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Recent advances and new biomarkers in ulcerative colitis

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Recent advances and new biomarkers in ulcerative colitis

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Editorial: Recent advances and new biomarkers in ulcerative colitis

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ulcerative colitis, biomarkers, fecal calprotectin, endoscopy, mucosal inflammation, steroid-refractory ulcerative colitis, quality of life, biological therapy

Editorial on the Research Topic
Recent advances and new biomarkers in ulcerative colitis

Recent advances in our understanding of ulcerative colitis (UC), one of the two major types of inflammatory bowel disease (IBD), have led to the identification of new biomarkers that may aid in the diagnosis, prognosis prediction, and treatment of this chronic inflammatory condition. Although biomarkers are objective measures of disease activity or response to therapy, it is still a long process to fully use them to guide clinical decision-making. In this editorial, we discuss papers published in the current Research Topic that demonstrate evidence for new biomarkers utilized in experimental UC and patient management.

Experimentally induced models of colitis remain a vital part of the UC research. Yu et al. investigated the GB1a active component of Garcinia Kola nuts as a therapeutic agent for ameliorating experimental UC. They have emphasized the need for biomarkers in the pathways involved in UC pathogenesis, such as NF-κB and Nrf2 signaling pathways. Furthermore, by evaluating the inflammation and oxidative stress through the expression of TNFa-induced inflammatory genes, they suggested that GB1a may regulate inflammation, oxidative stress and permeability (Yu et al.).

One of the most exciting recent advances in UC has been identifying new genetic risk factors for the disease. Genome-wide association studies (GWAS) have identified over 240 genetic loci associated with UC, many of which are involved in the immune response. These genetic findings have led to a better understanding of the underlying pathogenesis of UC. However, the heterogeneity of UC, in terms of disease severity and response to therapy, makes the application of biomarkers very challenging. While some patients have mild diseases that can be easily controlled with medication, others may have severe, refractory diseases requiring more aggressive treatment. Biomarkers that are reliable indicators of disease severity and response to therapy in one patient may not be as reliable in another. One of the most considerable advances is gene signature profiling for predicting- therapy responses in UC and other diseases. Feng et al. demonstrated that an artificial neural network may be used for development of combination random forest to predict primary non-response to infliximab. This study is significant for establishing the role of machine learning in constructing predictive models for therapy responses based on the molecular prognostic score system.

In addition to genetic markers, several new serum and fecal biomarkers have been identified in UC. Serum biomarkers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) have long been used to monitor disease activity in UC. However, these markers are not specific to UC and can be elevated in various inflammatory conditions. More recently, novel fecal markers (such as calprotectin, lactoferrin) have shown promise as more specific markers of UC activity. The role of fecal calprotectin was confirmed in 143 patients with UC by Chen et al., establishing a level of 164 μg/g as the level with 85.42% sensitivity and 73.68% specificity in predicting clinically active disease and mucosal healing at 154.5 μg/g with a sensitivity of 72.34% and specificity of 85.71%. Other fecal biomarkers such as M2-PK, and S100A12 have also shown promise as markers of UC activity but are less investigated. Shi et al. presented evidence from published systematic reviews and meta-analyses in their umbrella review. Markers, such as anti-neutrophil cytoplasmic antibodies, anti-neutrophil cytoplasmic antibodies, and imaging techniques (i.e., ultrasound and magnetic resonance enterography) validated their role for assessing disease activity (Shi et al.). This is also valid for other promising markers, such as trefoil factor 3 (1), which correlates with disease activity and predicts complete mucosal healing. Mucosal healing could be predicted by assessing mucosal vascular patterns under special imaging endoscopy called narrow-band. He et al. demonstrated that narrow-band imaging endoscopic staging of mucosal vascular patterns could predict histological healing and clinical recurrence of UC.

Less employed are the biomarkers in other biologic samples, such as urine. However, Gunawan et al. showed that urinary chemerin is a promising non-invasive marker for monitoring UC severity and clinical course. Other bright research fields not covered in current state-of-the-art papers collection are gut microbiome biomarkers.

Prediction of therapy response is one of the ultimate goals when discussing the effectiveness and safety of biological therapy for UC. As Zhou et al. showed in 146 patients with UC, the novel biomarker, the neutrophil-to-albumin ratio, was positively associated with the disease activity. Moreover, this ratio could discriminate initial responders to primary non-responders to infliximab induction therapy. Thus, it could be employed in diagnosing, monitoring and predicting treatment efficacy in UC patients (Zhou et al.).

In their systematic review and meta-analysis, Szemes et al. shed light on evaluating the long-term outcomes of cyclosporin and infliximab in steroid-refractory UC patients. Since they did find significant differences for colectomy-free survival in favor of infliximab in the first 3 years, no long-term differences were observed for severe adverse events or deaths. From all the 15 studies analyzed in the meta-analysis and 1,607 patients with steroid-refractory acute severe UC, authors did not find definitive evidence for any differences in cyclosporin and infliximab efficacy and safety in patients with severe acute UC (Szemes et al.).

The quality of life is part of UC patients' integrative management approach. Li et al. constructed a prediction model for IBD, focusing mainly on the factors that affect the quality of life, especially emotional function and systemic symptoms. Interestingly, annual household income, occupational stress and score on the IBD questionnaire were independent risk factors for UC recurrence.

In conclusion, recent advances in our understanding of UC have led to identifying new biomarkers that may aid in diagnose, prognosis prediction, and disease treatment. As our knowledge of the underlying pathogenesis of the heterogenous UC continues to evolve, we will need to address significant challenges such as low specificity, sensitivity and cost to extend the usefulness of new biomarkers in the clinical practice. Standardizing biomarker assays and developing clear guidelines will increase the reliability of these biomarkers. This requires validation in larger, multicenter studies, including a more diverse patient population.

Author contributions

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

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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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References

1. Nakov R, Velikova T, Nakov V, Gerova V, Tankova L. Trefoil factor 3 is highly predictive of complete mucosal healing independently and in combination with C-reactive protein in patients with ulcerative colitis. *J Gastrointestin Liver Dis*. (2019) 28:169–74. doi: 10.15403/jgld-177 PMID: 31204414

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SYSTEMATIC REVIEW
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Comparable Long-Term Outcomes of Cyclosporine and Infliximab in Patients With Steroid-Refractory Acute Severe Ulcerative Colitis: A Meta-Analysis

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Background: In steroid-refractory acute severe ulcerative colitis (ASUC), cyclosporine (CYS) or infliximab (IFX) may be considered as a second-line alternative to avoid colectomy. There are short-term data reported, but until now, there is no meta-analysis regarding long-term outcomes of CYS and IFX in patients with ASUC.

Aim: To compare long-term efficacy and safety of CYS and IFX in a meta-analysis.

Methods: Three electronic databases (PubMed, Embase, Cochrane Central Register of Controlled Trials) were searched for studies which compared CYS vs. IFX in adults with ASUC. Long-term colectomy-free rate from 1 to 10 years during CYS or IFX therapy was collected, last updated up to 22nd May 2019. Primary outcome was long-term colectomy-free rate, secondary outcomes were adverse events (AE), serious adverse events (SAE), and mortality. Long-term colectomy-free survival and safety measures were pooled with the random-effect model. Odds ratios (OR) with 95% confidence intervals (CI) were calculated.

Results: Data from 1,607 patients in 15 trials were extracted. In the first 3 years, pooled OR for colectomy-free survival was higher with IFX than with CYS (OR = 1.59, 95% CI: 1.11–2.29, $p = 0.012$; OR = 1.57, 95% CI: 1.14–2.18, $p = 0.006$; and OR = 1.75, 95% CI: 1.08–2.84, $p = 0.024$; at 1, 2, and 3 years, respectively). However, the significant difference remained undetected from the fourth year of follow-up and in subgroup of RCTs (OR = 1.35, 95% CI: 0.90–2.01, $p = 0.143$; OR = 1.41, 95% CI: 0.94–2.12, $p = 0.096$; and OR = 1.34, 95% CI: 0.89–2.00, $p = 0.157$; at 1, 2, and 3 years, respectively). No significant difference was detected regarding adverse events, serious adverse events and mortality between the groups. The neutral associations proved to be underpowered with trial sequential analysis.

Conclusion: However observational studies show IFX as a better choice, according to the RCTs, choosing either CYS or IFX as rescue therapy for ASUC, the long-term outcomes are not different, although further large RCTs are warranted.

Keywords: steroid-refractory, ulcerative colitis, cyclosporine, infliximab, colectomy, meta-analysis

INTRODUCTION

Ulcerative colitis (UC) is a lifelong inflammatory bowel disease that causes a continuous mucosal inflammation of the colon and occurs periodically in patients' life. Acute severe ulcerative colitis (ASUC) is a life-threatening condition which requires hospitalization and occurs in about 25% of patients with UC (1). ASUC is defined as patients with bloody diarrhea ≥6/day and any signs of systemic toxicity [pulse > 90/min, temperature > 37.8°C, hemoglobin < 105 g/l, erythrocyte sedimentation rate (ESR) > 30 mm/h, or C-reactive protein (CRP) > 30 mg/l] (2). In the case of ASUC, intravenous (IV) corticosteroids are the mainstay of first-line treatment, but up to 40% of the cases are resistant to this therapeutic modality (3). In steroid-refractory cases, second-line therapy is advised to be introduced to avoid colectomy. Cyclosporine (CYS) and infliximab (IFX) are widely used as rescue therapies.

Rationale

CYS is a calcineurin and cytochrome P450 inhibitor immunosuppressant blocking the transcription of cytokine genes (interleukin-2 and−4) in activated T cells, thereby reducing the inflammation in the intestine (4). In the 1990s, CYS was the first drug introduced as salvage therapy in steroid-refractory ASUC (5). In general, following 2 mg/kg/day IV CYS, 5 mg/kg oral CYS is recommended for up to 3 months as a bridge to an immunosuppressive agent [azathioprine (AZA) or 6-mercaptopurine (6-MP)] (6). Despite the fast response within 4–7 days and the reliable short-term effectiveness during CYS therapy, significant side effects may occur (7, 8). A close drug-level monitoring of CYS is required to avoid opportunistic infections, renal, vascular and neurological toxicity (9).

In the past 15 years, IFX, a chimeric IgG1 monoclonal antibody designed to bind tumor necrosis factor-alpha (TNFα) has become an alternative second-line therapeutic option in steroid-refractory ASUC (10). Regularly, a standard induction regimen of 5 mg/kg IFX is used, although recently accelerated dose intensification with 10 mg/kg IFX is often applied as well to counteract the increased intestinal clearance of IFX in ASUC (11). However, there is no data to support the benefit of 10 mg/kg. During IFX-linked immunosuppression, opportunistic infections, reactivation on latent tuberculosis or hepatitis may occur; therefore, careful screening is recommended before the initiation of IFX.

Abbreviations: AE, adverse events; ASUC, acute severe ulcerative colitis; AZA, azathioprine; CRP, C-reactive protein; CYS, cyclosporine; ESR, erythrocyte sedimentation rate; IFX, infliximab; IV, intravenous; OR, odds ratio; RCT, randomized controlled trial; SAE, serious adverse events; TNFα, tumor necrosis factor-alpha; TSA, trial sequential analysis; UC, ulcerative colitis.

Objectives and Research Question

In the treatment of steroid-refractory ASUC, two randomized controlled trials (RCTs) demonstrated equal short-term efficacy and safety of IFX and CYS (CYSIF, CONSTRUCT) (12, 13). These results were opposed by a previous meta-analysis of observational studies, where IFX was associated with significantly higher rates of treatment response and a lower 12 months colectomy-rate compared to that with CYS (14). A lately reported network meta-analysis with benefit-risk analysis also suggested that there is a rank order of efficacy for colectomy-free rates favoring IFX over CYS, although the difference between the treatments was small (15).

Since new studies have been released and long-term survival data have become available. Therefore, we aimed to summarize the currently available evidence on the long-term efficacy and safety of IFX and CYS in steroid-refractory ASUC.

METHODS

Study Design, Participants, Interventions, and Comparators

This meta-analysis was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (**Supplementary Table 1**) (16). The protocol for this study was registered in the International Prospective Register of Systematic Reviews (PROSPERO) *a priori* under number CRD42018115035.

Search Strategy

We searched MEDLINE via PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Embase (https://www.embase.com) and Cochrane Central Register of Controlled Trials (CENTRAL) (http://www.cochranelibrary.com) databases from inception up to 22nd May 2019.

Our search followed the PICO concept. Studies discussed a population (P) of patients with steroid-refractory ASUC who received IFX (I) or CYS (C) as salvage therapy. The primary outcome (O) was long-term colectomy-free survival rate, defined as the follow-up period exceeding 12 months after therapy initiation. Secondary outcomes were adverse events (AE), serious adverse events (SAE) and mortality. AE and SAE were categorized in accordance with the definitions of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use—Good Clinical Practice (ICH-GCP) consensus guidelines (17).

The following query combining Medical Subject Headings (MeSH) and free text terms were used'. ("colitis, ulcerative"[MeSH Terms] OR ("colitis"[All Fields] AND

"ulcerative"[All Fields]) OR "ulcerative colitis"[All Fields] OR ("ulcerative"[All Fields] AND "colitis"[All Fields])) AND ("infliximab"[MeSH Terms] OR "infliximab"[All Fields]) AND ("cyclosporine"[MeSH Terms] OR "cyclosporine"[All Fields] OR "cyclosporin"[All Fields]) AND ("colectomy"[MeSH Terms] OR "colectomy"[All Fields]). We imposed only "English-language" and "human" filters on the search.

Study Selection

After the database search, one author (KS) removed the overlapping records using a reference management software (EndNote X8, Clarivate Analytics, Philadelphia, PA, USA). Two investigators (KS and PS) independently screened titles, abstracts, and full-texts against the predefined eligibility criteria. Consensus involving a third party (PH) resolved discrepancies in each phase of selection.

We included any controlled studies (observational or experimental) that met the following criteria: (a) adult ASUC patients (aged ≥18 years) being refractory to IV or oral steroid treatment; (b) CYS and IFX was used as salvage therapy after 3–7 days of steroid treatment; (c) colectomy-free survival rate was assessed at 12 months or later; and (d) cytomegalovirus infection was not verified in the patients. There was no restriction for additional drugs used in UC treatment (e.g., AZA, 6-MP or methotrexate).

Data Extraction, Quality Assessment

The following data were extracted from each study (**Table 1**): first author, year of publication, study type (prospective/retrospective; randomized/non-randomized), drug regimen, the number of patients, age, gender distribution, rate of extensive colitis, concomitant, and maintenance therapy, follow-up period and the definition of ASUC. Intention-to-treat data were extracted from RCTs. If numerical data on long-term colectomy-free survival were not reported (13, 23, 26, 31), we extracted data from the Kaplan-Meier curves by identifying the values on the axes "x" and "y" with a software [GetData Graph Digitizer] according to the method proposed by Guyot et al. (32). Data collection was accomplished by two authors independently (KS and PS). Discrepancies were resolved by consensus. In the case of any disagreement, a third author was involved to resolve conflicts (PH).

We assessed the risk of bias of observational studies using the Newcastle-Ottawa scale (NOS) (**Table 2**) (33). There is a reliable "star system" that has three broad perspectives to secure a simple tool for quality assessment: selection and comparability of the groups, and the ascertainment of the outcome. The quality of the included RCTs was assessed with the Cochrane Risk of Bias Tool along seven domains (34). After the assessment, low, high and unclear risks of bias were indicated with green, red and yellow symbols.

Data Analysis

Data on colectomy-free survival were extracted with IFX and CYS. Odds ratios (ORs) were calculated with 95% confidence intervals using the random effects model with the DerSimonian–Laird estimation (35). Results of the meta-analysis were displayed graphically using Forest plots. All analyses were two-tailed and $p < 0.05$ was considered as significant.

Subgroup analyses were performed to examine different effects in a 10 years interval. We carried out subgroup analyses only for the first 4 years based on the study design because data from RCTs were lacking for longer follow-up. Heterogeneity was tested by using the Cochrane's Q and the I^2 statistics, where $I^2 = 100\% \times (Q - df)/Q$ and represents the magnitude of the heterogeneity (moderate: 30–60%, substantial: 50–90%, considerable: 75–100%) (34). All meta-analytical calculations were performed with Stata 15.1 data analysis and statistical software (Stata Corp LLC, College Station, TX, USA).

Trial Sequential Analysis

Trial sequential analysis (TSA) was performed to assess the risk of type-I error and to estimate the required information size for an adequate statistical power if only RCTs were included (36). TSA was interpreted with an overall five percent of risk of type-I error ($\alpha = 0.05$) and with a power of 80% (**Figures 1–3**). Crossing of the constructed cumulative Z-curves (blue) and the two-sided $Z = 1.96$ provides a traditionally significant result. To obtain reliable evidence, crossing of the trial sequential monitoring boundaries (red) is needed. We conducted TSA using Trial Sequential Analysis software 0.9 (Copenhagen Trials Unit, Denmark).

Quality of Evidence

The GRADE system was constructed for the assessment of the quality of the evidence for the main outcomes in a review (37). The rating extends from very low to high quality, wherein RCTs starting from a high, non-randomized studies starting from a low quality of evidence. After the assessment of study design, outcomes were tested against five criteria including risk of bias, inconsistency, indirectness, imprecision and publication bias. Finally, the overall quality of the evidence for each outcome was graded as high, moderate, low or very low. Grading was performed independently by two of the authors (KS and PS) and disagreements were discussed by involving a third party (AE).

RESULTS

Search Results

A total of 731 records were identified from the databases with our systematic search strategy (121 records in PubMed, 597 ones in EMBASE and 13 ones in CENTRAL) (as shown in the PRISMA flow diagram; **Figure 4**). Two additional articles were found from the reference lists of the included studies (19, 38). After the removal of duplicates, 594 records remained, 565 of which were excluded by titles and abstracts. The remaining 29 articles were assessed for eligibility by full-text and further 10 studies were excluded due to the following reasons: three studies reported only short-term follow-up data (39–41), one study did not report on the timing and rate of colectomy (38) and two studies were uncontrolled (42, 43). In two studies, the number of patients treated with CYS and IFX was not reported (44, 45), one study included patients pre-treated with either CYS or IFX (46) and one study evaluated patients with Crohn's colitis (47). Nineteen

TABLE 1 | Study characteristics.

References	Drug regimen (number of patients)	Age (years)	Male (%)	Extensive colitis (%)	Concomitant medication	Maintenance therapy	Follow-up period	Definition of ASUC
RANDOMIZED CONTROLLED TRIALS								
Laharie et al. (18)[a]	IFX (55): 5 mg/kg at 0, 2, 6 weeks	36 (26–51)[b]	28 (51%)	31 (55%)	AZA starting at day 7	AZA	7 years	Lichtiger score >10 + Mayo score
	CYS (60): 2 mg/kg/day IV for 1 week, then 4 mg oral for 3 months	39 (26–50)[b]	13 (22%)	34 (60%)	AZA starting at day 7	AZA		
Scimeca et al. (19)[a**]	IFX (17): 5 mg/kg at 0, 2, 6 weeks	39 ± 12[c]	not reported	13 (77%)	previous use: AZA/MP, steroid	AZA	1 year	Truelove and Witts score
	CYS (13): 5 mg/kg/day oral	39 ± 15[c]	not reported	11 (85%)	previous use: AZA/MP, steroid	AZA		
Williams et al. (13)**	IFX (135): 5 mg/kg at 0, 2, 6 weeks	39.3 ± 15.5[c]	89 (66%)	53 (39%)	AZA/6-MP started at week 4	AZA/6-MP + IFX	3 years	Truelove and Witts + Mayo score
	CYS (135): 2 mg/kg/day IV for 1 week, 5.5 mg/kg oral for 3 months	39.8 ± 15[c]	81 (60%)	62 (46%)	AZA/6-MP started at week 4	AZA/6-MP		
OBSERVATIONAL STUDIES								
Croft et al. (20)[a]	IFX (37): 5 mg/kg single-dose infusion	26 (20–43)[b]	15 (41%)	27 (73%)	AZA/6-MP/MTX	AZA/6-MP/MTX	1 year	Truelove and Witts score
	CYS (43): 4 mg/kg (1999–2003), 2 mg/kg (2003–2007), IV for 7 days, then oral for 3 months	28 (20–37)[b]	26 (60%)	32 (74%)	AZA/6-MP/MTX	AZA/6-MP/MTX		
Daperno et al. (21)[d]	IFX (6): 5 mg/kg at 0, 2 weeks	Not reported	Not reported	Not reported	Steroid	AZA	4 years	Truelove and Witts score
	CYS (15): oral 5 mg/kg/day	Not reported	Not reported	Not reported	Steroid	AZA		
Dean et al. (22)[d]	IFX (19): 5 mg/kg, max. 5 infusion	25 (16–85)[b]	11 (58%)	10 (53%)	AZA	AZA/6-MP/MTX	1 year	not reported
	CYS (19): 2 mg/k/day until response, then oral	31 (15–56)[b]	12 (39%)	9 (47%)	AZA	AZA		not reported
Duijvis et al. (23)[d]	IFX (22): 5 mg/kg IV at 0, 2, 6 weeks	35.5 ± 15.4[c]	14 (64%)	10 (45%)	AZA/6-MP/mesalazin	IFX	8 years	Mayo score
	CYS (33): 2 mg/kg/day IV until response, then oral for 3 months	37.7 ± 13.6[c]	17 (52%)	17 (52%)	AZA/6-MP/mesalazin	AZA/6-MP		
Kim et al. (24)[d]	IFX (33): 5 mg/kg IV at 0, 2, 6 weeks	44 (15–71)[b]	25 (76%)	12 (36%)	AZA	AZA + IFX	3 years	According to international criteria
	CYS (10): 2 mg/kg IV until response, then AZA	56 (22–72)[b]	3 (30%)	8 (80%)	AZA	AZA		
Mocciaro et al. (25)[d]	IFX (30): 5 mg/kg IV at 0, 2, 6 weeks	37 ± 16.6[c]	15 (50%)	20 (67%)	AZA	AZA	3 years	Truelove and Witts + Lichtiger score
	CYS (35): 2 mg/kg/day IV, if responded, switch to oral after 14 days (5 mg/kg)	34.9 ± 13.7[c]	15 (43%)	29 (83%)	AZA	AZA		
Naves et al. (26)[d]	IFX (30): 5 mg/kg IV at 0, 2, 6 weeks	38 (27–56)[b]	30 (100%)	21 (70%)	AZA/6-MP	IFX	6 years	Montreal severity score
	CYS (20): 2–4 mg/kg	42 (30–50)[b]	13 (65%)	14 (70%)	AZA	AZA		
Ordás et al. (27)[d]	IFX (131): 5 mg/kg IV at 0, 2, 6 weeks	40 (13–83)[b]	76 (58%)	91	AZA	AZA	5 years	According to international criteria
	CYS (377): 2–4 mg/kg, then 5–10 mg/kg oral	36 (9–83)[b]	217 (58%)	295	NA	AZA		

(Continued)

TABLE 1 | Continued

References	Drug regimen (number of patients)	Age (years)	Male (%)	Extensive colitis (%)	Concomitant medication	Maintenance therapy	Follow-up period	Definition of ASUC
Protic et al. (28)[d]	IFX (54): 5 mg/kg IV at 0, 2, 6 weeks	39 (16–90)[b]	47 (87%)	49 (65%)	NA	IFX	1 year	Truelove and Witts + Lichtiger score
	CYS (38): 2–4 mg/kg IV for 7 days, then 5 mg/kg oral				AZA	AZA		
Radojcic et al. (29)[d**]	IFX (13): not reported	Not reported	Not reported	Not reported	Not reported	Not reported	1 year	Not reported
	CYS (15): not reported	Not reported	Not reported	Not reported	Not reported	Not reported		
Sjöberg et al. (30)[d]	IFX (49): 5 mg/kg single-dose infusion	38 (17–60)[b]	30 (61%)	42 (44%)	AZA/6-MP/5-ASA	AZA/6-MP	1 year	Truelove and Witts score
	CYS (43): 4 mg/kg for 7 days, then oral 4 mg for 18 weeks	32 (17–72)[b]	21 (49%)	30 (70%)	AZA/6-MP	AZA/6-MP		
Song et al. (31)[d**]	IFX (97): not reported	Not reported	Not reported	Not reported	Not reported	Not reported	10 years	Truelove and Witts + Mayo score
	CYS (23): not reported	Not reported	Not reported	Not reported	Not reported	Not reported		

[a]*Prospective study.*
[b]*Median + interquartile range (IQR).*
[c]*Mean ± SD (standard deviation).*
[d]*Retrospective study.*
*ASUC, acute severe ulcerative colitis; IFX, infliximab; CYS, cyclosporine; AZA, azathioprine; 6-MP, 6-mercaptopurine; MTX, methotrexate; IV, intravenous; **, abstract form only.*

studies remained, but four additional studies were excluded from the quantitative synthesis because they investigated overlapping study population (12, 48–50). Thus, 15 studies fulfilled all inclusion criteria and were included in the meta-analysis.

Characteristics of the Studies Included

The main characteristics of the included studies are listed in **Table 1**. The studies were published between 2004 and 2018 and the follow-up period ranged at least from 1 year to maximum of 10 years. In the quantitative analysis, we used data from three RCTs (13, 18, 19) and 12 cohort studies (20–31). A total number of 1,607 patients with steroid-refractory ASUC were included, 879 of which (54.7%) were treated with CYS and the other 728 (45.3%) with IFX. The most common definitions of ASUC used in the studies were the Truelove and Witts criteria, the Mayo and the Lichtiger scores (7, 51, 52). Three of the 15 articles were published only in conference abstract form (19, 29, 31).

In most of the studies, the standard 2 mg/kg/day IV CYS regimen was applied, oral CYS was used for induction of remission only in two studies (19, 21). After the oral CYS bridging, AZA maintenance therapy was continued in all studies. Standard 5 mg/kg dose of IFX was administered in multiple IV infusions (at 0, 2, and 6 weeks) following the induction protocol. Only two studies reported a single infusion of IFX (20, 30). In the IFX treatment groups, AZA was the most commonly administered maintenance drug, albeit recent studies continued IFX (13, 23, 24, 26, 28). Due to the lack of available safety data during long-term follow-up in an RCT, the CYSIF trial (18), AE and SAE results reported in the original study were used in the meta-analysis (12).

