaccination with BNT162b2 vaccine, 3 weeks apart and a third (booster) dose 24 weeks after the second dose (**Figure 1**); (4) were at least 18 years of age or older. SARS-CoV-2 PCR was performed within 72 h of each vaccine dose, and if positive, patients were excluded. Patients were also excluded if they were infected or had symptoms of SARS-CoV-2 previously since the start of the pandemic. In addition, patients who received other vaccines than the BNT162b2 were excluded. Patients who received corticosteroids 2 weeks before the first dose of the vaccine up to the time of recruitment were also excluded. Finally, patients taking other immunomodulators such as methotrexate were also excluded.

Outcome Measures

Our primary outcome was the concentrations of SARS-CoV-2 antibodies, including Immunoglobulin G (IgG) and neutralizing antibodies 40–45 weeks after their first dose of BNT162b2 in patients with IBD receiving infliximab combination therapy. Data regarding the type and extent of IBD as well as duration of infliximab combination therapy were also recorded.

Laboratory Methods

Enzyme-linked immunosorbent assay (ELISA) kit (SERION ELISA agile SARS-CoV-2 IgG and IgA SERION Diagnostics, Würzburg, Germany) was used to measure plasma levels of SARS-CoV-2-specific IgG antibodies based on the manufacturer's protocol. Units of IgG levels were reported as binding antibody units (BAU)/ml. Values below 31.5 BAU/ml were considered negative or non-protective.

The SARS-CoV-2-Specific Neutralizing Antibodies levels in the plasma were quantified using a SARS-CoV-2-specific surrogate Virus Neutralization Test (sVNT) (SARS-CoV-2 sVNT kit, GenScript, USA, Inc.) based on the manufacturer's protocol. Determination of positive and negative thresholds was performed by following the manufacturer's recommendations. The results were interpreted by calculating inhibition rates

TABLE 1 | Baseline characteristics of participants.

Characteristic	Second dose, *N* = 81	Booster dose, *N* = 81
Mean Age (years)	35.2	35.6
Gender *n* (%)		
Female	38 (47%)	36 (45%)
Male	43 (53%)	45 (55%)
BMI (median)	24.7	25.8
Smoking *n* (%)	19 (23.0%)	17 (21.0%)
Co-morbidities *n* (%)		
Diabetes	3 (3.7%)	3 (3.6%)
Hypertension	7 (8.6%)	6 (7.1%)
Heart disease	5 (6.1%)	6 (7.1%)
Arthritis or any autoimmune disease	8 (9.8%)	9 (11%)
COPD	1 (1.2%)	1 (1.2%)
Kidney disease	2 (2.4%)	0 (0%)
Asthma	2 (2.5%)	1 (3.6%)
Hyperlipidemia	9 (11%)	9 (11%)
Duration of infliximab combination therapy (median, months)	12	13
Disease extent *n* (%)		
Ulcerative colitis (UC)	33 (41%)	36 (44%)
E1: ulcerative proctitis	5 (16%)	6 (18%)
E2: left sided colitis	11 (32%)	12 (33%)
E3: extensive colitis	17 (52%)	18 (49%)
Crohn's disease (CD)	48 (59%)	45 (56%)
L1: ileal	26 (54%)	23 (51%)
L2: colonic	5 (10%)	5 (11%)
L3: ileocolonic	17 (36%)	15 (34%)
L4: upper gastrointestinal	0 (0%)	2 (4%)
B1: inflammatory	21 (44%)	19 (43%)
B2: stricturing	13 (28%)	12 (27%)
B3: penetrating	14 (30%)	14 (30%)
Lab parameters		
CRP, mg/L (median)	6.3	6.2
Albumin, g/L (median)	40	42
ESR, mm/h	11	9
Stool fecal calprotectin, μg/g (median)	114	112

for samples per the following equation: Inhibition = (1– sample O.D/O.D negative control) × 100%. Neutralizing antibody levels below 20% were considered negative or non-protective.

Ethical Consideration

This study was reviewed and approved by the Ethical Review Board of Dasman Institute "Protocol # RA HM-2021-008" as per the updated guidelines of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and of the US Federal Policy for the Protection of Human Subjects. The study was also approved by the Ministry of Health of Kuwait (reference: 3799, protocol number 1729/2021). Subsequently, patient informed written consent was obtained before inclusion in the study.