Long-Term Colectomy-Free Survival

Fifteen, eight, five, and one studies reported 1, 3, 5, and 10 years colectomy-free survival rate. In the first 3 years, colectomy-free survival rate was higher with IFX compared to that with CYS (OR = 1.59, 95% CI: 1.11 −2.29, $p = 0.012$ for 1 year; OR = 1.57, 95% CI: 1.14–2.18, $p = 0.006$ for 2 years; and OR = 1.75, 95% CI: 1.08–2.84, $p = 0.024$ for 3 years), with moderate heterogeneity across the studies ($I^2 = 44.3\%$, $p = 0.033$; $I^2 = 0.0\%$, $p = 0.74$, and $I^2 = 42.6\%$, $p = 0.093$, respectively) (**Figure 5**). From the fourth year of follow-up, no significant difference regarding the colectomy-free rates was found between the two treatment groups (**Figure 6**). At 9 and 10 years of follow-up, only one small, retrospective study remained in the analysis, where the colectomy-free survival was higher with IFX compared to that with CYS (31).

However, separating the data of RCTs revealed that the significant association can only be seen if observational studies are included (ORs for observational studies = 1.84, 95% CI: 1.13–3.01, $p = 0.015$ in the first year; OR = 1.91, 95% CI: 1.11–3.28, $p = 0.020$ in the second year; and OR = 2.23, 95% CI: 1.00–4.96, $p = 0.049$ in the third year; ORs for RCTs = 1.35, 95% CI: 0.90–2.01, $p = 0.143$ in the first year; OR = 1.41, 95% CI: 0.94–2.12, $p = 0.096$ in the second year; and OR = 1.34, 95% CI: 0.89–2.00, $p = 0.157$ in the third year) (**Figure 5**). The heterogeneity remained substantial in the analysis from observational studies but was negligible if RCTs were included exclusively (in the first year I^2

TABLE 2 | Modified Newcastle-Ottawa Scale.

	Newcastle-Ottawa scale items	High-quality items carrying a low risk of bias (green)	Low-quality items carrying a high (red) or an unknown (yellow) risk of bias
Selection	Item 1: Representativeness of the initial study population—acute severe ulcerative colitis (ASUC)	Only patients with ASUC were included	Low: beside ASUC moderately severe UC cases were included. Unclear: no data on selection process.
	Item 2: Representativeness of the initial study population (ASUC)	Only patients with ASUC were included	Low: beside ASUC moderately severe UC cases were included. Unclear: no data on selection process.
	Item 3: Ascertainment of severity of ulcerative colitis	ASUCs was defined with objective scores (e.g., Lichtiger score, Mayo score)	Low: UC was defined with no objective scores Unclear: no data about objective severity score
	Item 4: Demonstration that outcome of interest was not present at start of study	The patients had no colectomy before and were treated with steroid as rescue therapy	Low: patients had any kind of colon resection before Unclear: no statement.
Comparability	Item 5: Study controls for age, sex	No significant difference was detected between patients treated with cyclosporine or infliximab regarding age.	Low: significant difference was detected between patients treated with cyclosporine or infliximab regarding age. Unclear: no comparison was performed based on age.
	Item 6: Study controls for extent of disease	No significant difference was detected between patients treated with cyclosporine or infliximab regarding extent of disease.	Low: significant difference was detected between patients treated with cyclosporine or infliximab regarding extent of disease. Unclear: no comparison was performed based on extent of disease.
Outcome	Item 7: Assessment of outcome	Colectomy-free survival rate or numbers of patients with colectomy were presented at least 1-year follow-up	Low: colectomy rate only available from the Kaplan-Meier curve Unclear: no statement
	Item 8: Adequacy of follow-up	At least 12 months follow-up period	Low: incomplete follow-up with explanations Unclear: incomplete follow-up without explanation of the loss.

= 52.6%, $p = 0.016$ and $I^2 = 0.0\%$, $p = 0.466$, respectively). TSA indicated that the analysis on colectomy-free survival at 1 year was underpowered, since the monitoring boundaries were not crossed, and the required information size was not reached (**Figure 1**). According to TSA, at least 1,502 patients would be required for drawing final conclusion while only 416 patients were included in the current analysis.

Based on our strict and consistent grading, the quality of the evidence for colectomy-free survival rates at 1, 3, 5, and 10 years proved to be low for the subgroups of RCTs and very low if non-randomized studies were included as well (**Table 3**).

Safety

Seven studies assessed AE (**Figure 7**) (12, 13, 20, 22, 25, 28, 30). Sixty-seven (18.1%) AEs were reported with CYS and 72 (18.9%) with IFX. The pooled OR of AEs was 0.93 (95% CI: 0.45–1.92, $p = 0.847$), demonstrating no significant difference between groups (**Figure 7**).

The cumulative Z-curve of the risk of AE during TSA reached but not crossed the conventional boundary (**Figure 2**). The number of participants included ($n = 385$) did not reach the required information size ($n = 749$), the cumulative Z-curve does not cross the monitoring boundary either.

Eight studies reported on SAE, such as opportunistic infections, sepsis, anaphylactic reaction and hepato- and nephrotoxicity (**Figure 7**) (12, 13, 19, 21, 24, 26–28). One hundred and three (15.5%) SAEs were reported with CYS and 72 (15.3%) with IFX. Rate of SAE was not elevated with IFX compared to that with CYS (OR = 1.27, 95% CI: 0.86–1.89, $p = 0.236$); although in the subgroup analysis of observational studies (21, 24, 26–28), IFX was associated with a higher SAE rate (OR = 1.80, 95% CI: 1.17–2.79, $p = 0.008$). However, in the three RCTs (13, 18, 19), no statistically significant difference could be detected between the two groups (OR = 0.81, 95% CI: 0.47–1.41, $p = 0.461$), data proved to be homogeneous ($I^2 = 0.0\%$, $p = 0.712$; $I^2 = 0.0\%$, $p = 0.781$, and $I^2 = 7.2\%$, $p = 0.374$ for observational and randomized studies and overall, respectively).

TSA of SAE showed that the number of patients in the analysis of RCTs did not reach the required information size and the cumulative Z-curve did not cross the monitoring boundary (**Figure 3**).

There was also no significant difference between treatment groups regarding mortality (OR: 0.79, 95% CI: 0.26–2.38, $p = 0.678$; $I^2 = 0.0\%$, $p = 0.411$) (**Figure 7**) (13, 18, 19, 27, 28).

The GRADE assessment of safety outcomes (AE, SAE, and mortality) showed low quality of evidence for the analyses of RCTs and very low quality of evidence for that of non-randomized studies (**Table 3**).

Risk of Bias Assessment

Assessments of the risk of bias of the included studies are shown in **Figure 8**. In the observational studies, the representativeness of the exposed and the selection of the non-exposed cohort was judged to be at high risk in multiple studies (23, 28, 30). In

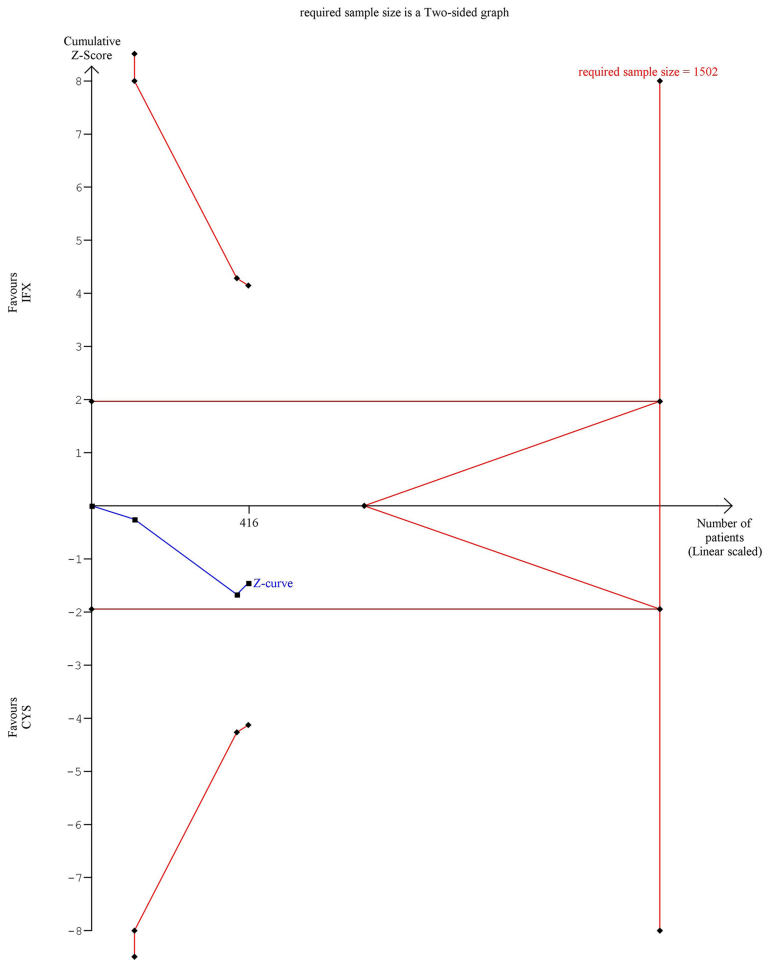

FIGURE 1 | Results of the trial sequential analysis of the risks of 1 year colectomy-free rate. The required sample size of 1,502 patients was estimated using $\alpha = 0.05$ (two-sided) and $\beta = 0.02$ (power of 80%). Crossing of the constructed cumulative Z-curves (blue) and the two-sided $Z = 1.96$ provides a traditionally significant result. To obtain reliable evidence, crossing of the trial sequential monitoring boundaries (red) is needed. In the case of 1 year colectomy-free rate outcome, the cumulative Z-curve (blue) did not crossed the conventional boundary and neither the trial sequential monitoring boundary nor the required sample size line was surpassed.

the studies of Daperno, Protic, and Radojcic, no comparison was performed between groups regarding age, sex, and extent of disease (21, 28, 29).

Among the RCTs, the studies of Williams and Laharie carried the lowest risk of bias (13, 18). As they were open trials, participants and personnel were not blinded; however, in the study of Williams, outcome assessment remained blinded. Because the study of Scimeca et al., was only published in a conference abstract form, almost all domains were judged as carrying "unclear" risk of bias (19).

DISCUSSION

Summary of Main Findings

ASUC is a medical emergency and should be managed in high-volume tertiary centers with a multidisciplinary approach. In patients failing to respond to parenteral corticosteroids, medical rescue therapy including CYS or IFX is needed. Recently, a meta-analysis has covered the short-term efficacy of the two drugs in treatment response and 12 months colectomy rates but failed to discuss long-term outcomes (14).

In our meta-analysis, we collected RCTs and observational studies to perform long-term statistics focusing on colectomy-free survival rates and drug safety. Our combined data from all the studies showed that there was a significantly higher colectomy-free survival rate with IFX compared to that with CYS. This difference was only seen within the first 3 years after rescue therapy was initiated and it disappeared after the fourth year of follow-up. Additionally, we performed a subgroup analysis by study design to reveal selection bias when comparing RCTs and observational studies. Higher colectomy-free survival was found in observational studies with IFX but not in RCTs. It should be noted that the level of heterogeneity was moderate to substantial in the analysis from observational studies whilst data from RCTs were homogenous. When evaluating safety outcomes, no significant difference was detected between CYS and IFX treatment groups regarding AE, SAE, and mortality. The neutral association calculated from RCTs proved to be underpowered

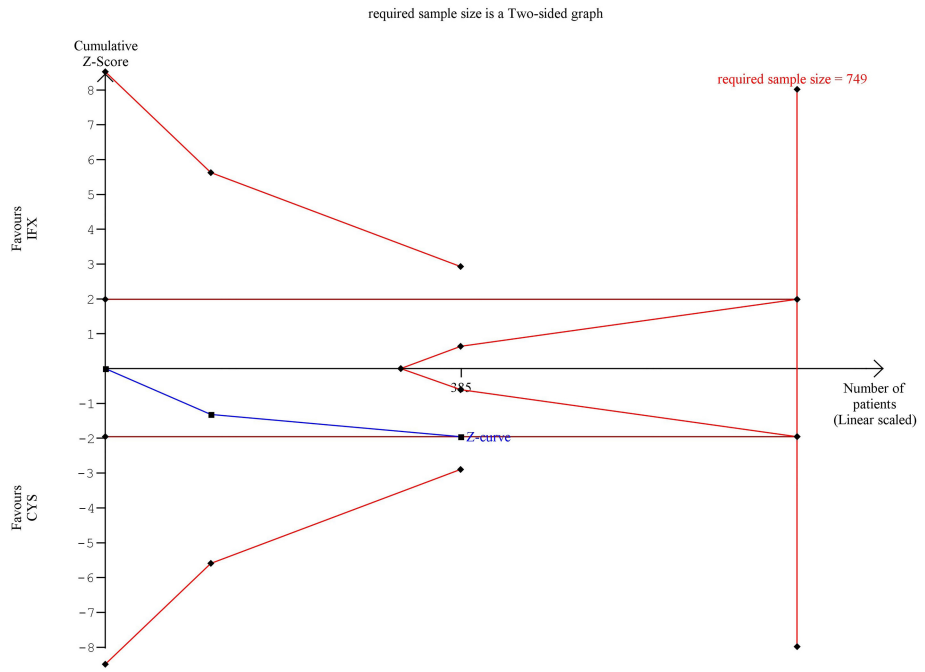

FIGURE 2 | Results of the trial sequential analysis of the risks of adverse events. The required sample size was calculated with $\alpha = 0.05$ (two-sided) and $\beta = 0.02$ (power of 80%). Although cumulative Z-curve (blue) reached the conventional boundary, it did not cross through. Number of participants (385) did not reach the information size (749) and the cumulative Z-curve does not cross the monitoring boundary either.

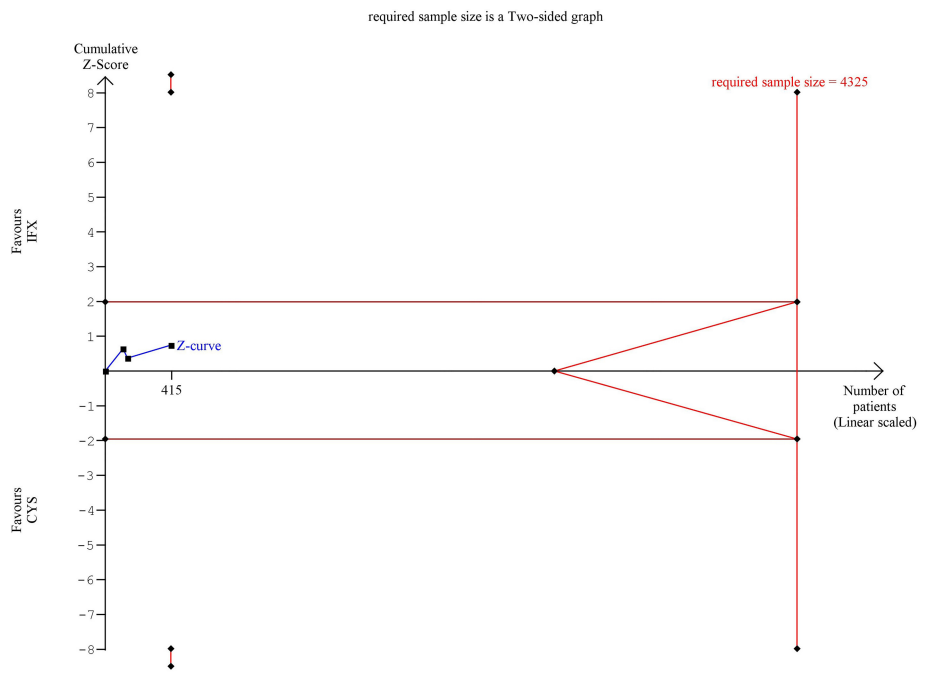

FIGURE 3 | Results of the trial sequential analysis of the risks of serious adverse events. The required sample size was calculated using $\alpha = 0.05$ (two-sided) and $\beta = 0.02$ (power of 80%). The cumulative Z-curve (blue) did not cross the monitoring boundary (red) and not reached the required information size (4,325 patients). There is insufficient information about the evidence of significance.

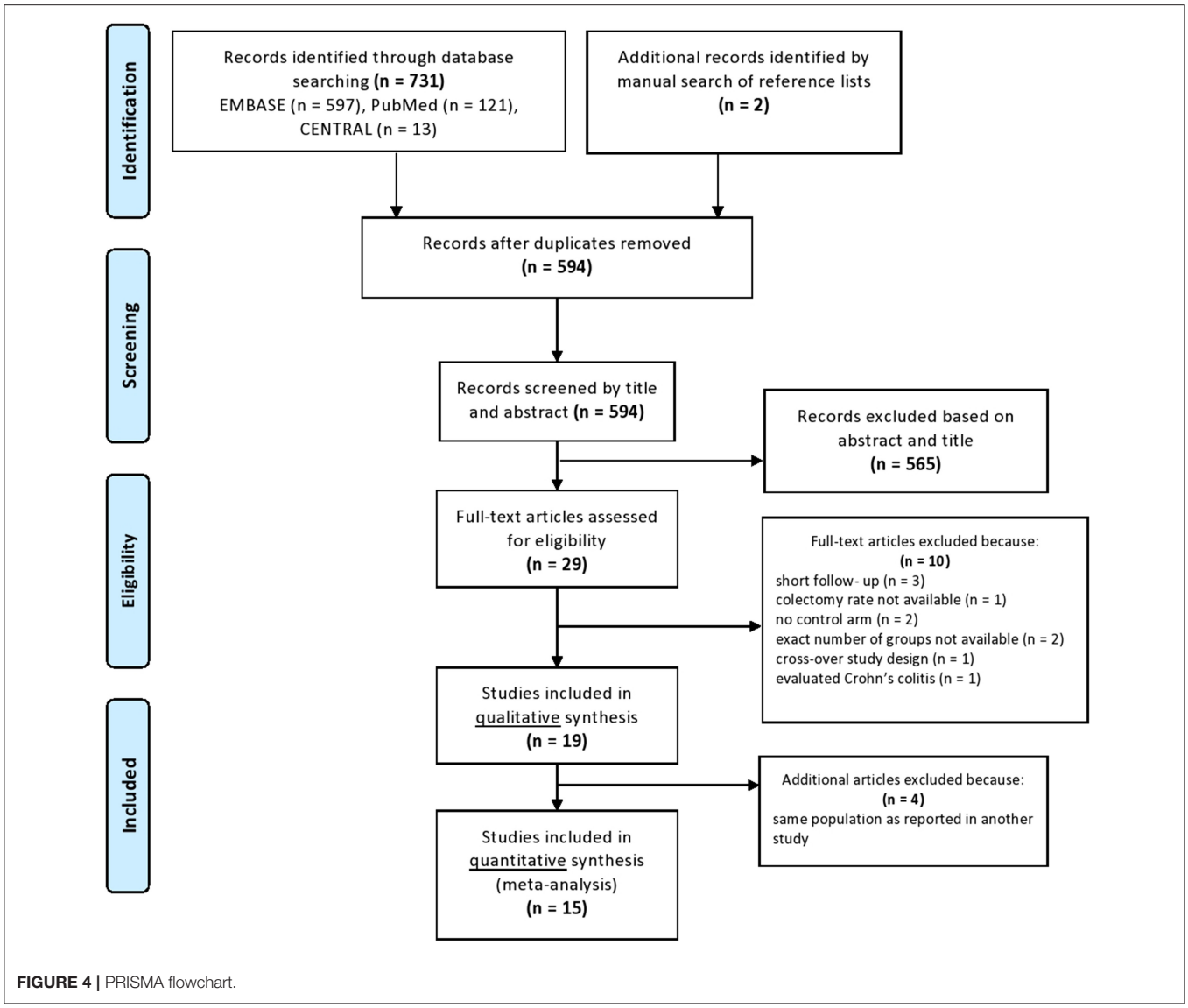

FIGURE 4 | PRISMA flowchart.

(indicated by TSA) and therefore insufficient to draw a final conclusion (36).

During ASUC treatment, early identification of steroid refractoriness and early introduction of rescue treatments are crucial to avoid morbidity and mortality. A variety of risk prediction tools have been developed to identify patients with ASUC being suitable for second-line medical therapy, these tools are used in clinical practice (such as the Oxford criteria and the Ho index) (53, 54). In a retrospective study, older age, severe endoscopic lesions, high CRP, low albumin levels and low serum IFX levels were identified as predictors of IFX failure in ASUC (30). Due to increased intestinal loss of IFX in ASUC, the serum IFX levels could be decreased; therefore, a modified IFX induction strategy can be considered (55). However, the results of other studies have opposed this association. Dose optimization based on IFX drug level monitoring may result in better patient outcomes (56, 57). In a retrospective study and meta-analysis, no association was found between accelerated IFX induction therapy and lower rates of colectomy in patients with ASUC, compared to standard induction therapy (58). The benefit of intensified induction regimen, i.e., shorter dosing intervals and/or higher doses of IFX is still not proven.

Limitations and Strengths

However, we are aware that our findings suffer from several limitations. First, most of the studies were non-randomized, retrospective studies, and the number of RCTs was low. Second, the use of maintenance therapy after the initial response was not uniform in all studies and must have contributed to the variation in the long-term outcomes. Third, the definitions of AE and SAE were often mixed together and were unclear in the reports; therefore, an internationally accepted guide has been adopted (17). Fourth, in two RCTs (13, 18), there is switch reported in some cases between CYS and IFX or IFX and CYS as third-line rescue therapy. The switch was necessary to avoid colectomy and achieve clinical remission. However, this can cause a difficulty defining the effect of the drug and may affect the long-term outcome.

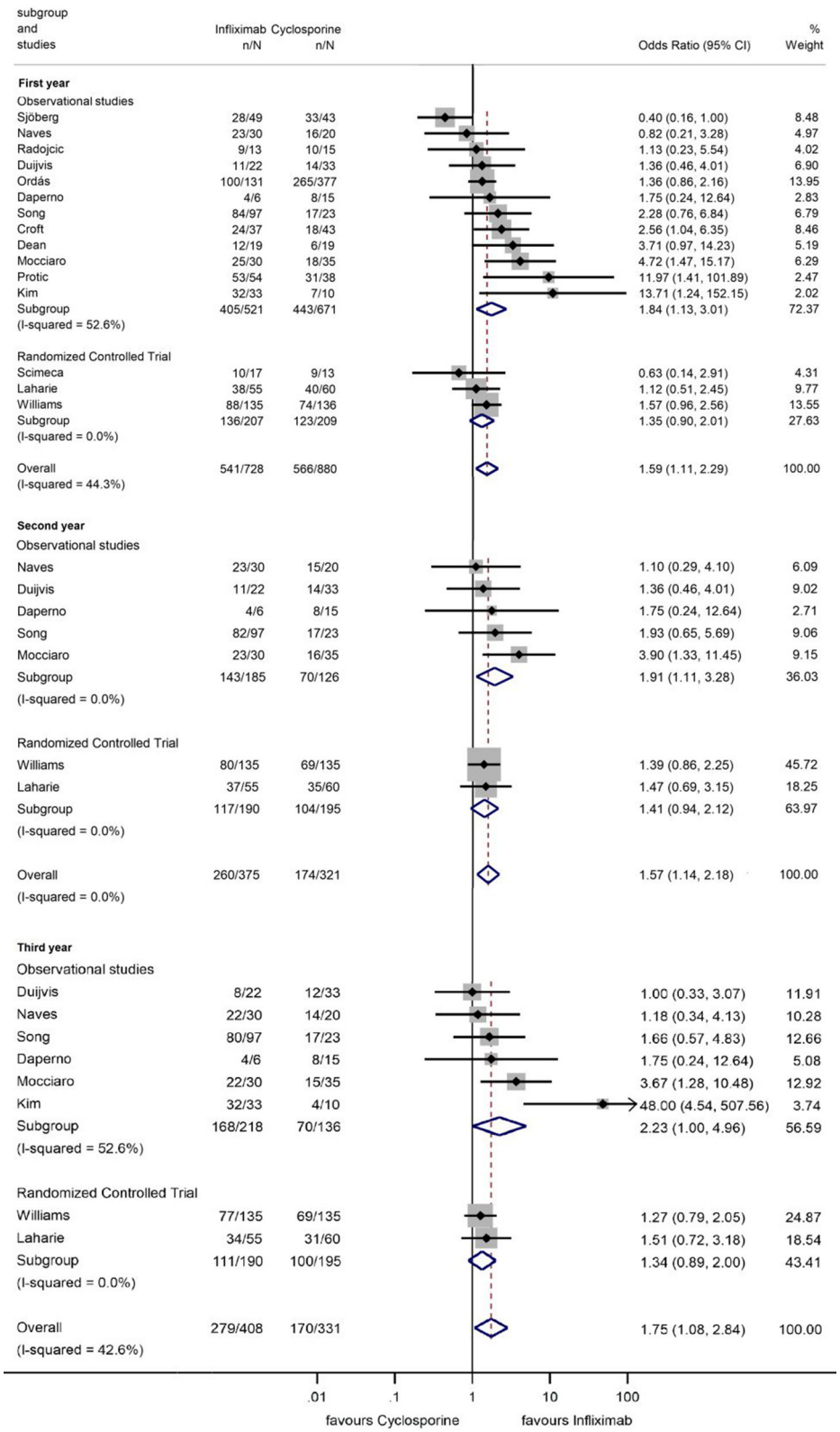

FIGURE 5 | Odds ratios of colectomy-free survival with infliximab (vs. cyclosporine) in the first, second, and third year in steroid-refractory acute severe ulcerative colitis.

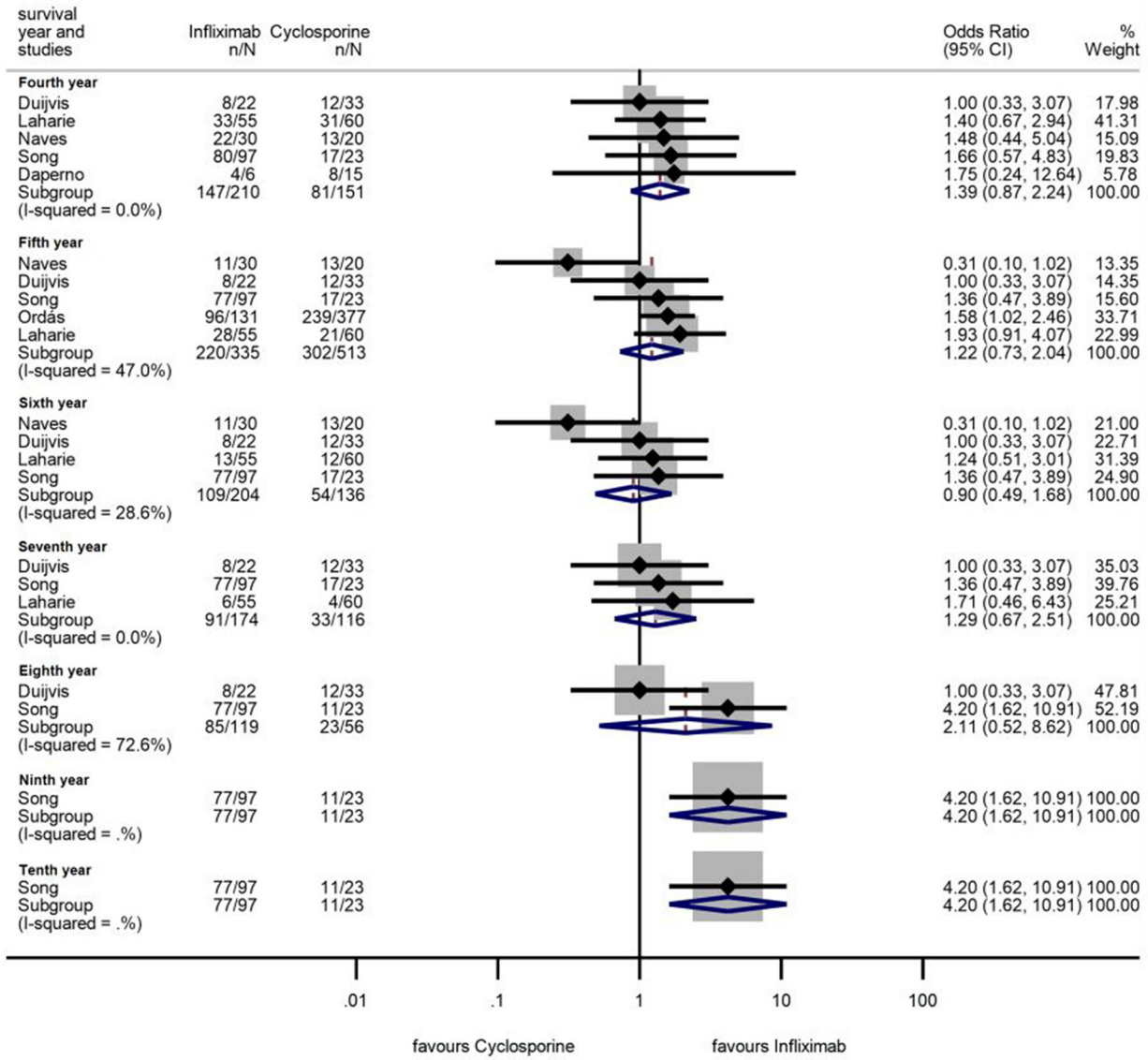

FIGURE 6 | Odds ratios of colectomy-free survival with infliximab (vs. cyclosporine) between the fourth and tenth year in steroid-refractory acute severe ulcerative colitis.

We deviated from the PROSPERO protocol regarding an important point. Originally, the primary outcome was planned to be the 5 years colectomy-free survival. However, we thought that investigating the same outcome at multiple time points may improve the clinical yield of the results.

Last, conference abstracts with limited information were also included in the meta-analysis, containing a high amount of unclear information and an increasing possibility of risk of bias.

There are several strengths of our meta-analysis that worth being highlighted. Altogether, a high number of patients with ASUC was investigated. Our meta-analysis is the first reporting more than 1 year colectomy free-survival rates with a high number of studies providing even seven or 10 years of colectomy-free survival data. Another strength of our meta-analysis is that the certainty of the evidence was examined for all outcomes according to the GRADE approach (37). Moreover, TSA was used to test whether the analyses are sufficiently powered; therefore, can be considered conclusive.

CONCLUSIONS

In summary, our meta-analysis has shown that there is no definitive evidence for any difference regarding long-term efficacy and safety between CYS and IFX in patients with steroid-refractory ASUC based on RCTs. Considering second-line treatment options in ASUC, the choice of drug depends on several factors other than efficacy and safety. Since the

TABLE 3 | Investigation of quality of the evidence for all included outcomes (GRADE).

Measured outcomes	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Other (upgrading factors*)	Quality of the evidence
Colectomy-free rate in the first year	Non-randomized studies (n = 12) (starts as low quality)	Data are from studies at low risk of bias	Low heterogeneity (I^2 = 0%)	Evidence that the studies found is no more restrictive then our PICO	Small sample sizes (<400 patients) (−1)	All results come from small studies (−1)	None	Very low •○○○
	RCTs (n = 3) (starts as high quality)	Data are from studies at low/unclear risk of bias	Low heterogeneity (I^2 = 0%)	Evidence that the studies found is no more restrictive then our PICO	Small sample sizes (<400 patients) (−1)	All results come from small studies (−1)	None	Low ••○○
Colectomy-free rate in the third year	Non-randomized studies (n = 6) (starts as low quality)	Data are from studies at low risk of bias	Moderate heterogeneity (I^2 > 60%) (−1)	Evidence that the studies found is no more restrictive then our PICO	Small sample sizes (<400 patients) (−1)	All results come from small studies (−1)	None	Very low •○○○
	RCTs (n = 2) (starts as high quality)	Data are from studies at low risk of bias	Low heterogeneity (I^2 = 0%)	Evidence that the studies found is no more restrictive then our PICO	Small sample sizes (<400 patients) (−1)	All results come from small studies (−1)	None	Low ••○○
Colectomy-free rate in the fifth year	Non-randomized studies (n = 4) (starts as low quality)	Data are from studies at low risk of bias	Moderate heterogeneity (I^2 > 60%) (−1)	Evidence that the studies found is no more restrictive then our PICO	Small sample sizes (<400 patients) (−1)	All results come from small studies (−1)	None	Very low •○○○
	RCTs (n = 1) (starts as high quality)	Data are from studies at low risk of bias		Evidence that the studies found is no more restrictive then our PICO	Small sample sizes (<400 patients) (−1)	All results come from small studies (−1)	None	Low ••○○
Colectomy-free rate in the tenth year	Non-randomized studies (n = 1) (starts as low quality)	Data are from studies at low risk of bias	NA	Evidence that the studies found is no more restrictive then our PICO	Small sample sizes (<400 patients) (−1)	All results come from small studies (−1)	None	Very low •○○○
	(No RCT in the tenth year)	NA	NA	NA	NA	NA	NA	NA
Adverse events	Non-randomized studies (n = 5) (starts as low quality)	Data are from studies at low/high risk of bias (−1)	Moderate heterogeneity (I^2 > 60%) (−1)	Evidence that the studies found is no more restrictive then our PICO	Small sample sizes (<400 patients) (−1)	All results come from small studies (−1)	None	Very low •○○○
	RCTs (n = 2) (starts as high quality)	Data are from studies at low risk of bias	Low heterogeneity (I^2 = 0%)	Evidence that the studies found is no more restrictive then our PICO	Small sample sizes (<400 patients) (−1)	All results come from small studies (−1)	None	Low ••○○
Serious adverse events	Non-randomized studies (n = 5) (starts as low quality)	Data are from studies at low/unclear risk of bias (−1)	Low heterogeneity (I^2 = 0%)	Evidence that the studies found is no more restrictive then our PICO	Small sample sizes (<400 patients) (−1)	All results come from small studies (−1)	None	Very low •○○○
	RCTs (n = 3) (starts as high quality)	Data are from studies at low/unclear risk of bias (−1)	Low heterogeneity (I^2 = 0%)	Evidence that the studies found is no more restrictive then our PICO	Small sample sizes (<400 patients) (−1)	All results come from small studies (−1)	None	Low ••○○
Mortality	Non-randomized studies (n = 2) (starts as low quality)	Data are from studies at low/high risk of bias (−1)	Low heterogeneity (I^2 = 0%)	Evidence that the studies found is no more restrictive then our PICO	Small sample sizes (<400 patients) (−1)	All results come from small studies (−1)	None	Very low •○○○
	RCTs (n = 3) (starts as high quality)	Data are from studies at low/unclear risk of bias (−1)	Low heterogeneity (I^2 >40%)	Evidence that the studies found is no more restrictive then our PICO	Small sample sizes (<400 patients) (−1)	All results come from small studies (−1)	None	Low ••○○

Including large effect, dose response, no plausible confounding factors, NA, non-applicable.

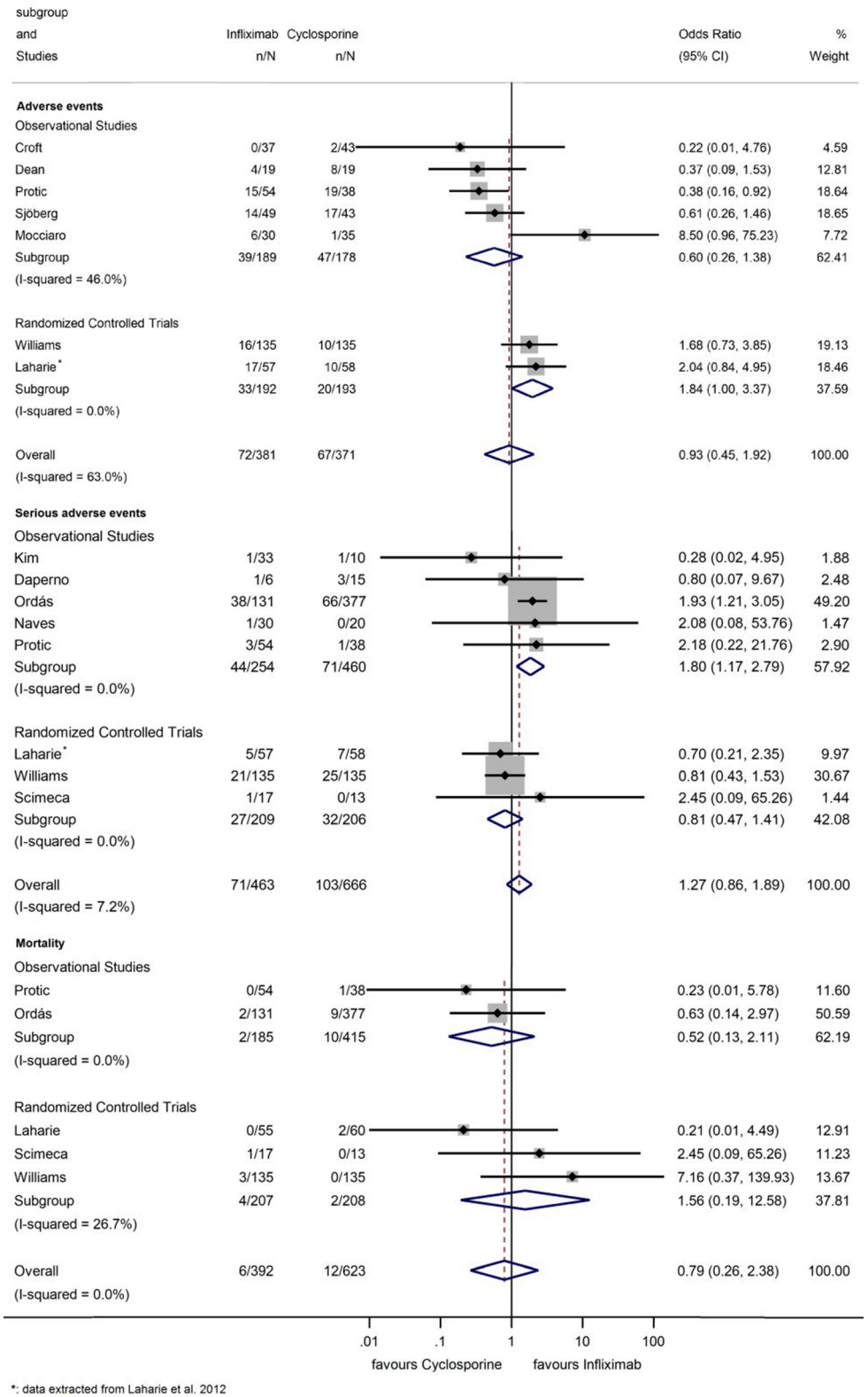

FIGURE 7 | Odds ratios of studies evaluating adverse events, serious adverse events, and mortality during infliximab treatment compared to the cyclosporine group in steroid-refractory acute severe ulcerative colitis.

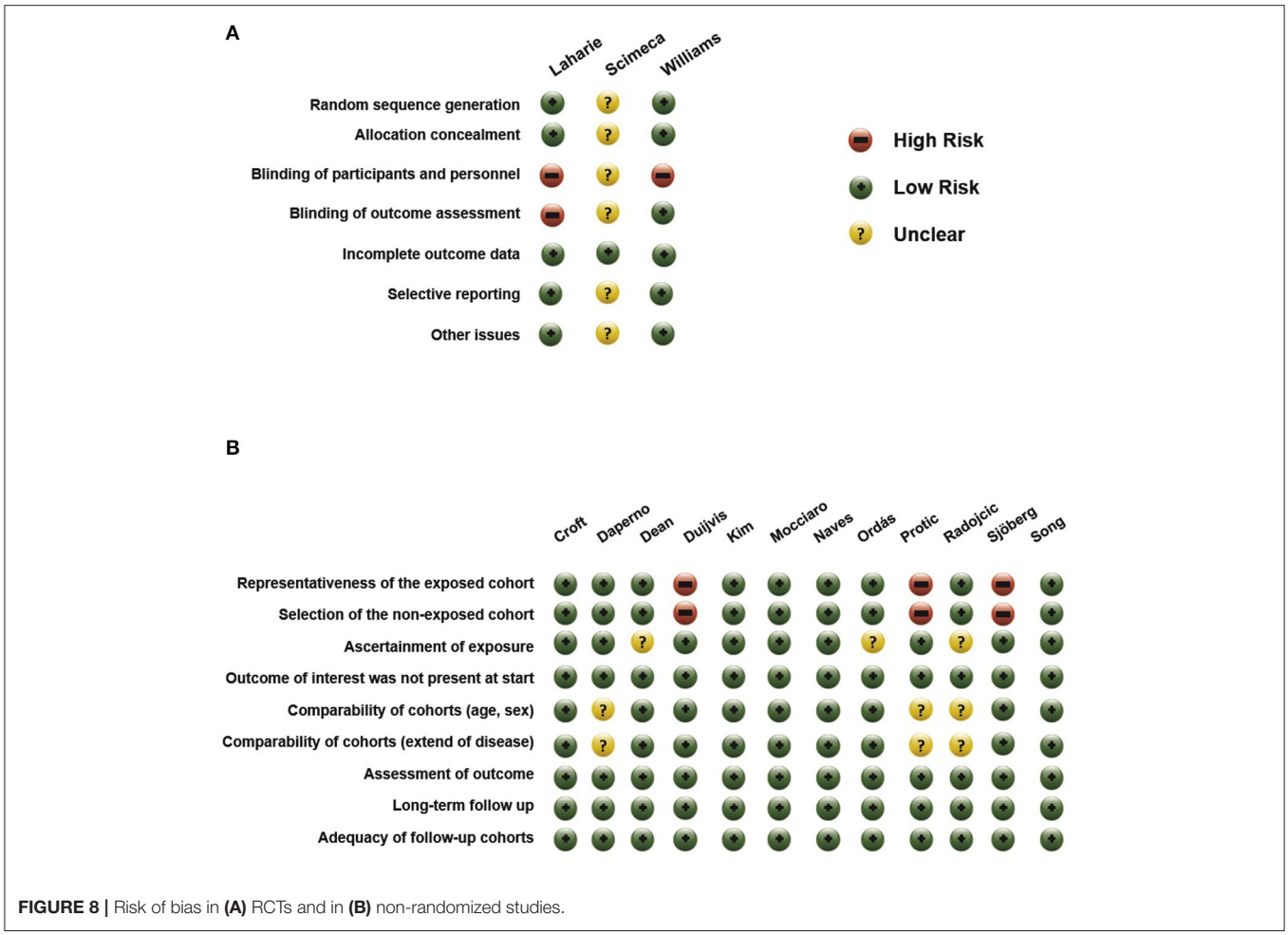

FIGURE 8 | Risk of bias in **(A)** RCTs and in **(B)** non-randomized studies.

introduction of IFX, as rescue therapy for ASUC and a proxy for CYS, the length of hospital stay and in-hospital costs have been reduced significantly (59). On the other hand, the total costs up to 3 months after initiation of rescue therapy were significantly higher in the IFX group (59). However, since 2013 lower-cost IFX biosimilars are available, which may result in large cost savings in the future. In addition to safety and efficacy, other components of evidence-based medicine, such as the experience of treating physicians and patient preferences, should also be highlighted. In thiopurine-naïve patients, CYS can be preferred as a bridge to thiopurine maintenance treatment. IFX is a reasonable option for patients who have previously failed thiopurine maintenance therapy. Results of TSA and the lack of high-quality evidence in our meta-analysis highlight that further large RCTs are warranted to decide which therapy is the preferable rescue therapy in ASUC.

DATA AVAILABILITY STATEMENT

The datasets analyzed in this article are not publicly available. Requests to access the datasets should be directed to KS, szemesk@gmail.com.

AUTHOR CONTRIBUTIONS

PS and KS designed the research. PS, KS, NF, and AS performed the research and statistical analyses, analyzed and interpreted the data. KS and PS wrote the article. BE, EM, KM, AE, and ZS made the critical revisions related to important intellectual content of the manuscript. PS and PH gave the final approval of the version of the article to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2019.00338/full#supplementary-material

REFERENCES

1. Dinesen LC, Walsh AJ, Protic MN, Heap G, Cummings F, Warren BF, et al. The pattern and outcome of acute severe colitis. *J Crohns Colitis.* (2010) 4:431–7. doi: 10.1016/j.crohns.2010.02.001
2. Harbord M, Eliakim R, Bettenworth D, Karmiris K, Katsanos K, Kopylov U, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. part 2: current management. *J Crohns Colitis.* (2017) 11:769–84. doi: 10.1093/ecco-jcc/jjx009
3. Jarnerot G, Rolny P, Sandberg-Gertzen H. Intensive intravenous treatment of ulcerative colitis. *Gastroenterology.* (1985) 89:1005–13. doi: 10.1016/0016-5085(85)90201-X
4. Herold KC, Lancki DW, Moldwin RL, Fitch FW. Immunosuppressive effects of cyclosporin A on cloned T cells. *J Immunol.* (1986) 136:1315–21.
5. Lichtiger S, Daniel H. Preliminary report: cyclosporin in treatment of severe active ulcerative colitis. *Lancet.* (1990) 336:16–9. doi: 10.1016/0140-6736(90)91521-B
6. Dulai PS, Jairath V. Acute severe ulcerative colitis: latest evidence and therapeutic implications. *Ther Adv Chronic Dis.* (2018) 9:65–72. doi: 10.1177/2040622317742095
7. Lichtiger S, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, et al. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J Med.* (1994) 330:1841–5. doi: 10.1056/NEJM199406303302601
8. Rolny P, Vatn M. Cyclosporine in patients with severe steroid refractory ulcerative colitis in the era of infliximab. *Review article Scand J Gastroenterol.* (2013) 48:131–5. doi: 10.3109/00365521.2012.733954
9. Lee J. Use of antioxidants to prevent cyclosporine a toxicity. *Toxicol Res.* (2010) 26:163–70. doi: 10.5487/TR.2010.26.3.163
10. Jarnerot G, Hertervig E, Friis-Liby I, Blomquist L, Karlen P, Granno C, et al. Infliximab as rescue therapy in severe to moderately severe ulcerative colitis: a randomized, placebo-controlled study. *Gastroenterology.* (2005) 128:1805–11. doi: 10.1053/j.gastro.2005.03.003
11. Brandse, JF, Mathot, RA, van der Kleij D, Rispens T, Ashruf Y, Jansen, JM, et al. Pharmacokinetic features and presence of antidrug antibodies associate with response to infliximab induction therapy in patients with moderate to severe ulcerative colitis. *Clin Gastroenterol Hepatol.* (2016) 14:251–8.e1–2. doi: 10.1016/j.cgh.2015.10.029
12. Laharie D, Bourreille A, Branche J, Allez M, Bouhnik Y, Filippi J, et al. Ciclosporin versus infliximab in patients with severe ulcerative colitis refractory to intravenous steroids: a parallel, open-label randomised controlled trial. *Lancet.* (2012) 380:1909–15. doi: 10.1016/S0140-6736(12)61084-8
13. Williams JG, Alam MF, Alrubaiy L, Clement C, Cohen D, Grey M, et al. Comparison of iNfliximab and ciclosporin in STeroid resistant ulcerative colitis: pragmatic randomised trial and economic evaluation (CONSTRUCT). *Health Technol Assess.* (2016) 20:1–320. doi: 10.3310/hta20440
14. Narula N, Marshall JK, Colombel JF, Leontiadis GI, Williams JG, Muqtadir Z, et al. Systematic review and meta-analysis: infliximab or cyclosporine as rescue therapy in patients with severe ulcerative colitis refractory to steroids. *Am J Gastroenterol.* (2016) 111:477–91. doi: 10.1038/ajg.2016.7
15. Komaki Y, Komaki F, Micic D, Yamada A, Suzuki Y, Sakuraba A. Pharmacologic therapies for severe steroid refractory hospitalized ulcerative colitis: a network meta-analysis. *J Gastroenterol Hepatol.* (2017) 32:1143–51. doi: 10.1111/jgh.13674
16. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg.* (2010) 8:336–41. doi: 10.1016/j.ijsu.2010.02.007
17. ICH. Guideline for good clinical practice E6 (R2). In: *The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).* (2019). Available online at: https://www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html (accessed May 5, 2019).
18. Laharie D, Bourreille A, Branche J, Allez M, Bouhnik Y, Filippi J, et al. Long-term outcome of patients with steroid-refractory acute severe UC treated with ciclosporin or infliximab. *Gut.* (2018) 67:237–43. doi: 10.1136/gutjnl-2016-313060
19. Scimeca D, Bossa F, Annese V, Biscaglia G, Caruso N. Infliximab vs oral cyclosporin in patients with severe ulcerative colitis refractory to intravenous steroids. A controlled, randomized study. United European Gastroenterology week. *Gut.* (2012) 61(Suppl. 3):A165.
20. Croft A, Walsh A, Doecke J, Cooley R, Howlett M, Radford-Smith G. Outcomes of salvage therapy for steroid-refractory acute severe ulcerative colitis: ciclosporin vs. infliximab. *Aliment Pharmacol Ther.* (2013) 38:294–302. doi: 10.1111/apt.12375
21. Daperno M, Sostegni R, Scaglione N, Ercole E, Rigazio C, Rocca R, et al. Outcome of a conservative approach in severe ulcerative colitis. *Dig Liver Dis.* (2004) 36:21–8. doi: 10.1016/j.dld.2003.04.001
22. Dean KE, Hikaka J, Huakau JT, Walmsley RS. Infliximab or cyclosporine for acute severe ulcerative colitis: a retrospective analysis. *J Gastroenterol Hepatol.* (2012) 27:487–92. doi: 10.1111/j.1440-1746.2011.06958.x
23. Duijvis NW, Ten Hove AS, Ponsioen CI, van den Brink, GR, Buskens, CJ, Bemelman, WA, et al. Similar short- and long-term colectomy rates with ciclosporin and infliximab treatment in hospitalised ulcerative colitis patients. *J Crohns Colitis.* (2016) 10:821–7. doi: 10.1093/ecco-jcc/jjw031
24. Kim, EH, Kim, DH, Park, SJ, Hong, SP, Kim, TI, Kim, WH, et al. Infliximab versus cyclosporine treatment for severe corticosteroid-refractory ulcerative colitis: a Korean, retrospective, single center study. *Gut Liver.* (2015) 9:601–6. doi: 10.5009/gnl14120
25. Mocciaro F, Renna S, Orlando A, Rizzuto G, Sinagra E, Orlando E, et al. Cyclosporine or infliximab as rescue therapy in severe refractory ulcerative colitis: early and long-term data from a retrospective observational study. *J Crohns Colitis.* (2012) 6:681–6. doi: 10.1016/j.crohns.2011.11.021
26. Naves JE, Llao J, Ruiz-Cerulla A, Romero C, Manosa M, Lobaton T, et al. Long-term comparative efficacy of cyclosporine- or infliximab-based strategies for the management of steroid-refractory ulcerative colitis attacks. *Inflamm Bowel Dis.* (2014) 20:1375–81. doi: 10.1097/MIB.0000000000000101
27. Ordás I, Domènech E, Mañosa M, García-Sánchez V, Iglesias-Flores E, Peñalva M, et al. Long-term efficacy and safety of cyclosporine in a cohort of steroid-refractory acute severe ulcerative colitis patients from the ENEIDA registry (1989–2013): a nationwide multicenter study. *Am J Gastroenterol.* (2017) 112:1709–18. doi: 10.1038/ajg.2017.180
28. Protic M, Seibold F, Schoepfer A, Radojicic Z, Juillerat P, Bojic D, et al. The effectiveness and safety of rescue treatments in 108 patients with steroid-refractory ulcerative colitis with sequential rescue therapies in a subgroup of patients. *J Crohns Colitis.* (2014) 8:1427–37. doi: 10.1016/j.crohns.2014.05.004
29. Radojcic M, Macrae F, Viney B Infliximab versus cyclosporine in severe, steroid-refractory ulcerative colitis; a comparison of clinical outcomes. *J Gastroenterol Hepatol.* (2014) 29:116–7.
30. Sjöberg M, Walch A, Meshkat M, Gustavsson A, Järnerot G, Vogelsang H, et al. Infliximab or cyclosporine as rescue therapy in hospitalized patients with steroid-refractory ulcerative colitis: a retrospective observational study. *Inflamm Bowel Dis.* (2012) 18:212–8. doi: 10.1002/ibd.21680
31. Song EM, Lee SH, Chang K, Hwang SW, Park SH, Yang DH, et al. Long-term outcomes of cyclosporine A and infliximab treatment for the management of steroid-refractory acute severe ulcerative colitis. *J Crohn's and Colitis.* (2018) 12:S182. doi: 10.1093/ecco-jcc/jjx180.294
32. Guyot P, Ades AE, Ouwens MJ, Welton NJ. Enhanced secondary analysis of survival data: reconstructing the data from published Kaplan-Meier survival curves. *BMC Med Res Methodol.* (2012) 12:9. doi: 10.1186/1471-2288-12-9
33. Wells GA, Shea B, O'Connell D. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses.* (2019). Available online at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (accessed May 5, 2019).
34. Higgins, JP, Altman, DG, Gotzsche, PC, Juni P, Moher D, Oxman, AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ.* (2011) 343:d5928. doi: 10.1136/bmj.d5928
35. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* (1986) 7:177–88. doi: 10.1016/0197-2456(86)90046-2
36. Thorlund K, Engstrom J, Wetterslev J. *User Manual for Trial Sequential Analysis (TSA).* Copenhagen: Copenhagen Trial Unit, Centre for Clinical Intervention Research (2019). Available online at: www.ctu.dk/tsa (accessed June 15, 2019).
37. Schünemann H, Brozek J, Guyatt G, Oxman A. *Handbook for Grading the Quality of Evidence and the Strength of Recommendations Using The GRADE Approach.* (2013). Available online at: https://gdt.gradepro.org/app/handbook/handbook.html#h.svwngs6pm0f2 (accessed May 5, 2019).