Statistics

We performed descriptive statistics to characterize both the second and third (booster) dose groups. The standard descriptive statistics were used to present the demographic characteristics of patients included in this study and their measured antibody levels. Analysis was conducted in R (19). Data are expressed as medians with interquartile range (IQR) unless otherwise indicated. Categorical variables were compared using the Fisher's exact test or Pearson's chi-squared test. The unpaired and paired continuous variables were compared with the Wilcoxon rank-sum test and Wilcoxon signed-rank exact test, respectively. A *P*-value of $\leq$0.05 is considered statistically significant.

Both groups were matched for age, sex, and time-since-first vaccine-dose using the optimal pair matching method. The technique attempts to choose matches that collectively optimize an overall criterion. The criterion used was the sum of the absolute pair distances in the matched sample. In addition, the percentage of positive IgG and neutralizing antibody levels was calculated in both groups. The χ^2 tests were used to assess whether the percentages of positive antibodies differed across categories of both groups.

RESULTS

Baseline Cohort Characteristics

Patients were recruited between January 1, 2022 and February 28, 2022. In total, 162 patients were recruited, and serology assays were performed to quantify SARS-CoV-2 antibody levels for all patients. The number of patients included in both the second dose group and third (booster) dose group was 81. The mean age was 35 years old in both groups, and body mass index (BMI) was lower in the second dose group compared to the third dose group (25.8 vs. 24.7 kg/m^2). In both groups, most patients had CD (>56%), and more than 20% of patients were smokers. The mean duration between the serology test and second dose of vaccine was 40 weeks, and the mean duration between the serology test and third (booster dose) was 16 weeks. The median duration of infliximab combination therapy was 12 months (see **Table 1**).

Outcomes

Median (IQR) SARS-CoV-2 IgG level was significantly lower in patients treated with infliximab combination therapy after the second dose [125 BAU/ml (43, 192)] compared to patients who received the third booster dose [207 BAU/ml (181, 234)] following vaccination with BNT162b2 ($P = 0.003$) (see **Figure 2**). Neutralizing antibody levels were also lower in patients who were treated with infliximab combination therapy after the second dose [80% (21, 95)] compared to patients who received the third booster dose [96% (93, 99)] following vaccination with BNT162b2 ($P \leq 0.001$) (see **Table 2** and **Figure 3**).

The percentage of patients who achieved positive SARS-CoV-2 IgG levels in patients who received the third (booster) dose was 96.3% (78 out of 81), whereas

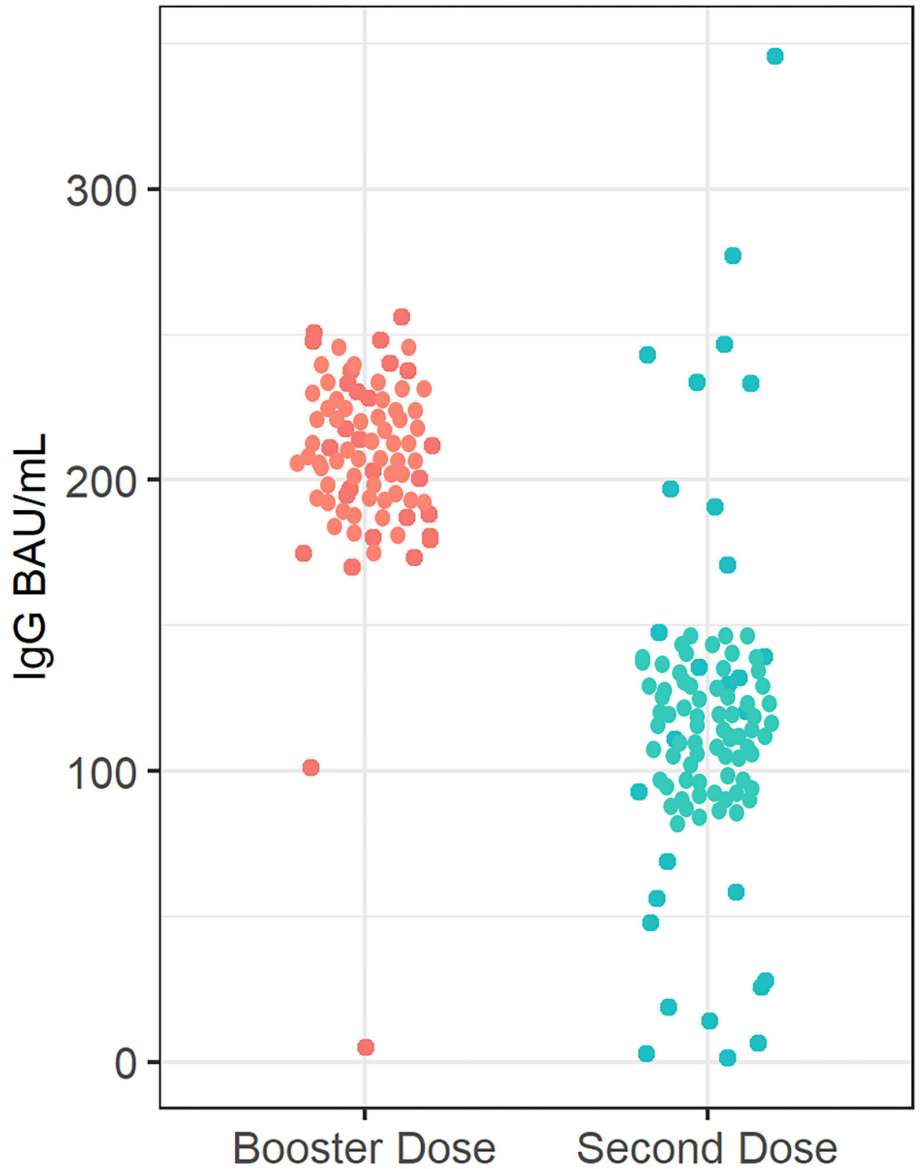

FIGURE 2 | Anti-SARS-CoV-2 -IgG antibody concentrations in patients receiving infliximab combination therapy after the second and third (booster) dose. The median anti-SARS-CoV-2 IgG concentration after receiving the second dose was 125 BAU/ml, whereas the median anti-SARS-CoV-2 -IgG antibody concentration was 207 BAU/ml after receiving the third (booster) dose.

TABLE 2 | Antibody responses in patients receiving infliximab combination therapy after third (booster) dose vs. second dose.

Type of antibody test	Second dose, $N = 81^a$	Booster dose, $N = 81^a$	*P*-value[b]
IgG BAU/ml	125 (43, 192)	207 (181, 234)	0.003
Neutralizing antibody (%)	80 (21, 95)	96 (93, 99)	<0.001

[a] *Median (IQR).*
[b] *Wilcoxon signed-rank exact test; random intercept logistic regression.*

the percentage of patients with positive SARS-CoV-2 IgG levels (>31.5 BAU/ml) in patients who received the second dose only was 86.4% (70 out 81) $P = 0.026$. The percentage of participants who received the third (booster) dose and achieved a positive SARS-CoV-2-neutralizing antibody level was 100%, and the percentage of patients was 88.9% (72 out 81) of the participants who received the second dose only ($P = 0.009$). Finally, four patients had 0 neutralizing antibody levels after the second dose (see **Figure 4**).