38. Lynch RW, Lowe D, Protheroe A, Driscoll R, Rhodes JM, Arnott ID. Outcomes of rescue therapy in acute severe ulcerative colitis: data from the United Kingdom inflammatory bowel disease audit. *Aliment Pharmacol Ther.* (2013) 38:935–45. doi: 10.1111/apt.12473
39. Govani SM, Waljee AK, Stidham RW, Higgins PD. Infliximab reduces 90 day colectomy rate versus cyclosporine for acute severe ulcerative colitis. *Gastroenterology.* (2014) 146:S464. doi: 10.1016/S0016-5085(14)61661-9
40. Nelson R, Liao C, Fichera A, Rubin DT, Pekow J. Rescue therapy with cyclosporine or infliximab is not associated with an increased risk for postoperative complications in patients hospitalized for severe steroid-refractory ulcerative colitis. *Inflamm Bowel Dis.* (2014) 20:14–20. doi: 10.1097/01.MIB.0000437497.07181.05
41. Yoshimura N, Tadami T, Kawaguchi T, Sako M, Saniabadi A, Takazoe M. Comparative short-term efficacy of cyclosporine, tacrolimus and infliximab in hospitalized patients with severe corticosteroid-refractory ulcerative colitis: a retrospective study. *Gastroenterology.* (2013) 144:S209–10. doi: 10.1016/S0016-5085(13)60738-6
42. Cunningham G, Macisaac M, Ding N, Spizzo P, Connell W, Wright E, et al. Colectomy rates remain high in the era of rescue therapy for acute severe ulcerative colitis: a single-center experience. *J Gastroenterol Hepatol.* (2017) 32:124. doi: 10.1111/jgh.13895
43. Chaparro M, Burgueño P, Iglesias E, Panés J, Muñoz F, Bastida G, et al. Infliximab salvage therapy after failure of ciclosporin in corticosteroid-refractory ulcerative colitis: a multicentre study. *Aliment Pharmacol Therapeut.* (2012) 35:275–83. doi: 10.1111/j.1365-2036.2011.04934.x
44. Motoya S, Miyakawa M, Nasuno M, Tanaka H. Short-and long-term outcomes of infliximab and calcineurin inhibitor treatment for steroidrefractory ulcerative colitis. *J Crohn's Colitis.* (2016) 10:S291–2. doi: 10.1093/ecco-jcc/jjw019.502
45. Patrick D, Ballard E, O'Rourke P, Howlett M, Radford-Smith, GL. Comparing colectomy rates in treatment naïve vs. Treatment experienced patients with acute severe ulcerative colitis (ASUC). *J Gastroenterol Hepatol.* (2015) 30:135. doi: 10.1111/jgh.13094
46. Leblanc S, Allez M, Seksik P, Flourie B, Peeters H, Dupas, JL, et al. Successive treatment with cyclosporine and infliximab in steroid-refractory ulcerative colitis. *Am J Gastroenterol.* (2011) 106:771–7. doi: 10.1038/ajg.2011.62
47. Shepherd S, Wright, EK, Holmes, JA, Brown, SJ, Lust M, Kamm, MA, et al. Outcomes of salvage therapy for acute severe colitis treatment in a single tertiary center: infliximab v. cyclosporine. *J Gastroenterol Hepatol.* (2014) 29:123. doi: 10.1111/jgh.12736-6
48. Protic M, Frei P, Radojicic, ZA, Schoepfer A, Juillerat P, Mottet C, et al. Comparative long-term outcomes of tacrolimus, cyclosporine and infliximab for steroid-refractory ulcerative colitis-week 52 results swiss IBD cohort study. *Gastroenterology.* (2013) 144:S428–9. doi: 10.1016/S0016-5085(13)61578-4
49. Duijvis N, Ten Hove A, Ponsioen C, Van Den Brink G, Te Velde A, D'Haens G, et al. Lower long-term colectomy rates with IFX than with CsA treatment in moderate to severe UC. *J Crohns Colitis.* (2015) 9:S53–4. doi: 10.1093/ecco-jcc/jju027.080
50. Seibold F, Protic M, Frei P, Mottet C, Juillerat P, Knezevic T, et al. Colectomy rate two years after sequential rescue treatments in steroid refractory ulcerative colitis. *Gastroenterology.* (2017) 152:S409. doi: 10.1016/S0016-5085(17)31597-4
51. Truelove SC, Witts LJ. Cortisone in ulcerative colitis; preliminary report on a therapeutic trial. *Br Med J.* (1954) 2:375–8. doi: 10.1136/bmj.2.4884.375
52. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N Engl J Med.* (1987) 317:1625–9. doi: 10.1056/NEJM198712243172603
53. Travis, SP, Farrant, JM, Ricketts C, Nolan, DJ, Mortensen, NM, Kettlewell, MG, et al. Predicting outcome in severe ulcerative colitis. *Gut.* (1996) 38:905–10. doi: 10.1136/gut.38.6.905
54. Ho, GT, Mowat C, Goddard, CJ, Fennell, JM, Shah, NB, Prescott, RJ, et al. Predicting the outcome of severe ulcerative colitis: development of a novel risk score to aid early selection of patients for second-line medical therapy or surgery. *Aliment Pharmacol Ther.* (2004) 19:1079–87. doi: 10.1111/j.1365-2036.2004.01945.x
55. Brandse, JF, van den Brink, GR, Wildenberg, ME, van der Kleij D, Rispens T, Jansen, JM, et al. Loss of infliximab into feces is associated with lack of response to therapy in patients with severe ulcerative colitis. *Gastroenterology.* (2015) 149:350–5.e2. doi: 10.1053/j.gastro.2015.04.016
56. Dotan I, Ron Y, Yanai H, Becker S, Fishman S, Yahav L, et al. Patient factors that increase infliximab clearance and shorten half-life in inflammatory bowel disease: a population pharmacokinetic study. *Inflamm Bowel Dis.* (2014) 20:2247–59. doi: 10.1097/MIB.0000000000000212
57. Seow CH, Newman A, Irwin SP, Steinhart AH, Silverberg MS, Greenberg GR. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. *Gut.* (2010) 59:49–54. doi: 10.1136/gut.2009.183095
58. Nalagatla N, Falloon K, Tran G, Borren, NZ, Avalos D, Luther J, et al. Effect of accelerated infliximab induction on short- and long-term outcomes of acute severe ulcerative colitis: a retrospective multicenter study and meta-analysis. *Clin Gastroenterol Hepatol.* (2019) 17:502–9.e1. doi: 10.1016/j.cgh.2018.06.031
59. Lowenberg M, Duijvis NW, Ponsioen C, van den Brink GR, Fockens P, D'Haens GR. Length of hospital stay and associated hospital costs with infliximab versus cyclosporine in severe ulcerative colitis. *Eur J Gastroenterol Hepatol.* (2014) 26:1240–6. doi: 10.1097/MEG.0000000000000187

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Clinical Value of Fecal Calprotectin in Predicting Mucosal Healing in Patients With Ulcerative Colitis

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Aim: This study aimed to evaluate the clinical significance of fecal calprotectin (FC) in assessment of ulcerative colitis (UC) patients' endoscopic patterns and clinical manifestation.

Methods: A total of 143 UC patients who received colonoscopy and 108 controls were included. After providing stool samples, patients underwent total colonoscopy. FC was measured by an enzyme-linked immunosorbent assay (ELISA). Clinical activity was based on the Mayo score. Endoscopic findings was scored by the Ulcerative Colitis Endoscopic Index of Severity (UCEIS). Correlation analysis and receiver-operator characteristic (ROC) analysis were carried out to determine the significance of measurements.

Results: The median (interquartile range, IQR) of FC levels was 211 (43–990) μg/g in UC and 87.5 (40.50∼181) μg/g in the control group. Fecal calprotectin correlated significantly with both Mayo and UCEIS scores (Spearman's r 0.670 and 0.592, $P < 0.01$). With a cut-off value of 164 μg/g for fecal calprotectin concentration, the area under the curve (AUC) in receiver operator characteristic analysis was 0.830, sensitivity was 85.42%, specificity was 73.68%, positive predictive value (PPV) was 62.12%, and negative predictive value (NPV) was 9.10% in predicting clinical active disease. Similarly, the power of FC to predict mucosal healing (MH) was modest. With a cut-off value of 154.5 μg/g, the AUC was 0.839, sensitivity was 72.34%, and specificity was 85.71%.

Conclusion: For evaluating the disease activity of UC, FC is a clinically relevant biomarker for both clinically active disease and MH in patients with UC. But the cut-off value still needs large and multicenter studies for confirmation.

Keywords: biomarkers, ulcerative colitis, fecal calprotectin, mucosal healing, clinical value

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INTRODUCTION

Ulcerative colitis (UC) is a chronic disease with a remitting and relapsing course. For evaluation of disease course and for monitoring treatment response, reliable tools are essential. Assessment of UC activity in clinic is usually based on a combination of clinical manifestations and laboratory tests. The current gold standard is colonoscopy because symptoms do not precisely reflect intestinal inflammation and mucosal healing (1). Endoscopic procedures, however, are unpleasant, sometimes painful, and time-consuming in China. Fecal calprotectin (FC) is a calcium-binding, cytosolic protein in neutrophils which has antimicrobial and antiproliferative properties.

Fecal calprotectin concentration reflects the increased migration of neutrophils through the inflamed bowel wall to the lumen (2). In stool, calprotectin is degradation-resistant, stable, and easily measurable by ELISA (3). The test has been used successfully to distinguish inflammatory from functional bowel disorders (4). Recent studies suggested that FC levels correlate well with endoscopic indices of UC activity including Matts' index (5), Sutherland criteria (6), Rachmilewitz index (7), and the Mayo endoscopic subscore (8). In addition, elevated FC may indicate an increased risk of disease relapse (9, 10).

Since longstanding active inflammation is also considered a risk factor for the development of tissue destruction, dysplasia, and cancer (11), healing of the mucosa may also lead to a reduction in those complications. For these reasons, mucosal healing has been brought into the treat to target era. The current study found that a subgroup of patients had persistently active endoscopic inflammation while in clinical remission (12). Obviously, a noninvasive biomarker to identify patients with MH is preferable in clinical settings. This could allow more regular assessment of inflammation and possibly lead to a reduced requirement for follow-up endoscopies.

In recent years, various biomarkers of MH have been explored such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Because in UC patients, inflammation is mainly confined to the colon and the rectum, it may be reasonable that a fecal marker is more accurate than a serum marker.

The aim of this study was to evaluate the clinical significance of FC in the assessment of UC clinical activity and MH. Additionally, cut-off levels were also determined for the clinical activity and MH.

METHODS

Patients

A total of 143 adult outpatients and inpatients with a previously confirmed diagnosis of UC referred for colonoscopy at the Departments of Gastroenterology of the First Affiliated Hospital of Zhejiang Chinese Medical University between May 2015 and December 2016 were included. They were diagnosed on the basis of clinical, endoscopic, and histologic criteria. A second cohort of 108 healthy volunteers served as controls. The disease extension was classified according to the Montreal classification (13). Exclusion criteria included pregnancy, colorectal cancer, history of bowel resection, long-term use of NSAIDs, or presence of comorbidities that could cause inflammatory reactions, active infection, incomplete colonoscopy (not reaching the cecum), and inability to provide stool samples.

Clinical disease severity was assessed according to Mayo scores. Clinical disease activity was divided into clinical remission (0–2), mild (3–5), moderate (6–10), and severe (11–12) according to the frequency of defecation, hematochezia, and findings of colonoscopy and physician's global assessment. The UCEIS score (14, 15), composed of vascular pattern (0–2), bleeding (0–3), and erosions and ulcers (0–3), was applied to evaluate endoscopic activity, while MH (16) was defined as UCEIS 0 or 1, and UCEIS 1 was limited to vascular patterns.

Study Protocol

Patients provided stool samples within the previous 7 days of the colonoscopy (prior to bowel preparation), and the stool samples were stored at −20°C until assay. After bowel preparation, patients underwent total colonoscopy, and UCEIS score was used to assess MH. The greatest score in any anatomical site was recorded.

Fecal Calprotectin Assays

Stools were collected within the previous 7 days of the colonoscopy, and immediately stored at −20°C. The stool samples were sent to Suzhou Herui IBD Project Center, and fecal calprotectin was measured in a blind manner using the PhiCal enzyme-linked immunosorbent assay (ELISA) Assay.

Statistics

For numerical variables, median and interquartile range (IQR) were calculated, and the Mann–Whitney *U*-test was applied. The Spearman correlation analysis between FC and clinical/endoscopic disease severity was carried out. The best cut-off for FC to predict clinical activity and MH were calculated by using receiver–operator characteristic (ROC) graphs. According to the cut-off levels, test significance including sensitivity (SENS), specificity (SPEC), positive–predictive value (PPV), negative predictive value (NPV), and accuracy rate (AR) were calculated. Two sided $P < 0.05$ were considered to be statistically significant.

RESULTS

Characteristics of the Participants

Overall, 143 UC patients and 108 controls were included in the study. Among the 143 UC patients (44% women), the mean age at the time of inclusion was 43.64 ± 13.62 years. While ulcerative colitis extent was limited to the rectum in 52

TABLE 1 | Baseline demographic variables of patients included in this study.

	UC	Control
N	143	108
Male/female	80/60	47/61
Age (Mean ± SD)	43.64 ± 13.62	48.53 ± 16.30
Age at diagnosis (years)		
A1 (≤16)	0	
A2 (17–40)	88 (61.54%)	
A3 (≥40)	55 (38.46%)	
Disease location		
Non	20 (13.99%)	
E1	52 (36.36%)	
E2	27 (18.88%)	
E3	44 (30.77%)	
Mayo grades		
Remission (≤2)	49 (34.27%)	
Mild activity (3–5)	46 (32.17%)	
Moderate activity (6–10)	41 (28.67%)	
Severe activity (11–12)	7 (4.90%)	

TABLE 2 | Median fecal calprotectin levels (interquartile range) in patients stratified according to the Mayo grades (μg/g).

Variable	N	FC (μg/g)			
		Median	Quartile	Min∼Max	Interquartile range (IQR)
Control	108	87.5	141	11∼1,560	40.50∼181
UC	143	211	947	17∼6,964	43∼990
Remission	49	38*	73	22∼5,321	30∼102.5
Mild activity	46	220.5	281	17∼5,235	87∼367.75
Moderate activity	41	1,138Δ	2359	26∼6,964	340.50∼2,699
Severe activity	7	2,481ΔΔ	2494	1,414∼6,324	1,573∼4,067

*$p < 0.05$ ($p = 0.002$), vs. the control; Δ$p < 0.05$ ($p = 0.000$), vs. the mild group; ΔΔ$p < 0.05$ ($p = 0.000$, $p = 0.033$, respectively), vs. the mild and moderate group.

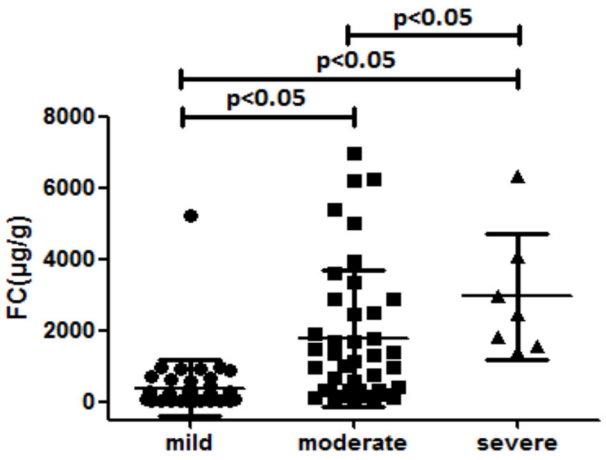

FIGURE 1 | Median fecal calprotectin levels (interquartile range) in patients stratified according to the Mayo grades (μg/g).

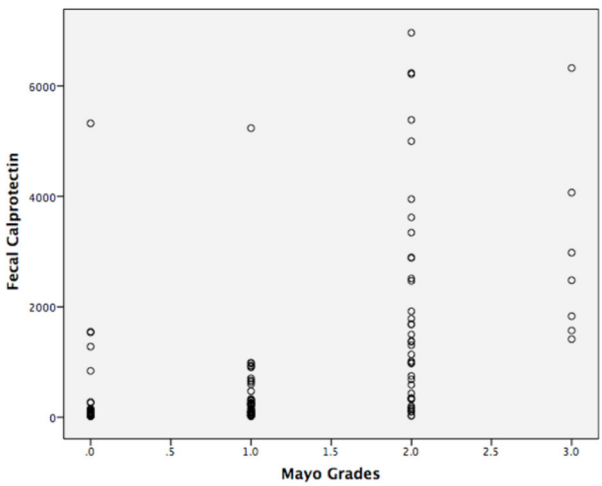

FIGURE 2 | Concentrations of fecal calprotectin and the Mayo grades of UC ($r = 0.670$, $p < 0.01$).

patients (36.36%), 27 patients (18.88%) had sigmoid/left colon involvement and 44 patients (30.77%) had pancolitis. Patients' characteristics are shown in **Table 1**. According to Mayo scores, 49 (34.27%) patients were in remission, 46 (32.17%) patients had mild, 41 (28.67%) patients had moderate, and 7 (4.90%) patients had severe disease activity. Overall, mucosal healing, defined as UCEIS score 0 or 1, was observed in 48 ulcerative colitis patients (33.57%).

In total, 108 controls were studied (56% women). Their median age was 48.53 ± 16.30 years. The median fecal calprotectin in this group was 87.5 (IQR 40.50–181) μg/g. The median (IQR) value for FC level of all patients was 211 (43–990) μg/g. There was a significant difference in the FC concentration between the UC and the controls ($P < 0.05$; **Table 2**). The FC concentration were 38 (30–102.5) μg/g, 220.5 (87–367.75) μg/g, 1,138 (340.50–2699) μg/g, and 2,481 (1573–4067) μg/g, respectively with each stage classified by Mayo scores. As seen in **Figure 1** and **Table 2**, there was a significant difference in FC levels between patients with mild disease and moderate disease ($P < 0.05$) as well as between moderate disease and severe disease ($P < 0.05$).

Correlation Analysis

The correlation analysis is shown in **Figures 2**, **3**. The Mayo grades and the UCEIS scores both correlated very well with the FC levels ($r = 0.670$, $P < 0.01$, and $r = 0.592$, $P < 0.01$, respectively).

ROC Curve Analysis

Using a ROC curve, we attempted to determine the best cut-off value of FC to detect clinical activity and MH. The area under the ROC curve to predict clinical activity and MH was 0.830 and 0.839, respectively (**Figures 4**, **5**). The best cut-off point to detect clinical activity was 164 μg/g (sensitivity 85.42%, specificity 73.68%, PPV 62.12%, NPV 9.10%, AR 77.62%). A cut-off value of 154.5 μg/g indicated MH, with sensitivity of 72.34%, specificity of 85.71%, PPV 90.67%, NPV 38.24%, and AR 76.92%.

DISCUSSION

In the present study, we assessed the correlation between fecal calprotectin level and clinical/endoscopic scores in UC and

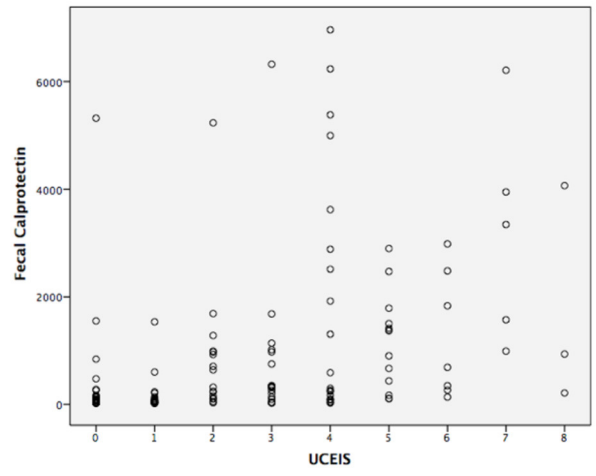

FIGURE 3 | Concentrations of fecal calprotectin and the UCEIS scores of UC ($r = 0.670$, $p < 0.01$).

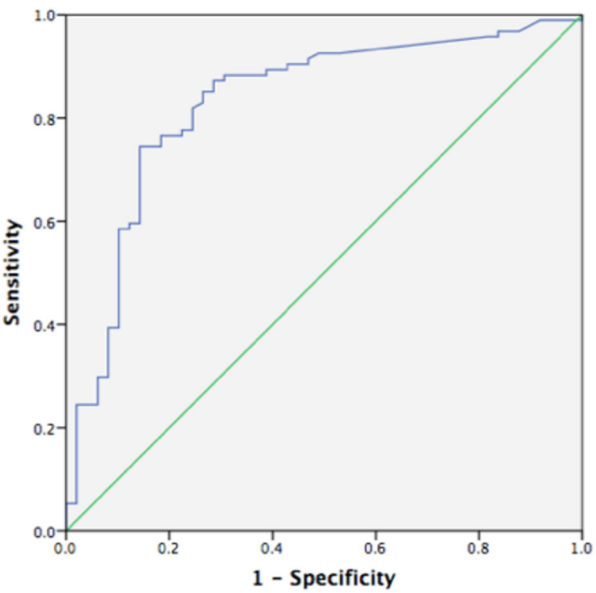

FIGURE 4 | Receiver operating curve analysis (ROC) of fecal calprotectin in detecting clinical activity (AUC 0.830, $p < 0.05$, 95%CI 0.755~0.904).

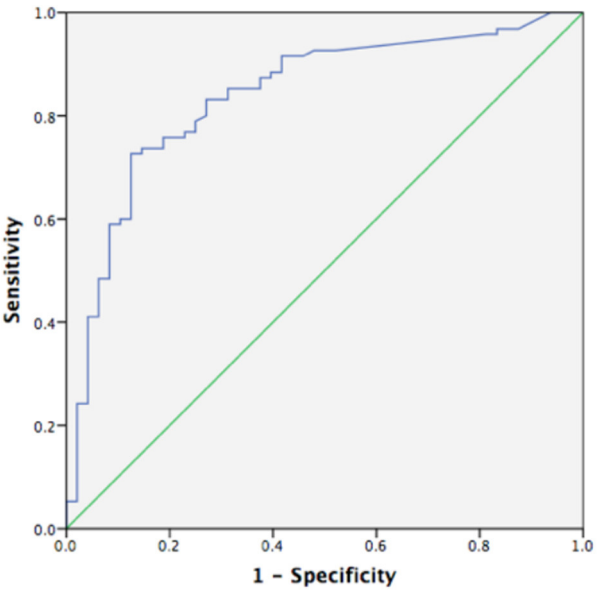

FIGURE 5 | Receiver operating curve analysis (ROC) of fecal calprotectin in MH (AUC 0.839, $p < 0.05$, 95%CI 0.769~0.909).

showed the performances of FC in detecting clinical activity and endoscopic mucosal healing.

Fecal calprotectin is an abundant protein in neutrophils, which infiltrates the mucosa during inflammation. Data support its use in differentiating inflammatory bowel disease (IBD) from irritable bowel syndrome (IBS) (17–20), evaluating abdominal discomfort (21). Several reports have shown that FC level correlates well with clinical, endoscopic, and histological parameters of disease activity (6, 19) in UC patients. To some extent, FC may reflect disease activity in UC better than in CD as some authors reported (22). FC determination may also be useful in predicting impending clinical relapse especially during the following 3 months in both CD and UC patients (23). FC is also useful in assessing treatment response (24–26).

In the management of patients with UC, endoscopy has an essential role in viewing and evaluating the severity of disease activity in the intestinal mucosa as well as assessing the efficacy of treatment modalities. However, discordance in clinical manifestations and endoscopic findings is not rare. Clinical indices are not reliable in assessing endoscopic MH and in predicting the disease course (27, 28). Evolving evidence indicates that MH is associated with lower risk of long-term complications (29–31). Therefore, currently, MH is of great interest to gastroenterologists and considered as an ideal therapeutic target. However, the exact definition of MH continues to be controversial and several scoring systems have been developed. In our study, we applied UCEIS to define MH as the remission stratum that corresponds to UCEIS 0 or 1. Further, we limited the UCEIS score 1 to a vascular pattern descriptor, so that score 1 of the bleeding descriptor and score 1 of the erosions and ulcers descriptor do not mean real MH. Arai et al. (32) recently reported that UCEIS is useful to predict clinical outcomes and long-term prognosis in UC patients with clinical remission. Consequently, FC had a good correlation with UCEIS. Additionally, we suggest that a definition of MH based on the UCEIS scores may be more relevant.

A recent systematic review (33) showed that fecal markers like FC are promising non-invasive indicators of MH. It is imperative that non-invasive markers become available for routine clinical use. In other words, this could allow more regular assessment of inflammation with subsequent timely clinical

decisions and possibly lead to a reduced requirement for follow-up endoscopies. Schoepfer's study (7), the largest study so far, described the diagnostic efficiency of FC to predict mucosal inflammation with sensitivity 93%, specificity 71%, PPV 91%, and NPV 81% using a cut-off 50 μg/g. Yamaguchi et al. (34) analyzed the correlation between FC with both Mayo endoscopic subscore 0 or Mayo endoscopic subscore 0 and 1 defining MH. Not surprisingly, specificity and PPV were greater when using the Mayo 0 score. Based on the interpretations of the ROC graphs, using UCEIS defining MH, we obtained a cut-off FC level of 154.5 μg/g to predict MH with sensitivity 72.34%, specificity 85.71%, and PPV 90.67%. It is not surprising that there has been no agreement regarding an appropriate cut-off level for FC to predict MH (35). Our results are reasonably comparable with these previously published data.

Our sample size could be considered as a limitation of our study. Second, using FC as a predictive tool for MH requires analysis from clinically quiescent patients. This is the biggest weaknesses in our study. Third, the FC levels have also been shown to be variable (36), to overcome this problem we ensured that all patients provided stool samples at least 1 week post biologic administrations. Combination of clinical symptoms and serum and fecal biomarkers is likely to be superior to one single parameter. Such analyses will require well-powered and multicenter studies.

In conclusion, fecal calprotectin could reflect the disease activity of UC and are rational fecal markers of intestinal inflammation for clinical application. FC is also a clinically relevant biomarker of MH in patients with UC, but the value of the cut-off still needs large and multicenter studies for confirmation.