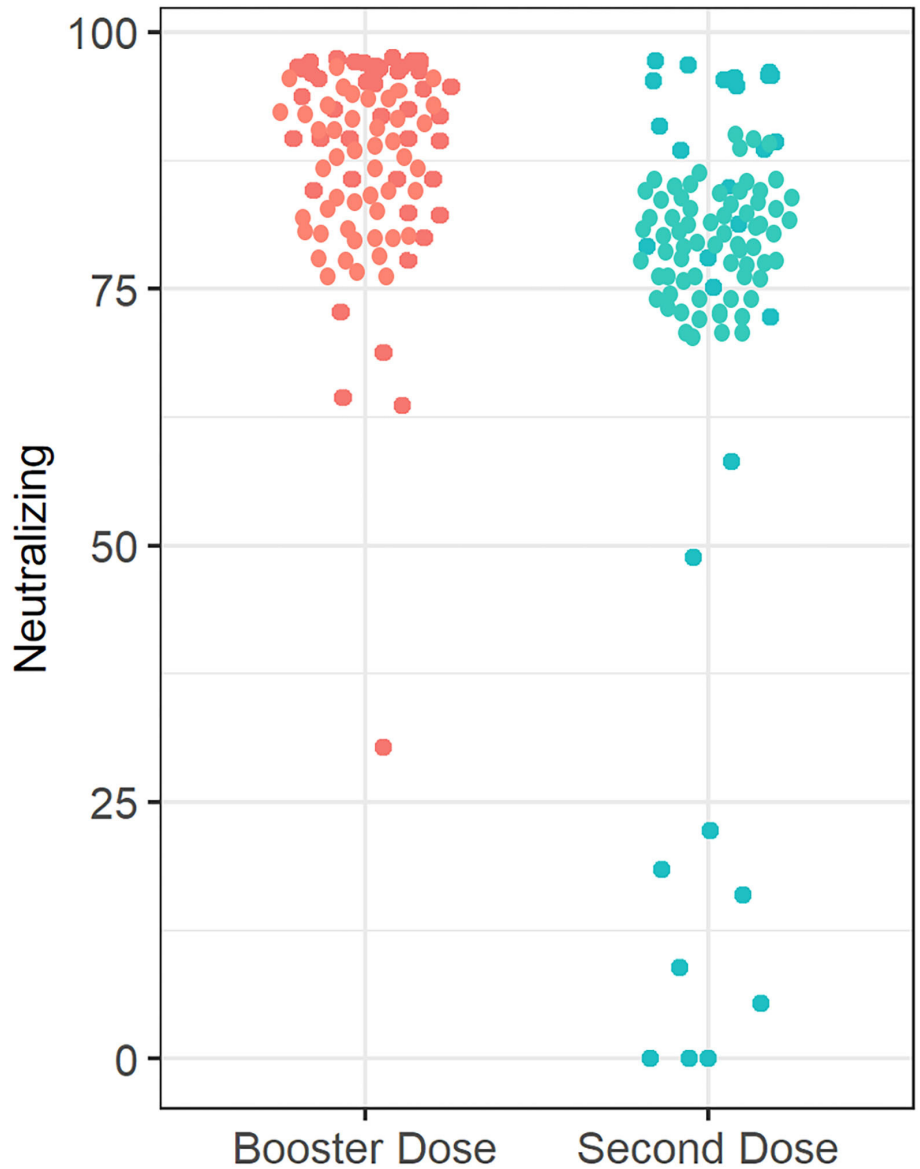

FIGURE 3 | Anti-SARS-CoV-2 neutralizing antibody concentrations in patients receiving infliximab combination therapy after the second and third (booster) dose. The median anti-SARS-CoV-2-neutralizing antibody concentration after receiving the second dose is 80%, whereas the median neutralizing antibody concentration was 96% after receiving the third (booster) dose.

DISCUSSION

In this study, we found that most patients with IBD on infliximab combination therapy had positive SARS-CoV-2 IgG and neutralizing antibody concentrations 40–45 weeks post BNT162b2 vaccination regardless of whether patients received a booster dose or not. However, SARS-CoV-2 IgG and neutralizing antibody concentrations were lower in patients who received two doses only compared to patients who received the third (booster) dose. Our study highlighted the importance of the booster dose in the IBD population.

A study by Levin et al. (20) found that 6 months after receiving the second dose of the BNT162b2 vaccine, the humoral response was substantially decreased among persons 65 years of age or older and persons with immunosuppression. Levin et al. also showed that SARS-CoV2 IgG and neutralizing antibody levels were lower in older men and participants with immunosuppression.

Another important finding of our study is that the percentage of participants who received the third (booster) dose and achieved positive SARS-CoV-2-neutralizing antibody level was significantly higher at 100%, relative to the percentage of patients who received the second dose only (88.9%). A study by Bergwerk

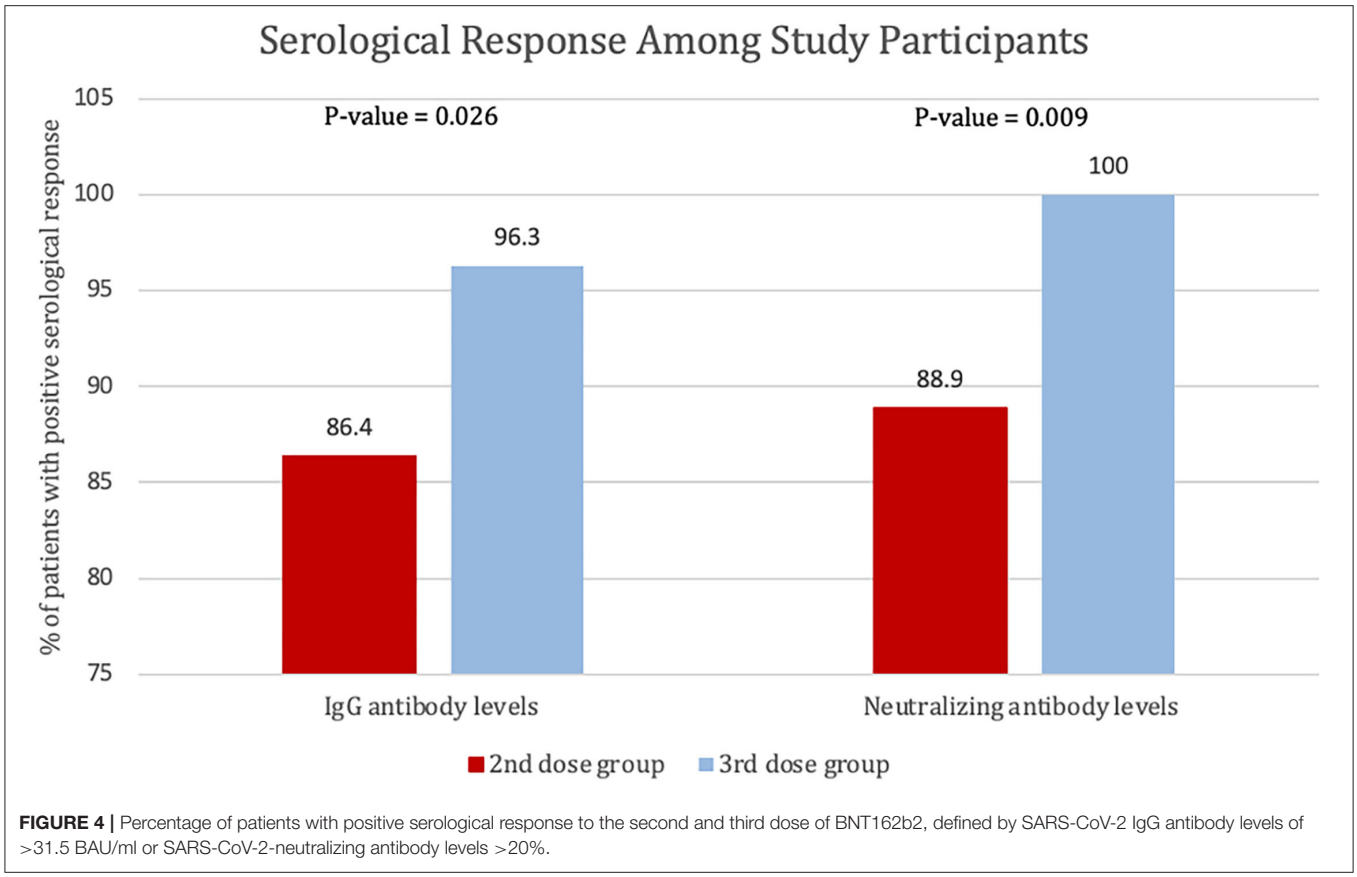

FIGURE 4 | Percentage of patients with positive serological response to the second and third dose of BNT162b2, defined by SARS-CoV-2 IgG antibody levels of >31.5 BAU/ml or SARS-CoV-2-neutralizing antibody levels >20%.

et al. (21) recently reported a correlation between neutralizing antibody titers and infectivity. Similar to our study, Deepak et al. (22) found that 88.7% (118 of 133) of patients with chronic inflammatory diseases such as IBD and rheumatoid arthritis treated with immunosuppressive medications achieved positive anti-SARS-CoV-2 IgG levels after two doses of the BNT162b2 or mRNA-1273 vaccine; however, in many cases, antibody levels were lower than in immunocompetent participants. Another study looked at the SARS-CoV-2 neutralization antibody levels after the second-vaccine dose. Similarly, the study found that 85% of infliximab-treated patients and 86% vedolizumab-treated patients achieved positive neutralizing antibody levels (23). These studies suggested that although the specific threshold of antibody levels that can confer protection against breakthrough infection is still unclear, neutralizing antibodies may still be used to determine the efficacy and protection of vaccines.