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 Ethics Committee of the First Affiliated Hospital of Zhejiang University of Traditional Chinese Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Y-HF designed the report. FC and YH collected the clinical data. BL contributed to revising the manuscript. FC wrote the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Gomes P, du Boulay C, Smith CL, Holdstock G. Relationship between disease activity indices and colonoscopic findings in patients with colonic inflammatory bowel disease. *Gut.* (1986) 27:92–5. doi: 10.1136/gut.27.1.92
2. Røseth AG, Schmidt PN, Fagerhol MK. Correlation between faecal excretion of indium-111-labelled granulocytes and calprotectin, a granulocyte marker protein, in patients with inflammatory bowel disease. *Scand J Gastroenterol.* (1999) 34:50–4. doi: 10.1080/00365529950172835
3. Røseth AG, Fagerhol MK, Aadland E, Schjønsby H. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. *Scand J Gastroenterol.* (1992) 27:793–8. doi: 10.3109/00365529209011186
4. Carroccio A, Iacono G, Cottone M, Di Prima L, Cartabellotta F, Cavataio F et al. Diagnostic accuracy of fecal calprotectin assay in distinguishing organic causes of chronic diarrhea from irritable bowel syndrome: a prospective study in adults and children. *Clin Chem.* (2003) 49(6 Pt 1):861–7. doi: 10.1373/49.6.861
5. Hanai H, Takeuchi K, Iida T, Kashiwagi N, Saniabadi AR, Matsushita I et al. Relationship between fecal calprotectin, intestinal inflammation, and peripheral blood neutrophils in patients with active ulcerative colitis. *Dig Dis Sci.* (2004) 49:1438–43. doi: 10.1023/B:DDAS.0000042243.47279.87
6. Xiang JY, Ouyang Q, Li GD, Xiao NP. Clinical value of fecal calprotectin in determining disease activity of ulcerative colitis. *World J Gastroenterol.* (2008) 14:53–7. doi: 10.3748/wjg.14.53
7. Schoepfer AM, Beglinger C, Straumann A, Trummler M, Renzulli P, Seibold F. Ulcerative colitis: correlation of the Rachmilewitz endoscopic activity index with fecal calprotectin, clinical activity, C-reactive protein, and blood leukocytes. *Inflamm Bowel Dis.* (2009) 15:1851–8. doi: 10.1002/ibd.20986
8. D'Haens G, Ferrante M, Vermeire S, Baert F, Noman M, Moortgat L et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. *Inflamm Bowel Dis.* (2012) 18:2218–24. doi: 10.1002/ibd.22917
9. Tibble JA, Sigthorsson G, Bridger S, Fagerhol MK, Bjarnason I. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. *Gastroenterology.* (2000) 119:15–22. doi: 10.1053/gast.2000.8523
10. Maiden L, Takeuchi K, Baur R, Bjarnason I, O'Donohue J, Forgacs I et al. Selective white cell apheresis reduces relapse rates in patients with IBD at significant risk of clinical relapse. *Inflamm Bowel Dis.* (2008) 14:1413–8. doi: 10.1002/ibd.20505
11. Rutter M, Saunders B, Wilkinson K, Rumbles S, Schofield G, Kamm M et al. Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology.* (2004) 126:451–9. doi: 10.1053/j.gastro.2003.11.010
12. Rosenberg L, Lawlor GO, Zenlea T, Goldsmith JD, Gifford A, Falchuk KR et al. Predictors of endoscopic inflammation in patients with ulcerative colitis in clinical remission. *Inflamm Bowel Dis.* (2013) 19:779–84. doi: 10.1097/MIB.0b013e3182802b0e
13. Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The montreal classification of inflammatory bowel disease: controversies, consensus, and implications. *Gut.* (2006) 55:749–53. doi: 10.1136/gut.2005.082909
14. Travis SP, Schnell D, Krzeski P, Abreu MT, Altman DG, Colombel JF et al. Developing an instrument to assess the endoscopic severity of ulcerative colitis: the ulcerative colitis endoscopic index of severity (UCEIS). *Gut.* (2012) 61:535–42. doi: 10.1136/gutjnl-2011-300486
15. Travis SP, Schnell D, Krzeski P, Abreu MT, Altman DG, Colombel JF et al. Reliability and initial validation of the ulcerative

colitis endoscopic index of severity. *Gastroenterology.* (2013) 145:987–95. doi: 10.1053/j.gastro.2013.07.024
16. Ikeya K, Hanai H, Sugimoto K, Osawa S, Kawasaki S, Iida T et al. The ulcerative colitis endoscopic index of severity more accurately reflects clinical outcomes and long-term prognosis than the mayo endoscopic score. *J Crohns Colitis.* (2016) 10:286–95. doi: 10.1093/ecco-jcc/jjv210
17. Sutherland AD, Gearry RB, Frizelle FA. Review of fecal biomarkers in inflammatory bowel disease. *Dis Colon Rectum.* (2008) 51:1283–91. doi: 10.1007/s10350-008-9310-8
18. Schoepfer AM, Trummler M, Seeholzer P, Seibold-Schmid B, Seibold F. Discriminating IBD from IBS: comparison of the test performance of fecal markers, blood leukocytes, CRP, and IBD antibodies. *Inflamm Bowel Dis.* (2008) 14:32–9. doi: 10.1002/ibd.20275
19. Gisbert JP, McNicholl AG. Questions and answers on the role of faecal calprotectin as a biological marker in inflammatory bowel disease. *Dig Liver Dis.* (2009) 41:56–66. doi: 10.1016/j.dld.2008.05.008
20. Mumolo MG, Bertani L, Ceccarelli L, Laino G, Di Fluri G, Albano E et al. From bench to bedside: fecal calprotectin in inflammatory bowel diseases clinical setting. *World J Gastroenterol.* (2018) 24:3681–94. doi: 10.3748/wjg.v24.i33.3681
21. Manz M, Burri E, Rothen C, Tchanguizi N, Niederberger C, Rossi L et al. Value of fecal calprotectin in the evaluation of patients with abdominal discomfort: an observational study. *BMC Gastroenterol.* (2012) 12:5. doi: 10.1186/1471-230X-12-5
22. Costa F, Mumolo MG, Ceccarelli L, Bellini M, Romano MR, Sterpi C et al. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. *Gut.* (2005) 54:364–8. doi: 10.1136/gut.2004.043406
23. Gisbert JP, Bermejo F, Pérez-Calle JL, Taxonera C, Vera I, McNicholl AG et al. Fecal calprotectin and lactoferrin for the prediction of inflammatory bowel disease relapse. *Inflamm Bowel Dis.* (2009) 15:1190–8. doi: 10.1002/ibd.20933
24. Wagner M, Peterson CG, Ridefelt P, Sangfelt P, Carlson M. Fecal markers of inflammation used as surrogate markers for treatment outcome in relapsing inflammatory bowel disease. *World J Gastroenterol.* (2008) 14:5584–9; discussion 5588. doi: 10.3748/wjg.14.5584
25. Sipponen T, Björkesten CG, Färkkilä M, Nuutinen H, Savilahti E, Kolho KL. Faecal calprotectin and lactoferrin are reliable surrogate markers of endoscopic response during Crohn's disease treatment. *Scand J Gastroenterol.* (2010) 45:325–31. doi: 10.3109/00365520903483650
26. Langhorst J, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP. and clinical indices. *Am J Gastroenterol.* (2008) 103:162–9. doi: 10.1111/j.1572-0241.2007.01556.x
27. Peyrin-Biroulet L, Reinisch W, Colombel JF, Mantzaris GJ, Kornbluth A, Diamond R, et al. Clinical disease activity, C-reactive protein normalisation and mucosal healing in Crohn's disease in the SONIC trial. *Gut.* (2014) 63:88–95. doi: 10.1136/gutjnl-2013-304984
28. Lahiff C, Safaie P, Awais A, Akbari M, Gashin L, Sheth S et al. The Crohn's disease activity index (CDAI) is similarly elevated in patients with Crohn's disease and in patients with irritable bowel syndrome. *Aliment Pharmacol Ther.* (2013) 37:786–94. doi: 10.1111/apt.12262
29. Frøslie KF, Jahnsen J, Moum BA, Vatn MH, IBSEN Group. Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort. *Gastroenterology.* (2007). 133:412–22. doi: 10.1053/j.gastro.2007.05.051
30. Ardizzone S, Cassinotti A, Duca P, Mazzali C, Penati C, Manes G et al. Mucosal healing predicts late outcomes after the first course of corticosteroids for newly diagnosed ulcerative colitis. *Clin Gastroenterol Hepatol.* (2011) 9:483–9.e3. doi: 10.1016/j.cgh.2010.12.028
31. Colombel JF, Rutgeerts P, Reinisch W, Esser D, Wang Y, Lang Y et al. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. *Gastroenterology.* (2011) 141:1194–201. doi: 10.1053/j.gastro.2011.06.054
32. Arai M, Naganuma M, Sugimoto S, Kiyohara H, Ono K, Mori K et al. The ulcerative colitis endoscopic index of severity is useful to predict medium- to long-term prognosis in ulcerative colitis patients with clinical remission. *J Crohns Colitis.* (2016) 10:1303–9. doi: 10.1093/ecco-jcc/jjw104
33. Boon GJ, Day AS, Mulder CJ, Gearry RB. Are faecal markers good indicators of mucosal healing in inflammatory bowel disease? *World J Gastroenterol.* (2015) 21:11469–80. doi: 10.3748/wjg.v21.i40.11469
34. Yamaguchi S, Takeuchi Y, Arai K, Fukuda K, Kuroki Y, Asonuma K et al. Fecal calprotectin is a clinically relevant biomarker of mucosal healing in patients with quiescent ulcerative colitis. *J Gastroenterol Hepatol.* (2016) 31:93–8. doi: 10.1111/jgh.13061
35. Kristensen V, Klepp P, Cvancarova M, Røseth A, Skar V, Moum B. Prediction of endoscopic disease activity in ulcerative colitis by two different assays for fecal calprotectin. *J Crohns Colitis.* (2015) 9:164–9. doi: 10.1093/ecco-jcc/jju015
36. Naismith GD, Smith LA, Barry SJ, Munro JI, Laird S, Rankin K et al. A prospective single-centre evaluation of the intraindividual variability of faecal calprotectin in quiescent Crohn's disease. *Aliment Pharmacol Ther.* (2013) 37:613–21. doi: 10.1111/apt.12221

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GB1a Ameliorates Ulcerative Colitis via Regulation of the NF-κB and Nrf2 Signaling Pathways in an Experimental Model

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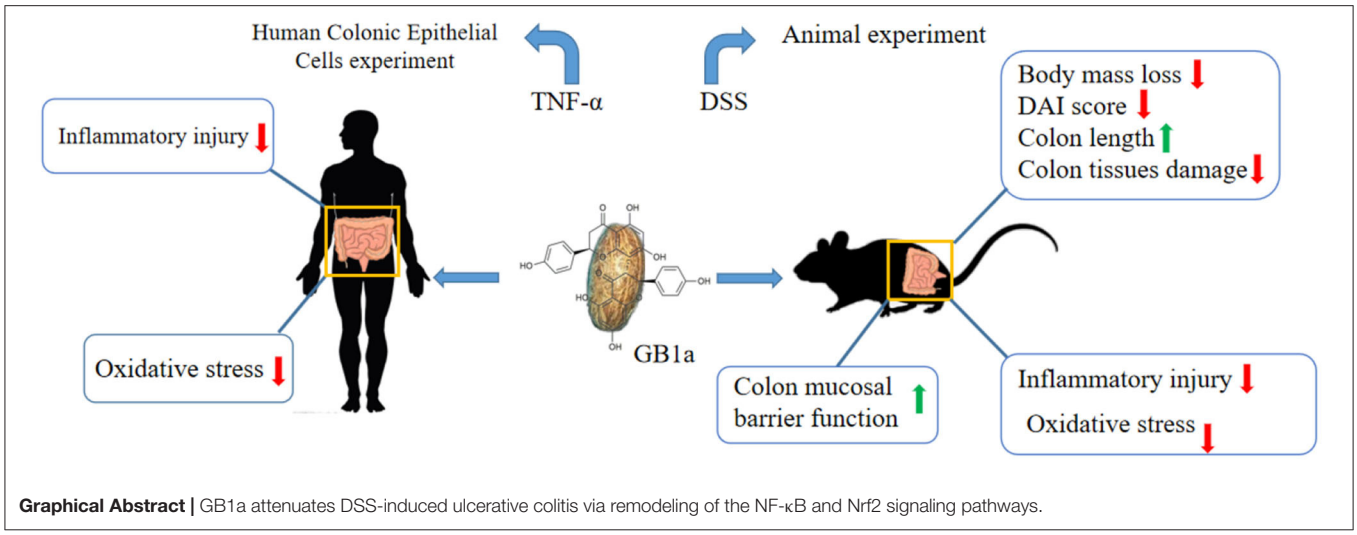
Ulcerative colitis (UC) is an inflammatory bowel disease. The intake of African Garcinia Kola nuts has been reported as a therapy for diarrhea and dysentery in the African population. However, the mechanism of action through which Garcinia Kola nuts act to ameliorates UC remains unknown. GB1a is the main active component of Garcinia Kola nuts. In this study, we explored the therapeutic effects and underlying mechanism of GB1a on dextran sodium sulfate (DSS)-induced UC. Human Colonic Epithelial Cells (HCoEpic) were challenged with TNF-α to test the effects of GB1a in protecting against oxidative stress and inflammation *in vitro*. Our data showed that GB1a significantly attenuated DSS-induced colonic inflammatory injury manifested as reversed loss of body weight and disease activity index (DAI) scores in UC mice. We also showed that GB1a improved the permeability of the intestinal epithelium by modulating the expression of tight junction proteins (ZO-1, Occludin). Mechanistically, GB1a may activate the Nrf2 antioxidant signaling pathway and suppress the nuclear translocation of NF-κB in reduced oxidative stress and expression of inflammatory genes induced by TNF-α in HCoEpic cells. Our study suggests that GB1a alleviates inflammation, oxidative stress and the permeability of the colonic epithelial mucosa in UC mice via the repression of NF-κB and activation of Nrf2 signaling pathway.

Keywords: ulcerative colitis, GB1a, inflammation, oxidative stress, colonic epithelial barrier

INTRODUCTION

Ulcerative colitis (UC), also known as non-specific ulcerative colitis, is a type of inflammatory bowel disease (IBD) that occurs in the rectum and colon (1). During the development of UC, many pathological lesions occur such as ulcers, crypt abscesses, small vessel inflammation, and reduced numbers of goblet and inflammatory cells (2). Inflammation and ulcerative lesions of the mucosa and submucosa are the main pathological features of UC (3). The main clinical symptoms of the disease include abdominal pain, bloody diarrhea, constipation, and fatigue. These symptoms have a major impact on the quality of life for patients and increase the risk of secondary infections and colon cancer in patients with long-term recurrence (4, 5).



Graphical Abstract | GB1a attenuates DSS-induced ulcerative colitis via remodeling of the NF-κB and Nrf2 signaling pathways.

The etiology of UC remains to be fully elucidated but is known to involve interactions between environmental, genetic, and immune factors leading to uncontrolled abnormal immune responses in the intestinal mucosa (6, 7). Studies have identified multiple molecular pathways that are involved in the pathogenesis of UC including the NF-κB pathway, oxidative stress, and the release of related inflammatory cytokines and pro-inflammatory mediators (8–10). Oxidative stress responses result in the infiltration of macrophages into the colon tissues of patients with UC leading to the production of high levels of reactive oxygen species (ROS) (11, 12). These changes act to increase the permeability of the intestinal epithelium and induce further damage in colon tissues leading to the development of intestinal inflammation (13).

Nuclear factor erythroid 2-related factors 2 (Nrf2) is a redox-sensitive transcription factor that protects cells from inflammation and oxidative stress by regulating the transcription of anti-oxidation and detoxification genes including glutathione S-transferase (GST), glutathione peroxidase (GPx), heme oxygenase-1 (HO-1), superoxide dismutase (SOD). Nrf2 enhances the ability of cells to remove electrophilic and reactive oxygen species (ROS) (14–16). Previous studies have shown Nrf2 knockout results in more severe damage in the colon of a UC mouse model which may be to excess generation of ROS generation and inflammatory cytokines (17, 18). Genetic or pharmacological activation of Nrf2 effectively protects mice against the DSS-induced symptoms of UC in mice via remodeling of the Nrf2/ARE and Nrf2/HO-1 pathways.

Previous studies have shown that abnormal activation of NF-κB plays a central role in regulating the release of cytokines in UC patients resulting in severe inflammation and immune response (19, 20). NF-κB and its inhibitor, IκB, stably bind in the cytoplasm. NF-κB dimers are released after degradation of the IκB protein as IκB kinase activation is stimulated by various extracellular factors. Subsequently, NF-κB is further activated by various post-translational modifications and combines with the promoter regions of target genes allowing the expression of downstream targets including TNF-α, IL-6, and IL-1β (21–25). Therefore, the effective suppression of NF-κB may provide a potential therapeutic approach in UC.

GB1a is a bioflavonoid that is extracted from the Garcinia Kola nuts, a tropical evergreen plant of the Garciniaceae and genus Garcinia. Garcinia Kola is widely used as an antioxidant, antibacterial, antiviral, antiulcer, and anti-inflammatory agent (26, 27). Previous studies have reported that flavonoids extracted from Garcinia Kola can reduce inflammation and increase antioxidant capacity by activating Nrf2, yet the active ingredients in the extract remain to be identified (28). In this study, we found that GB1a is effective on DSS-induced UC mice manifested by the recovery bodyweight and decreased DAI scores as well as the improvements in levels of damage in colon tissues. In this study, we explored the therapeutic effects and mechanism of GB1a on dextran sodium sulfate (DSS)-induced UC symptoms.

METHODS AND MATERIALS

Extract Preparation

Garcinia Kola nuts were obtained from Nigeria, Africa. Garcinia Kola nuts were cleaned using fresh tap water to remove dust, air-dried, and then crushed. Extraction was performed twice using 95% (v/v) ethanol and then the solution was evaporated to semi-dryness using a rotary vacuum evaporator at 45°C. The filter residue was added to pure water and refluxed for extraction twice for 1 h. The filtrate was combined and concentrated under reduced pressure to obtain the extract. All of the obtained extracts were dissolved in an appropriate amount of water for extraction and extracted three times with petroleum ether reagent to obtain a petroleum ether layer and a water layer. The water layer was extracted three times with n-butanol to obtain an n-butanol layer and a water layer. The n-butanol layer was concentrated under reduced pressure to obtain the extract.

For High-Performance Liquid Chromatography (HPLC) analysis, 1 mg of extract powder was dissolved in 1 ml of methanol and filtered through a 0.22 μm filter before HPLC

analysis. An Agilent 1260 HPLC (Agilent Technologies, Santa, Clara, CA, USA) equipped with a Zorbax Eclipse Plus C_{18} column (ZORBAX SB-C_{18}, 9.4 × 250 mm, 5 μm) was used for HPLC analysis and preparation. Chromatographic separation was performed at 30°C with a flow rate of 2.5 mL/min. The injection volume was 50 μL and the ultraviolet detection wavelength was set at 360 nm. The mobile phase consisted of methanol (A) and water (B). The gradient elution conditions of the mobile phase A were: 0–20 min, 53–65%; 20–21 min, 65%; 21–30 min, 65–70%; 30–31 min, 70%; 31–35 min, 70–53%; 35–40 min, 53%. After purification by HPLC, a single compound with a purity of 99.7% in the n-butanol extract layer was obtained (**Supplementary Figure 1A**). The identification and analysis of the hydrogen (^{1}H NMR) and carbon spectra (^{13}C NMR) showed that the compound was GB1a (**Supplementary Table 1** and **Supplementary Figures 1B,C**) (29).

Cell Protocols

Human Colonic Epithelial Cells (HCoEpic) were seeded in 96 well plates with 6 well replicates. After culturing for 24 h, the culture was changed to a medium containing GB1a (drug concentration gradient: 0, 2.5, 5.0, 10, 15, 20, 50, 100, 200, 400 μM) and the cells incubated for 24 h after administration. The culture medium was then aspirated and the cells were incubated with a pre-mixed medium containing CCK-8 (100 μL 1640 medium, 10 μL CCK-8 solution). The OD value was measured at 450 nm using a microplate reader. HCoEpic cells were harvested after incubation for 24 h with 30 ng·ml^{-1} TNF-α (TNF-α model group) or TNF-α plus 20 μM/40 μM GB1a (TNF-a+GB1a group). All experiments were performed in triplicate.

Animals

Male C57BL/6 mice (6–8 weeks old, 18–20 g) were purchased from the Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. Mice were maintained in a 12-h dark/light cycle environment with a room temperature of 23 ± 2°C and a relative humidity of 55 ± 5%. Mice had free access to a standard diet and purified water. All animal experiments were performed following protocols and guidelines approved by the Animal Ethics Committee of Guangzhou University of Chinese Medicine. All surgeries were performed under sodium pentobarbital anesthesia.

Induction of Colitis and Treatment Protocol

After 1 week of adaptive feeding, C57BL/6 mice were randomly divided into six groups (7 mice/group) as follows; Control, DSS, DSS+sulfasalazine (SASP, 300 mg·kg^{-1}), DSS+GB1a100, DSS+GB1a50, DSS+GB1a25 (GB1a, 100 mg·kg^{-1}, 50 mg·kg^{-1}, 25 mg·kg^{-1}). During the experimental periods, animals received a daily gavage of SASP (300 mg·kg^{-1}) or GB1a (25, 50, or 100 mg·kg^{-1}) in 0.5% carboxymethyl cellulose from day 1 to day 9. From day 3, for 2 h after administration of GB1a, mice were given 4% DSS (w/v) solution dissolved in sterile distilled water *ad libitum* for 6 days. GB1a and SASP administration continued until the end of the DSS treatment period.

Evaluation of Colitis

Daily observations were performed to assess the symptoms of colitis (body mass loss, the severity of diarrhea, rectal bleeding). The disease activity index (DAI) was evaluated as described (DAI = Score Weight loss (%) + Stool consistency + rectal bleeding) (30). At the end of the experiment, mice were anesthetized by i.p. administration of 10% chloral hydrate. The entire colon was excised and measured. Portions of the colon were fixed in 4% paraformaldehyde, embedded in paraffin and processed for routine hematoxylin and eosin (H&E) staining for examination under a light microscope. The histological scores of the H&E-stained colon specimens were blindly assessed by two pathologists. Histological sections were scored using a validated scoring system as previously described by Dou et al. (31). The remaining parts of the colons were stored at −80°C for further analysis.

Determination of Myeloperoxidase (MPO) Activity in Colon Tissues

Inflammation was assessed by measuring tissue myeloperoxidase (MPO) activity that is linearly related to neutrophil infiltration. MPO activity in the supernatant of the colon homogenate of mice was determined using an MPO assay kit according to the manufacturer's instructions (Nanjing jiancheng, Nanjing, China). The values were expressed as units per gram of tissue in each sample and calculated from the following formula: Myeloperoxidase (MPO) Activity (U/g) = (Measure OD value-control OD value)/11.3 × sampling volume (g).

Assessment of Serum Levels and Antioxidant Parameters

The serum levels of TNF-α and IL-6 were measured using ELISA assay kits (Abclonal Biotechnology Co., Ltd, Wuhan, China) according to the manufacturer's instructions. Assay kits (Nanjing jiancheng, Nanjing, China) were used to measure levels of malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD) in serum.

Mitochondrial DNA Copy Number

The mtDNA copy number was used as a marker for mitochondrial density using qPCR as previously reported (32, 33). Briefly, total DNA was isolated from HCoEpic cells using a Universal Genomic DNA Extraction kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The mitochondrial DNA copy number was calculated from the ratio of the mitochondrial-encoded gene COXII and the nuclear-encoded gene GAPDH. The primer sequences of the genes are shown in **Supplementary Table 2**.

Western Blotting Analysis

Protein was extracted from HCoEpic cells or mouse colon tissues samples. Equal concentrations of proteins were separated on a 10% SDS-polyacrylamide gel and transferred to polyvinylidenefluoride (PVDF) membranes. Western blotting was performed using specific antibodies (Anti-Nrf2, anti-HO-1,

anti-NF-κBp65, anti-ZO-1, anti-Occludin, anti-β-actin, and anti-LaminB) purchased from ABclonal (ABclonal, Biotechnology Co., Ltd.).

Measurement of ROS

HCoEpic cells were seeded in 6-well plates and treated as previously described. Cells were then incubated with DCFH-DA (5 uM) at 37°C for 0.5 h in the dark. Cells were washed three times with PBS and the fluorescence emission was detected using a fluorescence microscope.

Management of Fluorescein Isothiocyanate (FITC)-Dextran

At the end of the DSS treatment period, mice were given fluorescein isothiocyanate (FITC)-dextran solution (4 kDa, 600 mg/kg) by oral gavage. Blood samples were collected from the retinal vein after 4 h.

Statistical Analysis

All results presented in the figures are expressed as the mean ± SEM. The significant differences between multiple groups were detected using a one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) or a Dunnett's test (SPSS20.0). Data were evaluated using GraphPad Prism Version 7.0. *P*-values of <0.05 were considered statistically significant.

RESULTS

GB1a Exerts Anti-inflammatory Effects by Inhibiting the Nuclear Translocation of NF-κB *in vitro*

To investigate the anti-inflammatory function of GB1a, TNFα-incubated HCoEpic cells were used as previously described (34). Consistent with our hypothesis, GB1a displayed lower cytotoxicity (**Figure 1A**) and GB1a treatment effectively reduced the expression of pro-inflammatory genes including TNF-α, IL-1β, and IL-6 (**Figure 1B**). NF-κB is a the master regulator of proinflammatory gene expression. We showed that GB1a could reverse the TNF-α-induced the elevation of NF-κB p65 expression in HCoEpic cells in a dose-dependent manner by inhibiting the nuclear translocation of NF-κB induced by TNF-α (**Figures 1C,D**). To further explore the underlying mechanism, we performed docking analysis between GB1a and NF-κB. Our molecular docking data indicated a high binding affinity via hydrophilic interactions suggesting a potential role of GB1a in regulating NF-κB activity (**Figures 1E,F**). To confirm the mechanism is through the NF-κB signal pathway, we transfected the siRNA- NF-κBp65 on HCoEpi cells. As shown in **Supplementary Figure 2**, the expression of NF-κBp65 was significantly decreased after knocking down NF-κBp65 when compared with control group. In addition, knocking down NF-κBp65 significantly reduced the mRNA levels of TNF-α, IL-1β, and IL-6. As expected, GB1a significantly decreased the mRNA levels of NF-κB p65 and its downstream genes in HCoEpi cells. Altogether, these results demonstrated that GB1a reduced the expression level of NF-κBp65 in HCoEpi. Collectively, these data suggested a potential anti-inflammatory role of GB1a.