The effect of TNF antagonists on serological responses of COVID-19 vaccination is becoming well documented in the literature. One study (15) recruited 362 patients with IBD treated with different immunosuppressive treatment regimens and 121 healthy controls. The authors found that patients treated with infliximab or tofacitinib had lower anti-SARS-CoV-2 spike protein antibody concentrations after two doses of vaccine than healthy controls. On the other hand, reductions in antibody responses were not observed in patients with IBD being treated with thiopurines, ustekinumab, or vedolizumab compared to control participants. Surprisingly, patients treated with infliximab experienced a 10-times reduction in anti-SARS-CoV-2 spike protein antibody concentrations relative to the control group. Similarly, another study by Shehab et al. recruited 116 patients with IBD receiving infliximab combination therapy. The authors concluded that in patients with IBD receiving infliximab combination therapy, SARS-CoV2 IgG, IgA, and neutralizing antibody levels were lower than the healthy participants (16). Finally, Melmed et al. (23) published a study that assessed antibody titers in adults with IBD who received mRNA SARS-CoV-2 vaccination. The study included 582 participants with IBD receiving different immunosuppressive therapies who have been vaccinated with either BNT162b2 or mRNA-1273 vaccines. The authors found that the mean SARS-CoV-2 antibody levels at 8 weeks were the highest among those treated with anti-integrin and anti-interleukin-12/23 and lowest among those treated with anti-TNFs combination therapy or corticosteroids; however, the study was not powered to assess differences across medication subgroups. In our study, we also noticed wide variability in the range of IgG and neutralizing antibodies, which could be attributed to age and comorbidity differences among patients. However, the range was narrow after the booster dose, which supports the recommendation for a third dose.

Comparing the efficacy of frequently administered vaccines, such as Hepatitis B Virus (HBV) and Influenza, in IBD patients

can expand our understanding of viral vaccination interactions amid IBD therapies. Prat et al. reported considerably reduced seroprotective levels after HBV vaccinations (HBsAb $\geq$ 10 and 100 IU/L) among IBD patients receiving anti-TNF therapy (46.3%; $P < 0.01$) than those who did not. Furthermore, the report also mentioned that IBD patients receiving anti-TNF therapy combined with immunomodulators had worse outcomes, with 40.9% achieving levels adequate for protection ($P < 0.001$) (24). Similarly, Shirai et al. found that patients receiving anti-TNF therapies, such as infliximab, revealed decreased mean antibody titer after influenza vaccination. This was especially true in patients receiving infliximab combination therapy. Despite the overall reduction, patients still achieved sufficient levels of protection from a single influenza vaccine (25). These reports follow a comparable trend observed among COVID-19 vaccinated IBD patients. Like COVID-19 vaccines, HBV and Influenza vaccines among patients receiving anti-TNF therapy, especially infliximab (with or without combination therapy), exhibited lower seroprotective levels.

Our results build on growing literature confirming that most patients with IBD can mount humoral responses after the second dose of SARS-CoV-2 vaccination, with a small proportion generating poor or no response, which justifies current recommendations for this population to receive a booster dose of BNT162b2 vaccine. In addition, our pre-defined inclusion and exclusion criteria lower the risk of confounding bias, and patients were equally distributed and matched in terms of demographic characteristics such as age, sex, and BMI.

This study has some limitations. We cannot rule out that some of the included patients might have had silent COVID-19 at the time of recruitment, such as by doing SARS-CoV-2 N protein-specific IgG tests, which could have provoked a serological response (26). However, we did PCR testing before each vaccine dose and excluded any patients with current or previous symptoms of COVID-19. In addition, we only assessed positive IgG and neutralizing antibodies. However, we did not investigate cellular immunity, B-, and T-cell responses, which may also play a role in vaccine efficacy. We assessed infliximab with azathioprine or 6-mercaptopurine only. Further studies are needed to investigate the effect of other immunomodulators. Finally, we did not compare our data to a healthy control group.

CONCLUSION

Most patients with IBD on infliximab combination therapy had positive SARS-CoV-2 IgG and neutralizing antibody concentrations 40–45 weeks post BNT162b2 vaccination. However, SARS-CoV-2 IgG and neutralizing antibody concentrations were lower in patients who received two doses relative to patients who received the third (booster) dose. A longer follow-up study is needed to evaluate the decay in antibodies over time.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because local legal restrictions. Requests to access the datasets should be directed to mkh@moh.edu.kw.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Review Board of Dasman Institute Protocol # RA HM-2021-008 as per the updated guidelines of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and of the US Federal Policy for the Protection of Human Subjects. The study was also approved by the Ministry of Health of Kuwait (reference: 3799, protocol number 1729/2021). Subsequently, patient informed written consent was obtained before inclusion in the study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MS: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and submission of the manuscript. FA, AAlf, AAls, and UA: acquisition of data and drafting of the manuscript. PC and IA: statistical analysis. TT and AC: interpretation of data. AD, AAlb, and HA: data collection and supervision. MA-F, JA, and FA-M: critical revision of the manuscript for important intellectual content and study supervision. All authors contributed to the article and approved the submitted version.

FUNDING

This study was funded by the Kuwait Foundation for the Advancement of Sciences (KFAS) grant (RA HM-2021-008).

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