GB1a Activates the Nrf2 Pathway and Alleviate TNF-α-Induced Mitochondrial Injury *in vitro*

Previous studies (35, 36) have shown that long-term inflammation results in impaired mitochondrial function and the overproduction of ROS that drives the pathogenesis of UC damage. These data suggest that the suppression of chronic inflammation-induced colonic oxidative stress may colitis. We investigated the effects of GB1a on TNF-α-induced mitochondrial stress and intracellular redox status. GB1a treatment significantly upregulated genes involved in antioxidant pathways including Nrf2 and HO-1 (**Figure 2A**). In parallel to the enhanced mRNA levels, GB1a significantly promoted Nrf2 protein expression and nuclear translocation (**Figures 2B,C**). Molecular docking studies revealed that GB1a could bind to the inside of the Nrf2 domain based on the hydrogen, hydrophobic interactions and van der Waals forces (**Figures 2D,E**). Moreover, GB1a treatment effectively reversed TNF-α-induced mitochondrial loss in HCoEpic cells supported by improved mitochondrial biogenesis and ultrastructural features (**Figures 2F,G**). These changes led to the attenuation of redox imbalance supported by decreased ROS levels (**Figure 2H**). Collectively, our results showed that GB1a can directly interact with Nrf2 to facilitate the recruitment of coactivators suggesting that GB1a serves as an Nrf2 agonist.

GB1a Alleviates the Symptoms of DSS-Induced UC in Mice

Administration of GB1a results in a dose-dependent decrease in body mass compared to DSS-treated mice with concomitant improvements in the DAI score at a higher dose of GB1a (**Figures 3A,B**). DSS treatment caused significant shortening of the colon compared to the control group which was attenuated in a dose-dependent manner by GB1a post-treatment (**Figures 3C,D**). Histopathological examination of the mouse colon tissues showed that GB1a treatment effectively reversed the DSS-induced damage in a dosage dependent manner. These findings were supported by observations of a repaired mucosal structure, increased crypt numbers, and reduced inflammatory cell infiltration in the mucosa and submucosa (**Figures 3E,F**). Furthermore, the MPO activity assay results showed that GB1a intervention significantly reduced MPO activity in the colon tissues of UC mice (**Figure 3G**). In conclusion, these data indicated that GB1a has potential effects on the treatment of UC.

The Inhibitory Effects of GB1a Are Dependent on the NF-κB Signaling Pathway in UC Mice

Considering the anti-inflammatory effects of GB1a are mediated by inhibiting the activation of the NF-κB pathway *in vitro*, we hypothesized that GB1a also alleviates DSS-induced UC inflammatory damage through repression of the NF-κB pathway. As predicted, GB1a treatment reversed the DSS-induced increase of pro-inflammatory cytokines including TNF-α and IL-6 (**Figure 4A**). Also, GB1a treatment

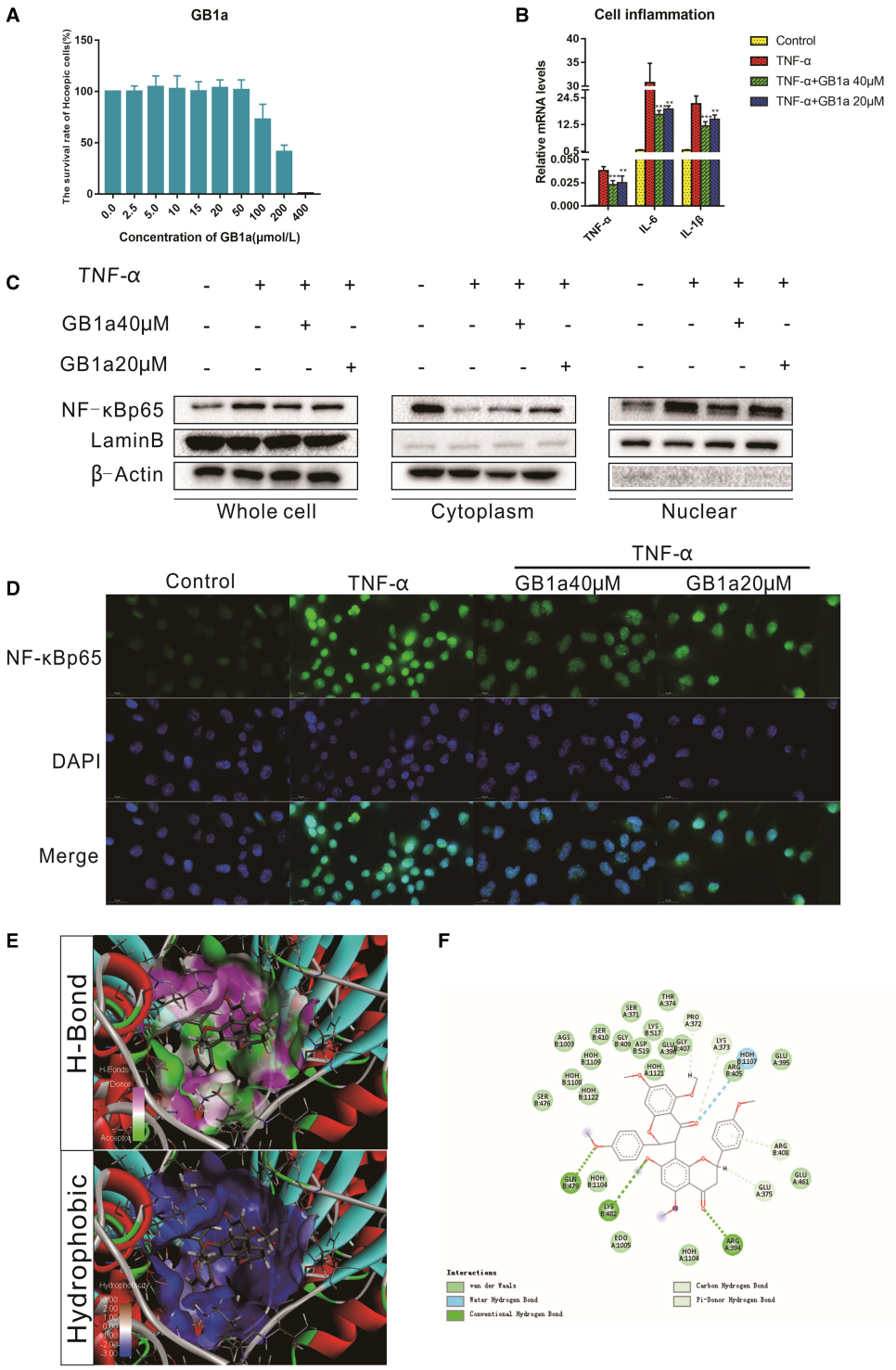


FIGURE 1 | The anti-inflammatory effects of GB1a are mediated by inhibition of NF-κB nuclear translocation *in vitro* **(A)** CCK8 analysis showed the cytotoxicity of GB1a on HCoEpic at different doses. **(B)** GB1a administration reduced the expression of intracellular pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β) in TNF-α-incubated HCoEpic in a dose-dependent manner. **(C)** GB1a treatment could inhibit NF-κB p65 protein expression in the nucleus and block NF-κB p65 translocation to the nucleus. **(D)** Immunofluorescence analysis of NF-κB (green) in HCoEpic. DAPI was used for nuclear staining (blue). **(E)** The 2D structure of the predicted binding of GB1a to NF-κB. **(F)** The molecular docking model of GB1a and NF-κB. Data are presented as means ± SD ($n = 5$/group). $^{**}p < 0.01$ and $^{***}p < 0.001$.vs the TNF-α-incubated group.

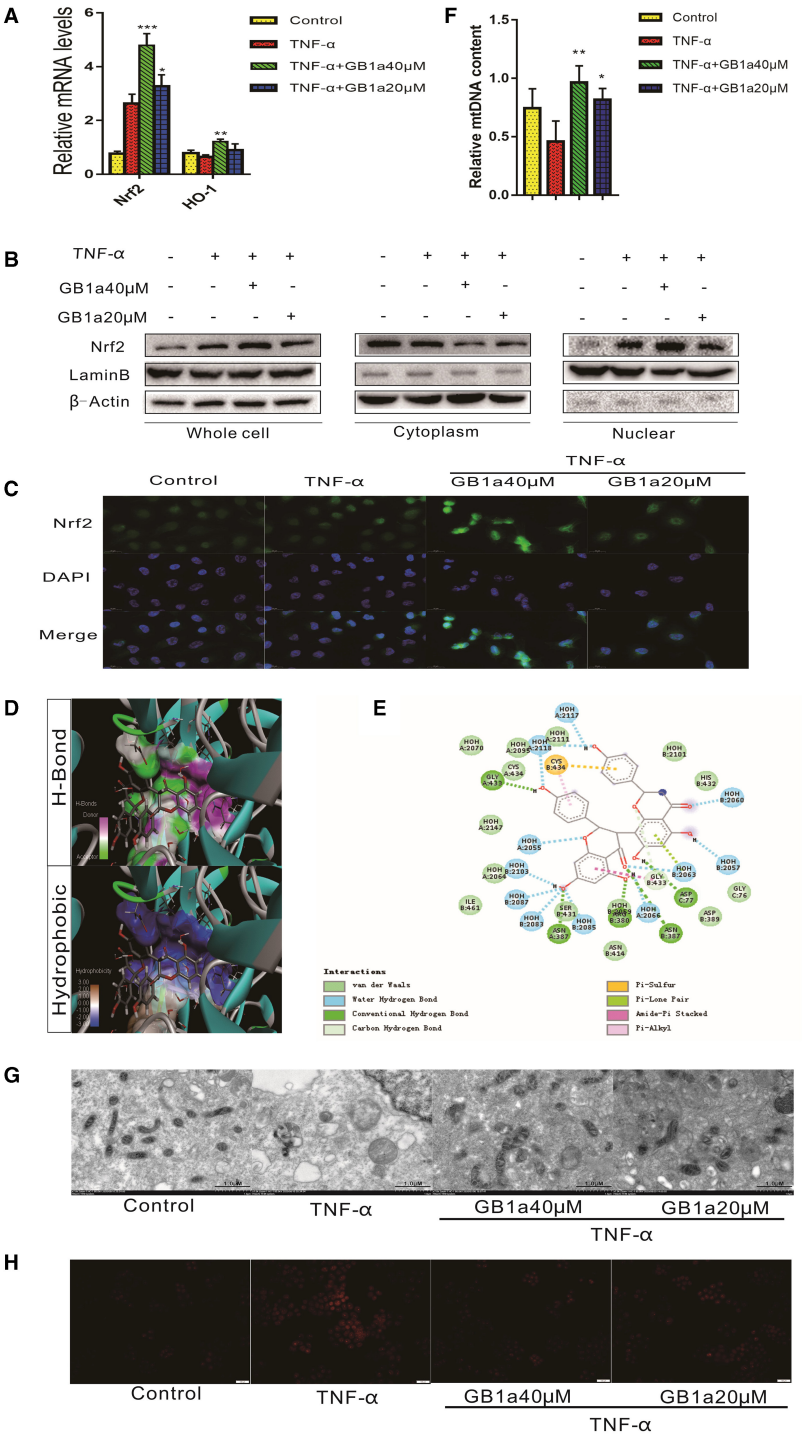


FIGURE 2 | GB1a activates the Nrf2 pathway and alleviates TNF-α-induced mitochondrial injury *in vitro*. **(A)** GB1a treatment upregulated the expression of Nrf2 and HO-1. **(B)** Western blotting results showed that GB1a advances Nrf2 translocation to the nucleus and promotes Nrf2 protein expression in the nucleus. **(C)** Immunofluorescence analysis of Nrf2 (green) in HCoEpic. DAPI was used for nuclear staining (blue). **(D)** The 2D structure of the predicted binding of GB1a to Nrf2. **(E)** The molecular docking model of GB1a and Nrf2. **(F,G)** GB1a treatment improved mitochondrial biogenesis **(F)** and morphology **(G)** in HCoEpic. **(H)** GB1a reduced the levels of ROS in TNF-α-incubated HCoEpic. Data are presented as means ± SD ($n = 5$/group). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. the TNF-α-incubated group.

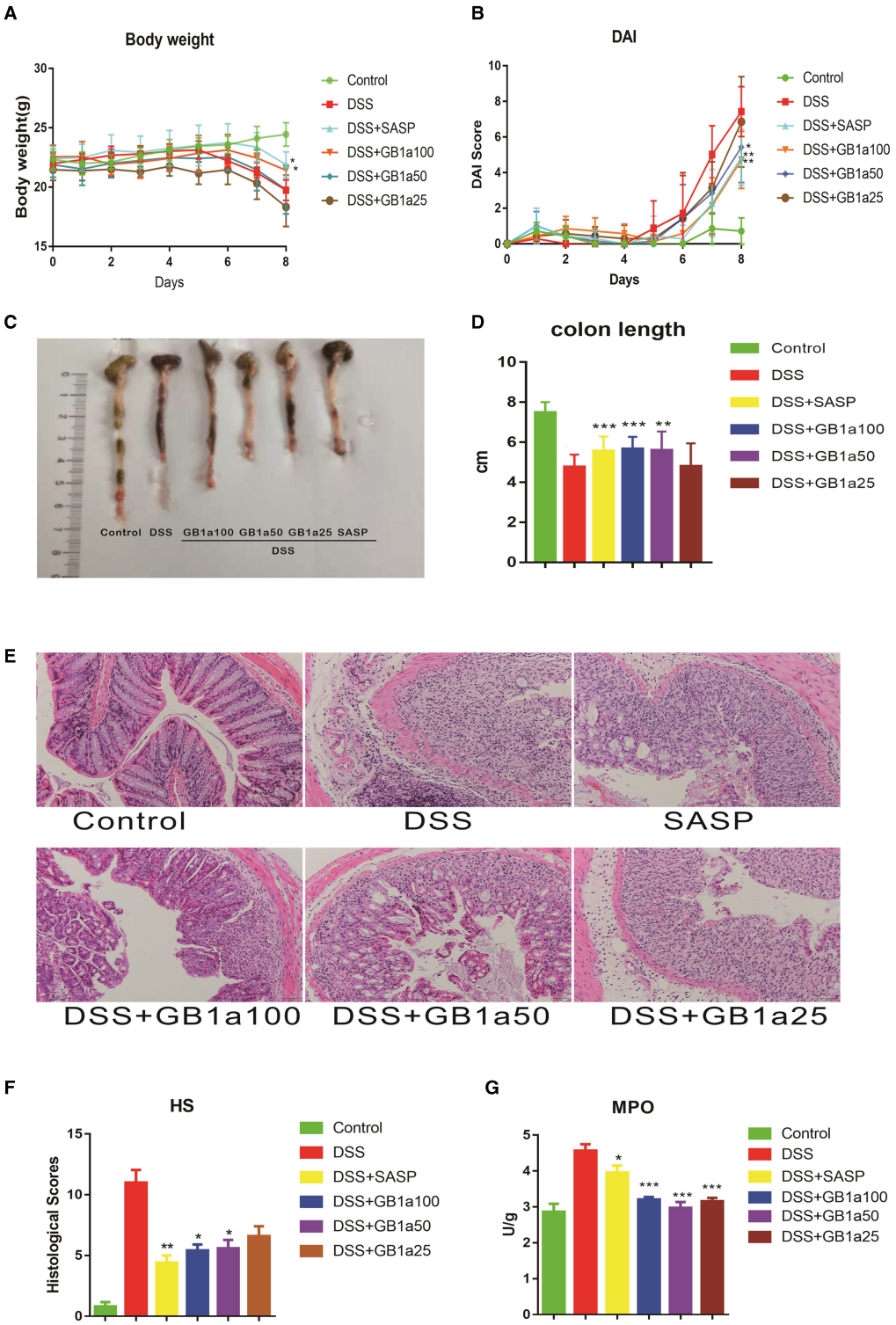


FIGURE 3 | GB1a alleviates the symptoms of DSS-induced UC in mice. **(A–D)** GB1a treatment ameliorated DSS-induced body weight loss **(A)**, DAI scores raising **(B)**, colon shortening **(C,D)**. **(E,F)** GB1a attenuates colonic pathological damage in UC mice **(E)** and decreased the histological scores of the colon tissues **(F)**. **(G)** GB1a intervention significantly reduced the serum MPO activity in UC mice. Data are presented as means ± SD ($n = 7$/group). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. the DSS-incubated group.

inhibited the DSS-induced elevation of IL-6 and TNF-α mRNA expression and inhibited the expression of chemokines including CCL5, CCL20, CXCL1 (**Figures 4B,C**). Finally, we measured the expression levels of NF-κBp65 in colon tissues by immunofluorescence and western blotting. Our results showed that GB1a inhibited NF-κBp65 expression and blocked NF-κBp65 translocation to the nucleus in DSS mice. These changes led to the amelioration of DSS-induced inflammation in the colon by inhibiting activation of the NF-κB pathway (**Figures 4D,E**).

The Effect of GB1a Activation on the Nrf2 Signaling Pathway in UC Mice

Based on the results of our *in vitro* experiments, we further investigated the dependency of the therapeutic effects of GB1a in UC mice via the activation of colonic Nrf2 pathways. We determined the levels of oxidants (MDA) and antioxidants (GSH, SOD) in the serum of mice and the mRNA expression of antioxidant genes (Nrf2, HO-1 and MarfK) in colon tissues. GB1a treatment significantly decreased the levels of the oxidant MDA and increased the level of the antioxidants GSH and SOD (**Figure 5A**). We also found the upregulated expression of the antioxidant genes, Nrf2, HO-1 and MarfK (**Figure 5B**). Immunofluorescence staining and western blotting analysis showed that GB1a administration significantly increased Nrf2 expression in colon tissues and promoted Nrf2 translocation to the nucleus (**Figures 5C,D**). Consistent with the activation of Nrf2 signaling, immunohistochemistry results showed that GB1a treatment significantly enhanced Nrf2 and HO-1 protein expression in inflamed colons compared to mice treated with DSS alone (**Figure 5E**). Taken together, these results suggest that GB1a exerts an anti-oxidant effect through the Nrf2 signaling pathway to improve the symptoms of UC.

The Protective Effect of GB1a on the Intestinal Mucosa

Damage to the intestinal mucosal barrier is an important cause of UC. We hypothesized that GB1a might have regulatory effects on DSS-induced tight junctions (TJ) molecules. Our results demonstrated that GB1a treatment increased the expression of ZO-1 and Occludin at the mRNA and protein levels (**Figures 6A,D**) and improved mucosal permeability and decreased the level of FITC in serum (**Figure 6B**). Immunohistochemistry staining results showed that GB1a treatment significantly enhanced ZO-1 and Occludin protein expression in inflamed colons compared to mice treated with DSS alone (**Figure 6C**) and were further verified by western blotting analysis (**Figure 6D**). Transmission Electron Microscope (TEM) revealed that GB1a (100 $mg{\cdot}kg^{-1}$) ameliorated DSS-induced loosening of the epithelial tight junction (TJ), increased colon space, caused the loss of microvilli, decreased desmosome density and decreased mitochondrial swelling. These changes acted to improve the integrity of the intestinal barrier (**Figure 6E**). In summary, our data indicated that GB1a treatment significantly repaired the damage of the intestinal mucosa by DSS-induced.

DISCUSSION

In this study, we showed that GB1a inhibited oxidative stress damage by activating the NRF2 pathway. GB1a regulated the balance between pro- and anti-inflammatory cytokines and maintained intestinal homeostasis by inhibiting activation of the NF-κB pathway. Also, GB1a reduced the permeability of the intestinal mucosa by repairing damage to the intestinal mucosal barrier and prevented endotoxins and bacteria from entering the blood circulation, ultimately relieving damage from the abnormal immune response.

Inflammatory bowel disease (IBD) includes UC and Crohn's disease (CD) (37). UC, also known as non-specific UC, is a chronic inflammatory disease where the main lesions occur in the colon. In 1859, the symptoms of UC were first described by Samuel Wilks (38) and included abdominal pain, diarrhea and bloody purulent stools. These symptoms are often accompanied by associated damage in the lymph nodes, skin, eyes, liver, and gallbladder (39).

Currently, the pathogenesis of UC is not fully understood. The process is thought to mainly involve genetic susceptibility, defects in the epithelial barrier, immune disorders and other environmental factors. Immune dysregulation is a key factor the affects the progression of the disease. Also, injury to the epithelial barrier injury is important in the pathogenesis of UC as pathogenic microorganisms and toxins can exacerbate ulcers by invading the intestinal tract.

Modern clinical approaches lack targeted drugs for the treatment of UC. Clinically, UC is mainly treated using strategies to regulate immune function, reduce intestinal mucosal edema and inhibit the production of inflammatory mediators. The main classes of drugs that are used to perform these functions are amino salicylic acid (5-ASA), adrenocorticosteroids, immunosuppressants, and inhibitors of inflammatory mediators. Whilst these drugs are effective, they have several side effects that impact the quality of life of patients.

Treatment with 5-ASA as a first-line therapy for UC may cause male infertility and folic acid deficiency. Also, various biological agents that target specific immune pathways have become recognized as potential treatments for UC (40, 41). Glucocorticoids are the most effective drugs used to inhibit acute active inflammation, yet their long-term use leads to hormone dependence and drug resistance (42). Immunosuppressive agents have more significant adverse reactions such as severe diarrhea, bone marrow suppression, hepatotoxicity and pancreatitis (43). Biological agents may be used to control the early stages of UC, however, these may result in adverse reactions including delayed allergic reactions, increased risk of infections and increased incidence of tumors (44). Therefore, there is an urgent need to develop more effective and safer alternative drugs in the treatment of UC.

Recently, researchers have identified natural compounds that are effective in the prevention and treatment of UC and other inflammatory diseases (45). These compounds are relatively non-toxic and have fewer side effects compared to established drugs. Natural products may have a high potential to improve the quality of life for patients with UC and also reduce the risk of

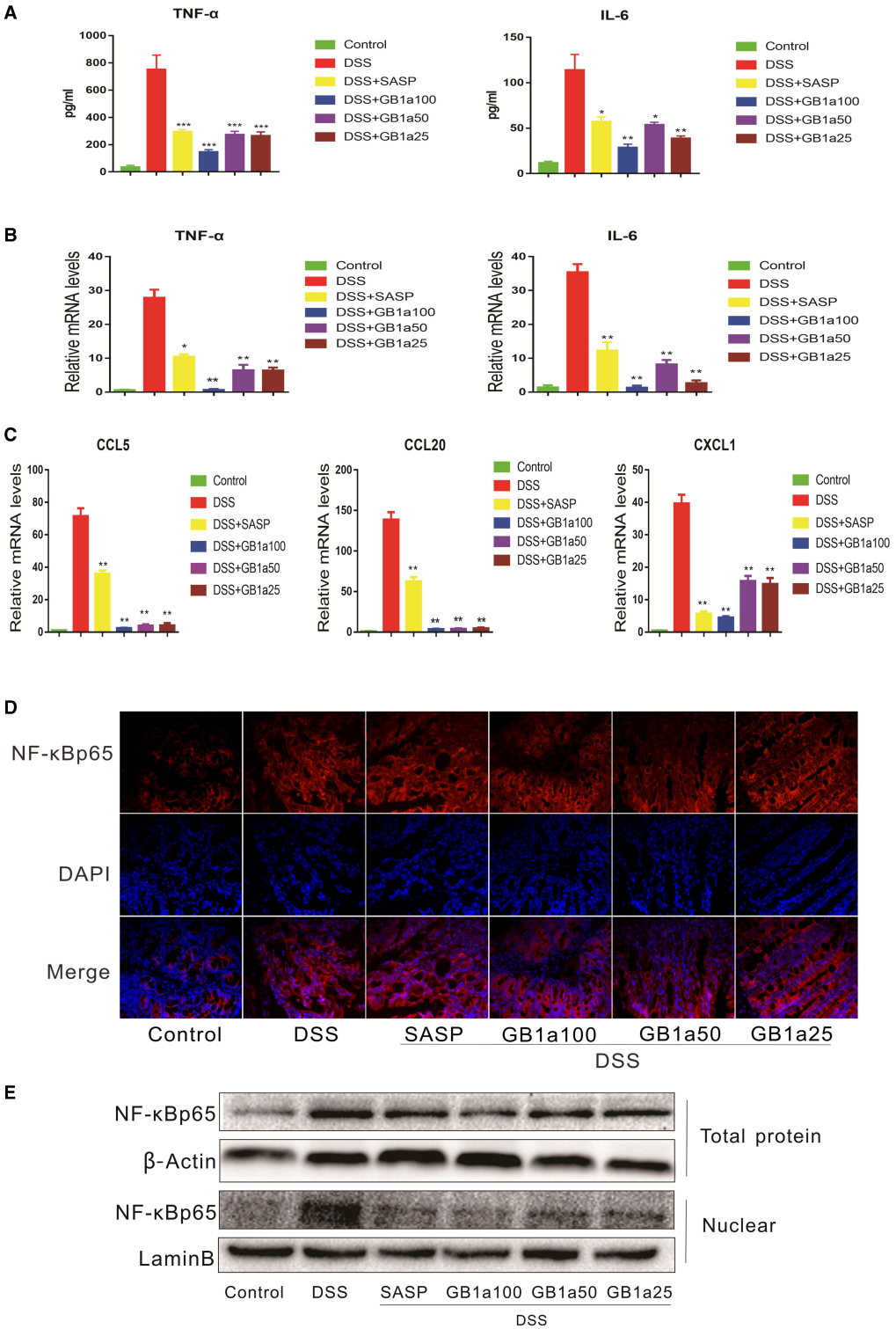


FIGURE 4 | The inhibitory effects of GB1a are dependent on the NF-κB signaling pathway in UC mice. **(A)** Treatment with GB1a down-regulated serum TNF-α and IL-6 levels in UC mice. **(B,C)** GB1a inhibited the expression of TNF-α, IL-6, CCL5, CCL20, CXCL1 in the colon tissue of UC mice. **(D)** Immunofluorescence analysis of NF-κBp65 (red) in the colon of mice. DAPI was used for nuclear staining (blue). **(E)** NF-κBp65 levels in whole and nuclear protein were analyzed by western blotting. Data are presented as means ± SD ($n = 7$/group). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. the DSS-incubated group.

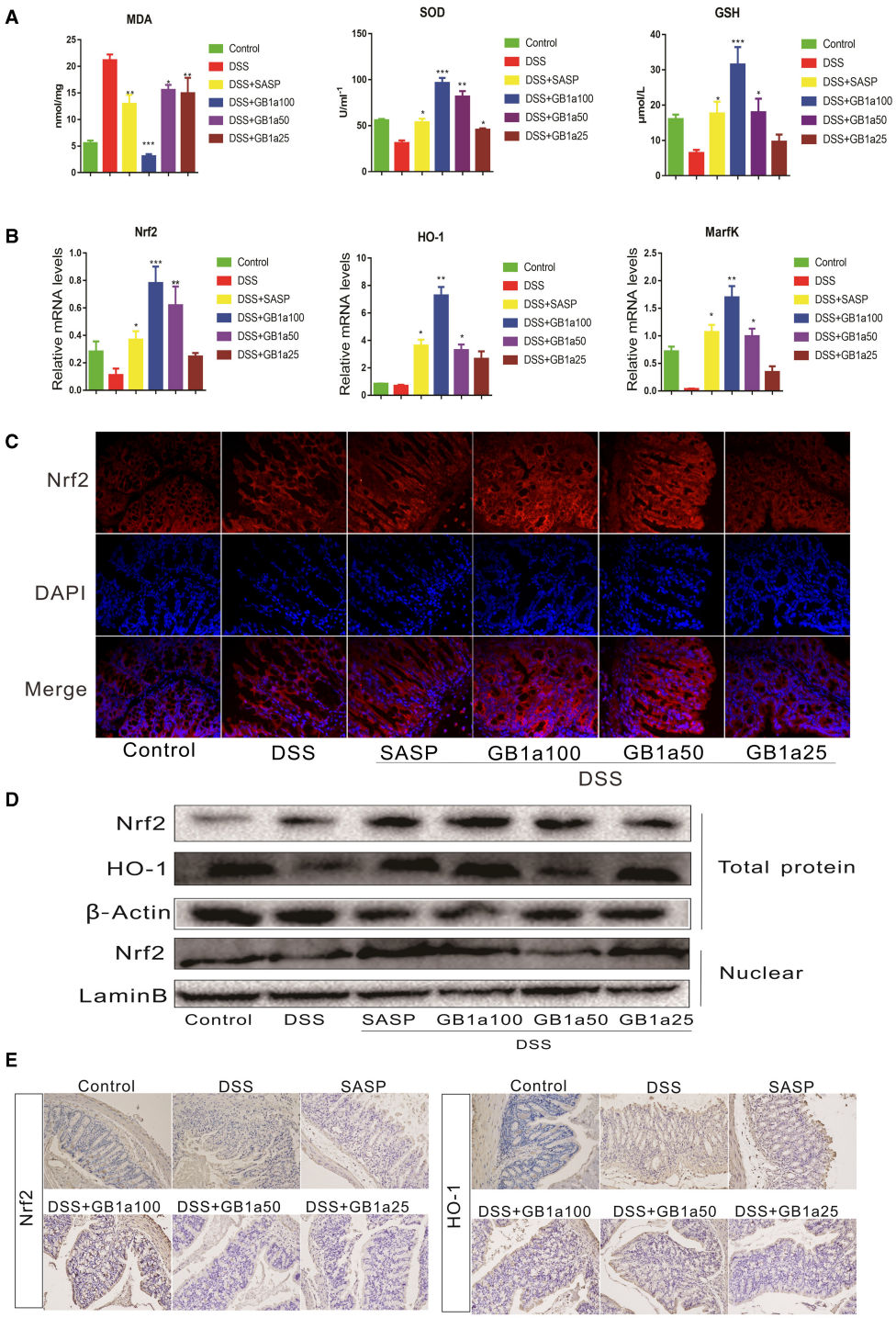


FIGURE 5 | The effects of GB1a on the activation of the Nrf2 signaling pathway in UC mice. **(A)** GB1a administration reduced the levels of oxidants (MDA) and increased the levels of antioxidants (GSH, SOD) in the serum of UC mice. **(B)** GB1a treatment upregulated the expression of antioxidant genes Nrf2, HO-1, and MarfK. **(C)** Immunofluorescence analysis of Nrf2 (red) in the colon of mice. DAPI was used for nuclear staining (blue). **(D)** GB1a administration increased the expression of Nrf2 and HO-1 proteins and promoted Nrf2 translocation to the nucleus. **(E)** Representative images of immunohistochemical staining of Nrf2 and HO-1 in mice colon sections. Data are presented as means ± SD ($n = 7$/group). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. the DSS-incubated group.

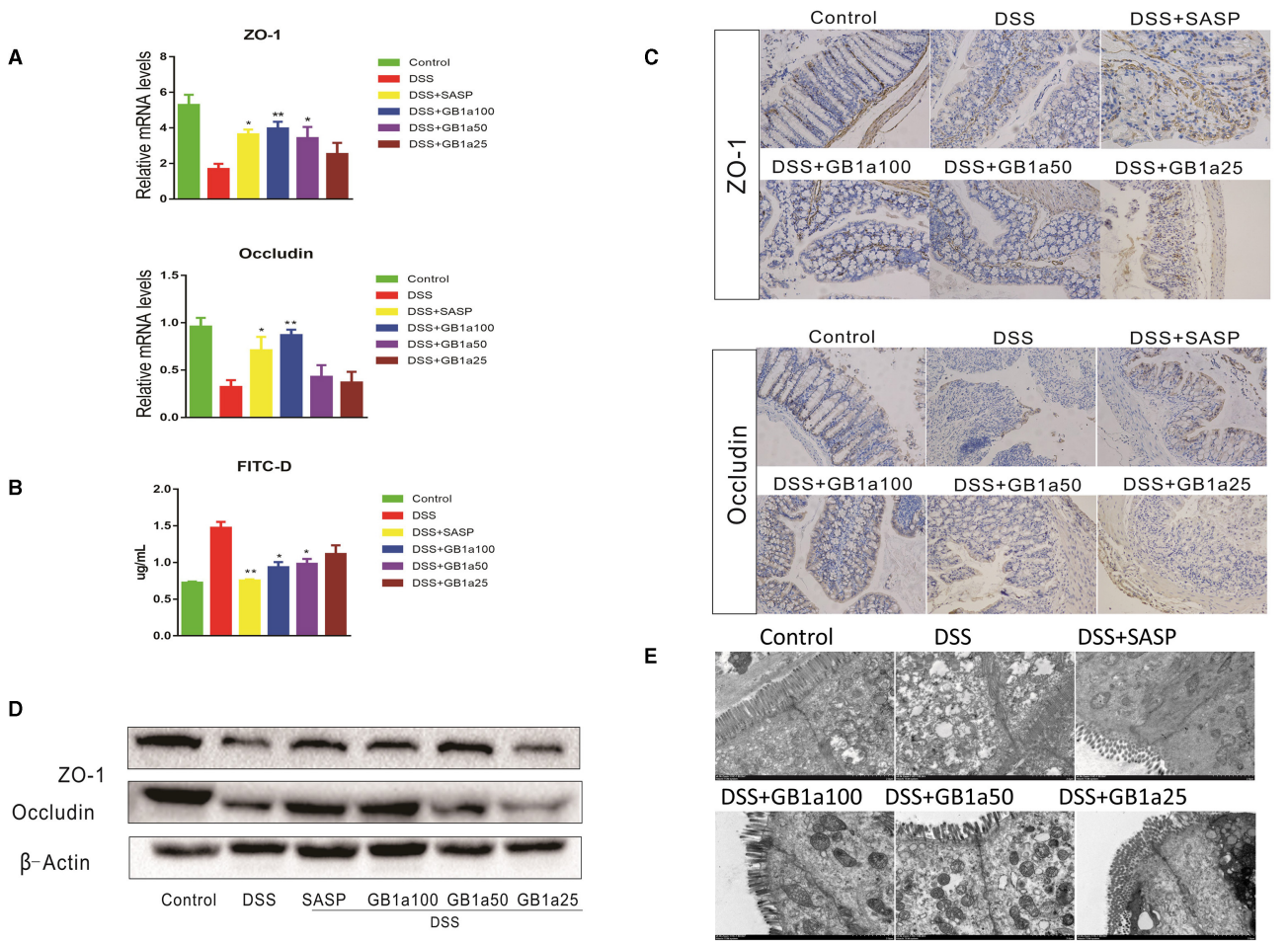


FIGURE 6 | The protective effect of GB1a on the intestinal mucosa **(A)** GB1a treatment upregulated the expression of genes ZO-1 and Occludin. **(B)** GB1a administration reduced the levels of fluorescein isothiocyanate (FITC)-dextran in the serum of UC mice and improved mucosal permeability in the colon. **(C)** Representative images of immunohistochemical staining of ZO-1 and Occludin and Claudin2 in mouse colon sections. **(D,E)** Ultrastructural morphology of the intestinal mucosal barrier. Data are presented as means ± SD ($n = 7$/group). $^{*}p < 0.05$ and $^{**}p < 0.01$. vs the DSS-incubated group.

cancer. Garcinia Kola Heckel is a flowering plant that produces a natural product with known anti-inflammatory, antioxidant, antiviral, antiulcer, and anti-bacterial activities (46–48). The crude extract of Garcinia kola is known to have protective effects against acetic acid-induced UC in rats. Kolaviron, a diflavonoid compound extracted from Garcinia Kola, has been shown to improve DSS-induced UC in rats through anti-inflammatory and antioxidant effects (49). In the current study, the biflavonoid compound, GB1a, was extracted from the seeds of Garcinia Kola (26, 27). GB1a is one of the most important active ingredients found in Garcinia Kola and has been reported to have analgesic, anti-inflammatory, antimalarial and antioxidant activities (50–52), however, it has not been evaluated in the treatment of UC.

Although the exact pathogenesis of UC remains unclear, accumulating evidence indicates that anti-oxidative and inflammatory pathways play significant roles (53, 54). Natural compounds are involved in the inflammatory and immune responses of the UC intestine. The integrity and repair of the mucosal barrier in the colon are critically important in improving the symptoms of UC (45, 55) and developing effective treatments for UC (56).

A major feature of UC is the development of severe inflammation in response to impaired immune responses in which NF-κB signaling pathways play a central role (57, 58). The release of related inflammatory cytokines and pro-inflammatory mediators after activation of the NF-κB pathway plays a crucial role in UC. These changes include elevated levels of TNF-α, IL-6, and IL-1β along with decreased levels of anti-inflammatory cytokines such as IL-10 (8, 9). In the current study, natural compounds had pronounced inhibitory effects on the NF-κB pathway by reducing the expression of inflammatory factors and promoting the expression of anti-inflammatory factors that acted to improve the symptoms of UC.

Curcumin is a natural hydrophobic polyphenol that has a variety of pharmacological effects in UC (59). Curcumin has been

shown to down-regulate the expression of pro-inflammatory cytokines (IL-1, IL-6, IL-8, and TNF-α) by regulating the NF-κB/IκB pathway and reduce inflammatory cell infiltration in several experimental models. Saikosaponin-d improves dextran sulfate sodium-induced colitis by inhibiting activation of NF-κB signaling and regulating the intestinal microbiota in mice. Also, nutmeg reduces TNF-α, IL-6, and IL-1β levels in LPS induced mouse serum by blocking nuclear translocation of endotoxin shock and inhibits binding in LPS stimulated macrophages (60).

Cardamonin is another natural compound that blocks the nuclear translocation of NF-κBp65 in a mouse model of endotoxin shock. Cardamonin can reduce the levels of TNF-α, IL-6, and IL-1β secretion in LPS-induced mouse blood serum and inhibits NF-κB DNA-binding in LPS-stimulated macrophage cells (61). Our results revealed that GB1a administration decreased the expression of TNF-α, IL-6 mRNA and repressed NF-κBp65 protein expression and nuclear translocation by inhibiting activation of the NF-κB pathway. These data demonstrate that GB1a can effectively reduce inflammatory damage and highlight the potential for the therapeutic application of GB1a in the treatment of UC.

Neutrophil infiltration, free radical formation and increased oxidative stress are known biological mechanisms of UC (62, 63). Oxidative stress plays a key role in the development of many diseases and is usually accompanied by the production of a large amounts of oxygen free radicals. This directly causes oxidative damage to macromolecules such as DNA, proteins and lipids, destroying cell membranes, and other cellular structures. By stimulating the expression of cytokines and adhesion molecules, oxidative damage mediates inflammation, and the immune response to enhance tissue damage. The presence of oxygen free radicals can also indirectly activate apoptotic signaling pathways through the inhibition of mitochondrial function (64). During the development of UC, the oxidative burst of infiltrating macrophages leads to the production of large amounts of reactive oxygen species in the inflamed tissues of patients. This oxidative burst leads to the destruction of colon tissue and decreases epithelial permeability causing intestinal inflammation (13).

The nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway is a defense system that regulates the expression of antioxidant proteins and the transcription of genes encoding detoxification enzymes. *In vivo* studies have shown that the Nrf2 signaling pathway also plays an important role in the improvement of UC (65–67). In our study, for the first time, we report that GB1a supplementation can effectively improve mitochondrial and oxidative stresses by reducing ROS in a Nrf2 dependent manner suggesting a strong link between Nrf2 and oxidative stress during the progression of UC.

The persistent inflammatory response in patients with UC compromises the integrity of the colonic mucosa through sustained cytokine release (9). The mechanical barrier of the intestinal mucosa is particularly important in the treatment of UC. The main structure of the mechanical barrier is formed by tight junction proteins (TJs) that are composed of claudin, zos and connexins (68). Also, the intestinal mucosal barrier plays a vital role in maintaining the barrier function to protect against intestinal allergens, toxins, and pathogens (59).

During the development of UC, the destruction of the intestinal mucosal barrier activates intestinal inflammation to promote the development of colon cancer (69). Previously, it has been shown that changes in the composition of colon mucus in UC promotes damage to the colon mucosal barrier. This leads to immune activation of symbiotic microbial communities and promotes the progression of UC diseases (70, 71). The results presented in this study demonstrate that GB1a can effectively increase the expression of the tight junction protein ZO-1 and Occludin in UC mice. The serum FITC content of the UC mice decreased after GB1a treatment which effectively alleviated the permeability of the colon mucosa toward maintaining the normal physiological function of the colon mucosa.

In summary, this is the first study to demonstrate the protective effects of GB1a on DSS-induced mouse UC. The underpinning molecular mechanisms of GB1a are potentially associated with the activation of Nrf2, protection of intestinal mucosa and the inhibition of NF-κB-mediated proinflammatory signaling.

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 animal study was reviewed and approved by Animal Ethics Committee of Guangzhou University of Traditional Chinese Medicine.

AUTHOR CONTRIBUTIONS

YY, CZ, XL, CD, and WG performed the experiments and data analysis and wrote the manuscript. CL and QWu contributed to the study design and acquisition and analysis of data. QWa, QX, and XH contributed to the drafting of the manuscript. JS designed the experiments, provided funding support, and performed a critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.654867/full#supplementary-material

REFERENCES

1. Eom T, Kim YS, Choi CH, Sadowsky MJ, Unno T. Current understanding of microbiota- and dietary-therapies for treating inflammatory bowel disease. *J Microbiol.* (2018) 56:189–98. doi: 10.1007/s12275-018-8049-8
2. Sclano G. Asthma, nasal polyposis and ulcerative colitis: a new perspective. *Clin Exp Allergy.* (2002) 32:1144–9. doi: 10.1046/j.1365-2745.2002.01460.x
3. Karlinger K, Gyorke T, Mako E, Mester A, Tarjan Z. The epidemiology and the pathogenesis of inflammatory bowel disease. *Eur J Radiol.* (2000) 35:154–67. doi: 10.1016/S0720-048X(00)00238-2
4. Bonovas S, Lytras T, Nikolopoulos G, Peyrin-Biroulet L, Danese S. Systematic review with network meta-analysis: comparative assessment of tofacitinib and biological therapies for moderate-to-severe ulcerative colitis. *Aliment Pharmacol Ther.* (2018) 47:454–65. doi: 10.1111/apt.14449
5. Duijvestein M, Battat R, Vande Casteele N, D'Haens GR, Sandborn WJ, Khanna R, et al. Novel therapies and treatment strategies for patients with inflammatory bowel disease. *Curr Treat Options Gastroenterol.* (2018) 16:129–46. doi: 10.1007/s11938-018-0175-1
6. Brown SJ, Mayer L. The immune response in inflammatory bowel disease. *Am J Gastroenterol.* (2007) 102:2058–69. doi: 10.1111/j.1572-0241.2007.01343.x
7. Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol.* (2006) 3:390–407. doi: 10.1038/ncpgasthep0528
8. Sanchez-Fidalgo S, Cardeno A, Sanchez-Hidalgo M, Aparicio-Soto M, de la Lastra CA. Dietary extra virgin olive oil polyphenols supplementation modulates DSS-induced chronic colitis in mice. *J Nutr Biochem.* (2013) 24:1401–13. doi: 10.1016/j.jnutbio.2012.11.008
9. Chiou YS, Ma NJ, Sang S, Ho CT, Wang YJ, Pan MH. Peracetylated (-)-epigallocatechin-3-gallate (AcEGCG) potently suppresses dextran sulfate sodium-induced colitis and colon tumorigenesis in mice. *J Agric Food Chem.* (2012) 60:3441–51. doi: 10.1021/jf300441p
10. Pandurangan AK, Mohebali N, Norhaizan ME, Looi CY. Gallic acid attenuates dextran sulfate sodium-induced experimental colitis in BALB/c mice. *Drug Des Devel Ther.* (2015) 9:3923–34. doi: 10.2147/DDDT.S86345
11. Pavlick KP, Laroux FS, Fuseler J, Wolf RE, Gray L, Hoffman J, et al. Role of reactive metabolites of oxygen and nitrogen in inflammatory bowel disease. *Free Radic Biol Med.* (2002) 33:311–22. doi: 10.1016/S0891-5849(02)00853-5
12. Tanida S, Mizoshita T, Mizushima T, Sasaki M, Shimura T, Kamiya T, et al. Involvement of oxidative stress and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in inflammatory bowel disease. *J Clin Biochem Nutr.* (2011) 48:112–6. doi: 10.3164/jcbn.10-41
13. Forman HJ, Davies KJ, Ursini F. How do nutritional antioxidants really work: nucleophilic tone and para-hormesis versus free radical scavenging *in vivo*. *Free Radic Biol Med.* (2014) 66:24–35. doi: 10.1016/j.freeradbiomed.2013.05.045
14. Cho HY, Reddy SP, Yamamoto M, Kleeberger SR. The transcription factor NRF2 protects against pulmonary fibrosis. *FASEB J.* (2004) 18:1258–60. doi: 10.1096/fj.03-1127fje
15. Saw CL, Yang AY, Guo Y, Kong AN. Astaxanthin and omega-3 fatty acids individually and in combination protect against oxidative stress via the Nrf2-ARE pathway. *Food Chem Toxicol.* (2013) 62:869–75. doi: 10.1016/j.fct.2013.10.023
16. Uruno A, Motohashi H. The Keap1-Nrf2 system as an *in vivo* sensor for electrophiles. *Nitric Oxide.* (2011) 25:153–60. doi: 10.1016/j.niox.2011.02.007
17. Khor TO, Huang MT, Kwon KH, Chan JY, Reddy BS, Kong AN. Nrf2-deficient mice have an increased susceptibility to dextran sulfate sodium-induced colitis. *Cancer Res.* (2006) 66:11580–4. doi: 10.1158/0008-5472.CAN-06-3562
18. Osburn WO, Karim B, Dolan PM, Liu G, Yamamoto M, Huso DL, et al. Increased colonic inflammatory injury and formation of aberrant crypt foci in Nrf2-deficient mice upon dextran sulfate treatment. *Int J Cancer.* (2007) 121:1883–91. doi: 10.1002/ijc.22943
19. Hegazy SK, El-Bedewy MM. Effect of probiotics on pro-inflammatory cytokines and NF-kappaB activation in ulcerative colitis. *World J Gastroenterol.* (2010) 16:4145–51. doi: 10.3748/wjg.v16.i33.4145
20. Ma J, Yin G, Lu Z, Xie P, Zhou H, Liu J, et al. Casticin prevents DSS induced ulcerative colitis in mice through inhibitions of NF-kappaB pathway and ROS signaling. *Phytother Res.* (2018) 32:1770–83. doi: 10.1002/ptr.6108
21. Baldwin AS Jr. The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu Rev Immunol.* (1996) 14:649–83. doi: 10.1146/annurev.immunol.14.1.649
22. Kwon DJ, Bae YS, Ju SM, Youn GS, Choi SY, Park J. Salicortin suppresses lipopolysaccharide-stimulated inflammatory responses via blockade of NF-kappaB and JNK activation in RAW 264.7 macrophages. *BMB Rep.* (2014) 47:318–23. doi: 10.5483/BMBRep.2014.47.6.200
23. Fan X, Zhang Y, Dong H, Wang B, Ji H, Liu X. Trilobatin attenuates the LPS-mediated inflammatory response by suppressing the NF-kappaB signaling pathway. *Food Chem.* (2015) 166:609–15. doi: 10.1016/j.foodchem.2014.06.022
24. Amirshahrokhi K. Febuxostat attenuates ulcerative colitis by the inhibition of NF-kappaB, proinflammatory cytokines, and oxidative stress in mice. *Int Immunopharmacol.* (2019) 76:105884. doi: 10.1016/j.intimp.2019.105884
25. Zhu L, Gu P, Shen H. Protective effects of berberine hydrochloride on DSS-induced ulcerative colitis in rats. *Int Immunopharmacol.* (2019) 68:242–51. doi: 10.1016/j.intimp.2018.12.036
26. Adegbehingbe OO, Adesanya SA, Idowu TO, Okimi OC, Oyelami OA, Iwalewa EO. Clinical effects of Garcinia kola in knee osteoarthritis. *J Orthop Surg Res.* (2008) 3:34. doi: 10.1186/1749-799X-3-34
27. Kabangu K, Galeffi C, Aonzo E, Nicoletti M, Messana I. A new biflavanone from the bark of Garcinia kola1. *Planta Med.* (1987) 53:275–7. doi: 10.1055/s-2006-962704
28. González-Gallego J, Sánchez-Campos S, Tu nón MJ. Anti-inflammatory properties of dietary flavonoids. *Nutr Hosp.* (2007) 22:287–93.
29. Djoufack GLN, Valant-Vetschera KM, Schinnerl J, Brecker L, Lorbeer E, Robien W. Xanthones, Biflavanones and Triterpenes from *Pentadesma grandifolia* (Clusiaceae): structural determination and bioactivity. *Nat Prod Commun.* (2010) 5:1055–60. doi: 10.1177/1934578X1000500714
30. Detel D, Pugel EP, Pucar LB, Buljevic S, Varljen J. Development and resolution of colitis in mice with target deletion of dipeptidyl peptidase IV. *Exp Physiol.* (2012) 97:486–96. doi: 10.1113/expphysiol.2011.061143
31. Dou W, Zhang J, Li H, Kortagere S, Sun K, Ding L, et al. Plant flavonol isorhamnetin attenuates chemically induced inflammatory bowel disease via a PXR-dependent pathway. *J Nutr Biochem.* (2014) 25:923–33. doi: 10.1016/j.jnutbio.2014.04.006
32. Hunter SE, Jung D, Di Giulio RT, Meyer JN. The QPCR assay for analysis of mitochondrial DNA damage, repair, and relative copy number. *Methods.* (2010) 51:444–51. doi: 10.1016/j.ymeth.2010.01.033
33. Cheng B, Gao W, Wu X, Zheng M, Yu Y, Song C, et al. Ginsenoside Rg2 ameliorates high-fat diet-induced metabolic disease through SIRT1. *J Agric Food Chem.* (2020) 68:4215–26. doi: 10.1021/acs.jafc.0c00833
34. Fairaq A, Goc A, Artham S, Sabbineni H, Somanath PR. TNFalpha induces inflammatory stress response in microvascular endothelial cells via Akt- and P38 MAP kinase-mediated thrombospondin-1 expression. *Mol Cell Biochem.* (2015) 406:227–36. doi: 10.1007/s11010-015-2440-0
35. Wang Z, Li S, Cao Y, Tian X, Zeng R, Liao D-F, et al. Oxidative stress and carbonyl lesions in ulcerative colitis and associated colorectal cancer. *Oxid Med Cell Longev.* (2016) 2016:9875298. doi: 10.1155/2016/9875298
36. Rimessi A, Previati M, Nigro F, Wieckowski MR, Pinton P. Mitochondrial reactive oxygen species and inflammation: molecular mechanisms, diseases and promising therapies. *Int J Biochem Cell Biol.* (2016) 81:281–93. doi: 10.1016/j.biocel.2016.06.015
37. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the

21st century: a systematic review of population-based studies. *Lancet.* (2018) 390:2769–78. doi: 10.1016/S0140-6736(17)32448-0
38. Wilks S. Morbid appearances in the intestine of Miss Bankers. *Med Times Gazette.* (1859) 2:264–5.
39. Xu CT, Meng SY, Pan BR. Drug therapy for ulcerative colitis. *World J Gastroenterol.* (2004) 10:2311–7. doi: 10.3748/wjg.v10.i16.2311
40. Sandborn WJ, Ghosh S, Panes J, Vranic I, Su C, Rousell S, et al. Tofacitinib, an oral Janus kinase inhibitor, in active ulcerative colitis. *N Engl J Med.* (2012) 367:616–24. doi: 10.1056/NEJMoa1112168
41. Stidham RW, Lee TC, Higgins PD, Deshpande AR, Sussman DA, Singal AG, et al. Systematic review with network meta-analysis: the efficacy of anti-tumour necrosis factor-alpha agents for the treatment of ulcerative colitis. *Aliment Pharmacol Ther.* (2014) 39:660–71. doi: 10.1111/apt.12644
42. Bianchi Porro G, Cassinotti A, Ferrara E, Maconi G, Ardizzone S. Review article: the management of steroid dependency in ulcerative colitis. *Aliment Pharmacol Ther.* (2007) 26:779–94. doi: 10.1111/j.1365-2036.2007.03334.x
43. Stein RB, Hanauer SB. Comparative tolerability of treatments for inflammatory bowel disease. *Drug Saf.* (2000) 23:429–48. doi: 10.2165/00002018-200023050-00006
44. Quezada SM, McLean LP, Cross RK. Adverse events in IBD therapy: the 2018 update. *Expert Rev Gastroenterol Hepatol.* (2018) 12:1183–91. doi: 10.1080/17474124.2018.1545574
45. Yuan Z, Yang L, Zhang X, Ji P, Hua Y, Wei Y. Huang-Lian-Jie-Du decoction ameliorates acute ulcerative colitis in mice via regulating NF-kappaB and Nrf2 signaling pathways and enhancing intestinal barrier function. *Front Pharmacol.* (2019) 10:1354. doi: 10.3389/fphar.2019.01354
46. Delalastra CA, Martin MJ, Lacasa C, Motilva V. Antiulcerogenicity of the flavonoid fraction from bidens aurea - comparison with ranitidine and omeprazole. *J Ethnopharmacol.* (1994) 42:161–8. doi: 10.1016/0378-8741(94)90081-7
47. Izzo AA, Dicarlo G, Mascolo N, Capasso F, Autore G. Antiulcer effect of flavonoids - role of endogenous Paf. *Phytother Res.* (1994) 8:179–81. doi: 10.1002/ptr.2650080313
48. Reyes M, Martin C, Alarcon de la Lastra C, Trujillo J, Toro MV, Ayuso MJ. Antiulcerogenicity of the flavonoid fraction from Erica andevalensis Cabezudo-Rivera. *Z Naturforsch C J Biosci.* (1996) 51:563–9. doi: 10.1515/znc-1996-7-816
49. Farombi EO, Adedara IA, Ajayi BO, Ayepola OR, Egbeme EE. Kolaviron, a natural antioxidant and anti-inflammatory phytochemical prevents dextran sulphate sodium-induced colitis in rats. *Basic Clin Pharmacol Toxicol.* (2013) 113:49–55. doi: 10.1111/bcpt.12050
50. Bittar M, de Souza MM, Yunes RA, Lento R, Delle Monache F, Cechinel Filho V. Antinociceptive activity of I3,II8-binaringenin, a biflavonoid present in plants of the guttiferae. *Planta Med.* (2000) 66:84–6. doi: 10.1055/s-0029-1243118
51. Burda S, Oleszek W. Antioxidant and antiradical activities of flavonoids. *J Agric Food Chem.* (2001) 49:2774–9. doi: 10.1021/jf001413m
52. Tajuddeen N, Van Heerden FR. Antiplasmodial natural products: an update. *Malar J.* (2019) 18:404. doi: 10.1186/s12936-019-3026-1
53. Piechota-Polanczyk A, Fichna J. Review article: the role of oxidative stress in pathogenesis and treatment of inflammatory bowel diseases. *Naunyn Schmiedebergs Arch Pharmacol.* (2014) 387:605–20. doi: 10.1007/s00210-014-0985-1
54. Sangaraju R, Nalban N, Alavala S, Rajendran V, Jerald MK, Sistla R. Protective effect of galangin against dextran sulfate sodium (DSS)-induced ulcerative colitis in Balb/c mice. *Inflamm Res.* (2019) 68:691–704. doi: 10.1007/s00011-019-01252-w
55. Wang J, Zhang C, Guo C, Li X. Chitosan ameliorates DSS-induced ulcerative colitis mice by enhancing intestinal barrier function and improving microflora. *Int J Mol Sci.* (2019) 20:5751. doi: 10.3390/ijms20225751
56. Merga Y, Campbell BJ, Rhodes JM. Mucosal barrier, bacteria and inflammatory bowel disease: possibilities for therapy. *Dig Dis.* (2014) 32:475–83. doi: 10.1159/000358156
57. Peng L, Gao X, Nie L, Xie J, Dai T, Shi C, et al. Astragalin attenuates Dextran Sulfate Sodium (DSS)-induced acute experimental colitis by alleviating gut microbiota dysbiosis and inhibiting NF-kappaB activation in mice. *Front Immunol.* (2020) 11:2058. doi: 10.3389/fimmu.2020.02058
58. Papoutsopoulou S, Burkitt MD, Bergey F, England H, Hough R, Schmidt L, et al. Macrophage-specific NF-kappaB activation dynamics can segregate inflammatory bowel disease patients. *Front Immunol.* (2019) 10:2168. doi: 10.3389/fimmu.2019.02168
59. Li P, Wu M, Xiong W, Li J, An Y, Ren J, et al. Saikosaponin-d ameliorates dextran sulfate sodium-induced colitis by suppressing NF-kappaB activation and modulating the gut microbiota in mice. *Int Immunopharmacol.* (2020) 81:106288. doi: 10.1016/j.intimp.2020.106288
60. Kim YJ, Ko H, Park JS, Han IH, Amor EC, Lee JW, et al. Dimethyl cardamonin inhibits lipopolysaccharide-induced inflammatory factors through blocking NF-kappaB p65 activation. *Int Immunopharmacol.* (2010) 10:1127–34. doi: 10.1016/j.intimp.2010.06.017
61. Hatziieremia S, Gray AI, Ferro VA, Paul A, Plevin R. The effects of cardamonin on lipopolysaccharide-induced inflammatory protein production and MAP kinase and NFkappaB signalling pathways in monocytes/macrophages. *Br J Pharmacol.* (2006) 149:188–98. doi: 10.1038/sj.bjp.0706856
62. Behera JP, Mohanty B, Ramani YR, Rath B, Pradhan S. Effect of aqueous extract of Aegle marmelos unripe fruit on inflammatory bowel disease. *Indian J Pharmacol.* (2012) 44:614–8. doi: 10.4103/0253-7613.100389
63. Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev.* (2008) 88:1243–76. doi: 10.1152/physrev.00031.2007
64. Runkel ED, Liu S, Baumeister R, Schulze E. Surveillance-activated defenses block the ROS-induced mitochondrial unfolded protein response. *PLoS Genet.* (2013) 9:e1003346. doi: 10.1371/journal.pgen.1003346
65. Chaparala A, Tashkandi H, Chumanevich AA, Witalison EE, Windust A, Cui T, et al. Molecules from American ginseng suppress colitis through nuclear factor erythroid-2-related factor 2. *Nutrients.* (2020) 12:1850. doi: 10.3390/nu12061850
66. Piotrowska M, Swierczynski M, Fichna J, Piechota-Polanczyk A. The Nrf2 in the pathophysiology of the intestine: molecular mechanisms and therapeutic implications for inflammatory bowel diseases. *Pharmacol Res.* (2020) 163:105243. doi: 10.1016/j.phrs.2020.105243
67. El-Baz AM, Khodir AE, Adel El-Sokkary MM, Shata A. The protective effect of Lactobacillus versus 5-aminosalicylic acid in ulcerative colitis model by modulation of gut microbiota and Nrf2/Ho-1 pathway. *Life Sci.* (2020) 256:117927. doi: 10.1016/j.lfs.2020.117927
68. Salomon N, Lang A, Gamus D. [Curcumin add-on therapy for ulcerative colitis]. *Harefuah.* (2015) 154:56–8, 66.
69. Park KI, Kim DG, Lee BH, Ma JY. Fermented herbal formulas KIOM-MA128 Ameliorate IL-6-induced intestinal barrier dysfunction in colon cancer cell line. *Mediators Inflamm.* (2016) 2016:6189590. doi: 10.1155/2016/6189590
70. Johansson ME, Gustafsson JK, Holmen-Larsson J, Jabbar KS, Xia L, Xu H, et al. Bacteria penetrate the normally impenetrable inner colon mucus layer in both murine colitis models and patients with ulcerative colitis. *Gut.* (2014) 63:281–91. doi: 10.1136/gutjnl-2012-303207
71. van der Post S, Jabbar KS, Birchenough G, Arike L, Akhtar N, Sjovall H, et al. Structural weakening of the colonic mucus barrier is an early event in ulcerative colitis pathogenesis. *Gut.* (2019) 68:2142–51. doi: 10.1136/gutjnl-2018-317571

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Novel Gene Signatures Predicting Primary Non-response to Infliximab in Ulcerative Colitis: Development and Validation Combining Random Forest With Artificial Neural Network

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Background: While infliximab has revolutionized the treatment of ulcerative colitis, primary non-response is difficult to predict, which limits effective disease management. The study aimed to establish a novel genetic model to predict primary non-response to infliximab in patients with ulcerative colitis.

Methods: Publicly available mucosal expression profiles of infliximab-treated ulcerative colitis patients (GSE16879, GSE12251) were utilized to identify potential predictive gene panels. The random forest algorithm and artificial neural network were applied to further screen for predictive signatures and establish a model to predict primary non-response to infliximab.

Results: A total of 28 downregulated and 2 upregulated differentially expressed genes were identified as predictors. The novel model was successfully established on the basis of the molecular prognostic score system, with a significantly predictive value (AUC = 0.93), and was validated with an independent dataset GSE23597 (AUC = 0.81).

Conclusion: Machine learning was used to construct a predictive model based on the molecular prognostic score system. The novel model can predict primary non-response to infliximab in patients with ulcerative colitis, which aids in clinical-decision making.

Keywords: ulcerative colitis, infliximab, predictive model, machine learning, primary non- response

INTRODUCTION

While the exact pathogenesis of ulcerative colitis (UC) remains unclear, factors including genetic predisposition, environmental factors, intestinal barrier defects, and dysregulation of the immune system all contribute to the disease (1, 2). Five-amino salicylates, corticosteroids, and azathioprine are conventionally used to induce and maintain clinical remission based on the severity and location of UC (3). Nevertheless, the clinical benefits of these traditional therapeutic drugs are limited due to their lack of specificity.

Infliximab (IFX), a monoclonal antibody against human tumor necrosis factor alpha (TNF-α), has revolutionized the treatment of UC. At present, IFX is generally used for moderate to severe UC, with the advantage of promoting mucosal healing, reducing the probability of surgery, and improving the prognosis (4). However, the response rate to IFX differs among patients. It has been reported that up to 30% of patients show primary non-response (PNR) to IFX, which means they receive no clinical benefit from IFX and effective disease treatment is often delayed (5, 6). Therefore, it is important to construct a reliable model to predict non-response to IFX in the early stages of the disease.

Currently, there are few effective tools able to accurately predict PNR due to the complexity of the IFX treatment mechanism. Rapid advances in the field of bioinformatics offer new approaches for predictions with clinical application in addition to therapeutic drug monitoring, serological antibodies, and C-reactive protein levels. Detecting genetic signatures in array data is a robust way to predict clinical response based on the hypothesis that single nucleotide polymorphisms (SNPs) related to the pathogenesis of disease or mechanism of drug action may determine the relationship between genes and drug therapeutic effect (7).

Machine learning techniques, including random forest (RF) and artificial neural network (ANN), have been successful in biomarker discovery and in studies spanning multiple disease types (8–10). With the development of machine learning, the most significant differentially expressed genes (DEGs) can be selected and converted to statistical models to guide clinicians to reasonable and effective therapeutic options (11). In our previous study, we identified the TNFRSF1B SNP variation as a predictor for secondary non-response to IFX in Crohn's disease. However, a comprehensive analysis of the genetic predictors of IFX response in patients with UC is still lacking. Thus, the aim of this study was to develop and validate a genetic model based on machine learning to predict IFX PNR in patients with UC.

MATERIALS AND METHODS

Study Design and Processing

In order to predict IFX PNR prior to treatment, three sets of mucosal array profiles at the baseline (week 0) in IFX-treated UC patients were employed in this study (GSE16879, GSE12251, and GSE23597). The training datasets comprised GSE16879 and GSE12251, and GSE23597 was selected as the validation dataset. In this study, whether patients responded to IFX was defined according to the Mayo endoscopic subscore and histological score for UC. The therapeutic effect of IFX in all study participants was evaluated within 14 weeks of starting treatment; PNR was diagnosed if patients showed no endoscopic improvement in this time. RF was used to further screen the top-30 DEGs that contributed the most to the prediction of PNR to IFX in UC patients. Subsequently, gene expression scores were calculated according to the expression data of DEGs from all samples. The weight values of the top-30 DEGs were attained by developing an ANN model. Then, we used the weight values and gene expression scores to build a molecular prognostic score (mPS) system, and GSE23597 was used as a validation dataset to prove the efficacy of the novel predictive model. The study flowchart is shown in **Figure 1**. Institutional review board approval is not needed for this study.

Abbreviations: UC, Ulcerative Colitis; IFX, Infliximab; TNF-α, Tumor necrosis factor alpha; PNR, Primary Non-Response; SNPs, Single Nucleotide Polymorphisms; RF, Random Forest; ANN, Artificial Neural Network; DEGs, Differentially expressed genes; mPS, molecular Prognostic Score; AUC, The area under the receiver operating characteristic curve; LOR : Loss of response.

Datasets and Identification of DEGs

The raw data of the datasets used in our study were downloaded from the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/). These three datasets were derived from the same microarray platform, GPL570 [(HGU133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Arrays]. Analysis and verification were conducted on the expression profiles of IFX-treated UC patients at the baseline, which were extracted from diseased rectal biopsies within a week prior to the first intravenous infusion of 5 mg infliximab per kg body weight (12, 13). According to the clinical information provided by the datasets, these patients were classified as responders and non-responders.

The raw data were processed and standardized using R software (version 4.0.1). The datasets for gene expression were normalized using the RMA algorithm. Multiple probes associated with the same gene were deleted and summarized for further analysis. The Limma package was used to remove the batch effect by building linear models, and to identify DEGs. The significant DEGs of the training dataset were identified with the threshold: false discovery rate <0.05, and $|\log2 \text{ (Fold Change)}| > 1$. A volcano plot was generated to visualize the DEGs.

Gene Ontology and Pathway Enrichment Analysis

Metascape (http://metascape.org) was used to perform pathway enrichment and biological process annotation, providing comprehensive and detailed information for each gene (14). In this study, Metascape was used to carry out gene ontology and pathway enrichment analysis in order to identify the functional biological terms and signaling pathways of significant DEGs in the training dataset. Only terms with $P < 0.01$ and a count of enriched genes ≥ 3 were considered significant. All the significant terms were then grouped into clusters based on their membership similarities, and the most enriched term was chosen to represent the cluster.

Screening DEGs Predicting PNR to IFX With Machine Learning

The Random Forest package in R version 4.0.1 was applied to further screen out 30 DEGs that contributed the most to the prediction of PNR to IFX in UC patients. The top 30 is a common selection criterion based on the algorithm requirements of RF packages and has been widely used in similar studies (15, 16). Subsequently, the expression data of the 30 DEGs were converted into a score table named "Gene Score," according to the diagnosis of UC (17). The specific conversion rules are as follows: If the

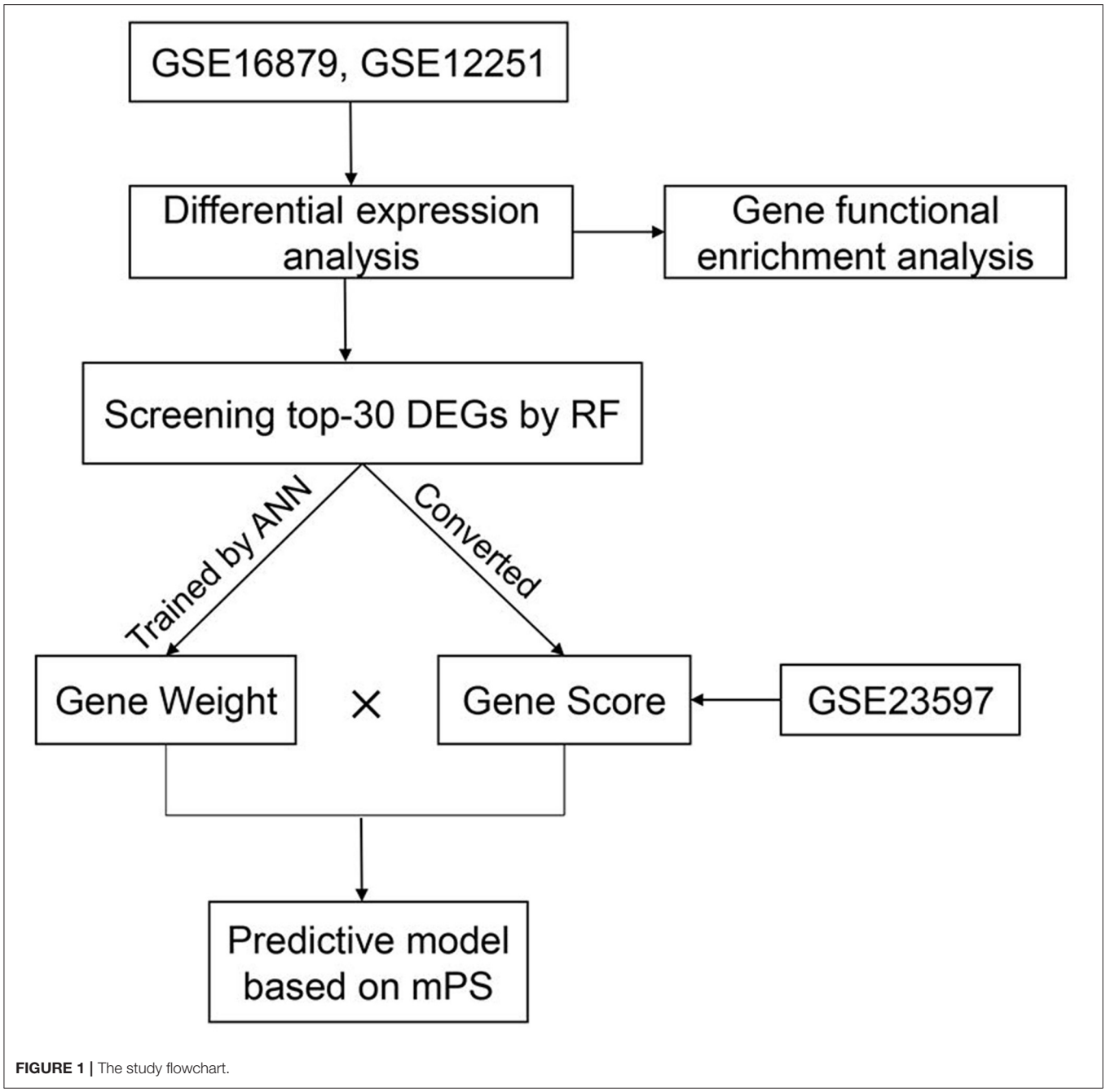

FIGURE 1 | The study flowchart.

expression value of an upregulated gene in a certain sample is higher than the median expression value of the gene in all samples, its expression value will be converted to 1, otherwise 0. If the expression value of a downregulated gene is higher, its expression value will be converted to 0, otherwise 1. As for the therapeutic effect of IFX in UC patients, responders are converted to 1 and non-responders are converted to 0. Above all, the Gene Score is composed of 46 lines of samples, 30 columns of DEGs, and column of response to IFX (response/non-response).

Finally, we used the Python-based Keras library to establish an ANN forecast model. The therapeutic result of IFX was designated as y, and the Gene Score of each of the top-30 DEGs was designated as x. The ANN was composed of one input layer, one hidden layer, and one output layer. In the hidden layer, we set ten hidden nodes and exploited rectified linear unit as an activation function. In the output layer, we set two nodes (response/non-response) and the activation function of each node was a softmax function. The cross-entropy error was set as a loss function and the Adam method was used to optimize the value of each weight. After training, we selected the maximum weight value of a certain DEG in the hidden layer named "Gene Weight" (18).

Development and Validation of the Predictive Model

The construction of the model to predict PNR to IFX in UC patients was based on the mPS system. As an innovative scoring system, mPS was created in 2019, and is effective in the prediction of overall survival of breast cancer patients and the diagnosis of UC (17, 18). The mPS of each sample was calculated by summation of "Gene Score" × "Gene Weight" for all top-30 DEGs (18).

The array data in GSE23597 were used to validate the effectiveness of the mPS scoring system based on the training dataset. According to the conversion rules, we obtained an updated "Gene Score," and calculated the summation of "Gene Score" × "Gene Weight." The area under the receiver operating characteristic curve (AUC) was used to evaluate the predictive value of this model, and it was calculated using the ROCR package in R (version 4.0.1). If the AUC value was higher than 0.8, it was considered an excellent discrimination. If the AUC value was higher than 0.9, it was considered an outstanding discrimination (19).

RESULTS

Determination of Sample Group

UC patients initially treated with IFX induction therapy in GSE16879 and GSE12251 were enrolled into the training dataset. The expression profiles at the baseline (week 0) were used for further analysis. The response to IFX was defined as complete mucosal healing with a Mayo endoscopic subscore of 0 or 1 and a grade 0 or 1 on the histological score for UC (12, 20). Non-responders were patients who did not achieve healing, although some presented with minor endoscopic or histologic improvement (12, 20). The response to IFX was assessed 4 weeks after the first IFX therapy in the GSE16879 dataset and 8 weeks in the GSE12251 dataset.

The validation dataset was UC patients initially treated with IFX in GSE23597, and the response to IFX was assessed 8 weeks after the first IFX therapy (13, 21). The specific information of the training and validation datasets we selected is shown in **Table 1**.

Identification of DEGs

A total of 104 DEGs were identified in the training dataset (**Figure 2**). The expression status of all DEGs is shown in the volcano plot, from which we can observe that most of the DEGs were downregulated in responders and upregulated in non-responders. Only six of them (HEPACAM2, C10orf99, HSD11B2, ADH1C, PKIB, and CHP2) were upregulated in responders and downregulated in non-responders.

Functional Enrichment Analysis of DEGs in the Training Dataset

To further understand the functions and metabolic pathways associated with these DEGs, enrichment analysis was performed using Metascape. The Metascape analysis showed the top 20 clusters in which DEGs were significantly enriched (**Figure 3**). The top enriched gene ontology terms in biological process were "myeloid leukocyte activation," and "leukocyte chemotaxis." Interestingly, in KEGG pathway analysis, DEGs were mainly involved in the "IL-17 signaling pathway" and "JAK-STAT signaling pathway." These enriched terms and pathways were upregulated in non-responders, and their inhibitors can be considered as alternative treatment strategies for these patients.

Top 30 DEGs Screened by RF

The expression data of 104 DEGs were included in the RF classifier (**Figure 4A**). The top 3 response-related genes were IL13RA2, TNFRSF11B, and STC1. Except for C10orf99 and ADH1C, the other 28 genes were downregulated in responders and upregulated in non-responders. The heat map (**Figure 4B**) shows the expression status of the top 30 DEGs.

ANN-based Establishment of the mPS

The ANN algorithm was used to optimize the weight value of each gene after the expression data of the 30 DEGs were converted into "Gene Score." The Gene Weight of each gene is shown in **Table 2**. The mPS was calculated by summation of "Gene Score" × "Gene Weight" for all 30 DEGs. Then, we set the mPS of 46 samples as predicted values and set the response of UC patients to IFX as true values. Using the ROCR package (R version 4.0.1), the AUC of our model was found to be 0.93, indicating that our model achieved outstanding predictive power (**Figure 5A**).

Validation of the Predictive Model

An independent dataset (GSE23597) was used to test whether the model we built could predict the therapeutic effect of IFX in the training dataset as well as any other independent cohort. Similarly, we used RF to screen out the top 30 DEGs of the validation set. These DEGs were the same as those of the training set, demonstrating the scalability and robustness of RF. Then, we calculated the "Gene Score" and mPS of GSE23597 in the same way as the training set. The AUC of the validation model was 0.81, confirming the validity and stability of our model (**Figure 5B**).

DISCUSSION

Infliximab has demonstrated efficacy in the treatment of moderate to severe UC, achieving mucosal healing even in steroid-refractory patients (21). However, PNR to IFX emerges due to genetic factors, as it relates to disease pathogenesis and the mechanism of action of this type of therapy (22). It is necessary to develop a simple and effective method to rapidly identify UC patients who exhibit IFX PNR. In the present study, an innovative model was established and validated to predict PNR to IFX in UC patients on the basis of machine learning and a new clinical prediction scoring system called "mPS," which has already proven useful in the prediction of malignant diseases.

Previous research on patients with inflammatory bowel disease (IBD) has identified several genes (IL13RA2, TNFRSF11B, STC1, IL-6, and IL-11) that constitute potential biomarkers that can identify patients with limited response to IFX, which is consistent with our study (12, 23). However, a systematic, robust, and reliable approach that can be applied to clinical decision-making has not yet been developed. In

TABLE 1 | The information of training/validation datasets.

Dateset ID	Platform	UC	response	Non-response
GSE16879	GPL570	24	8	16
GSE12251	GPL570	22	12	10
GSSE23597	GPL570	28	21	7

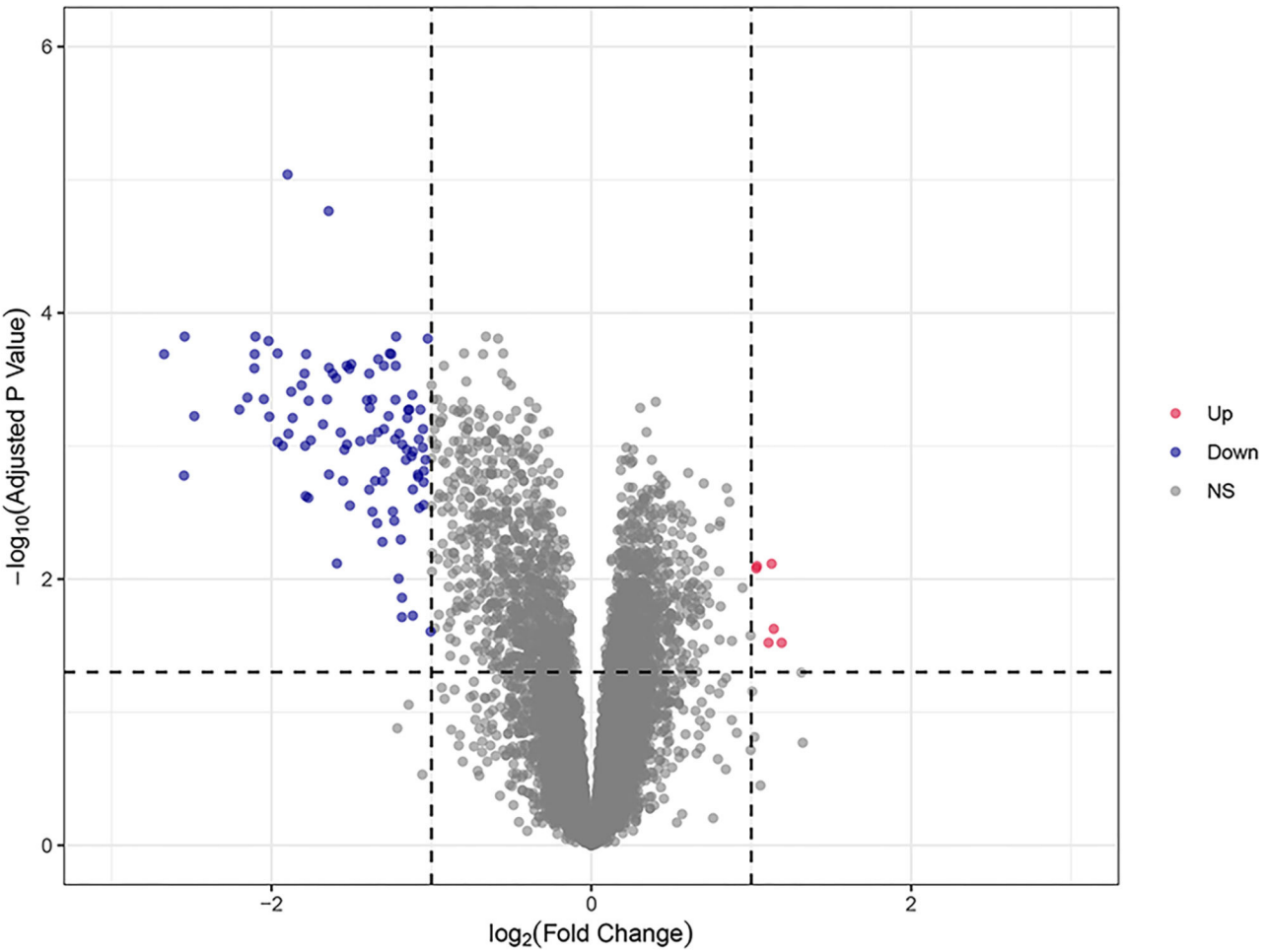

FIGURE 2 | The volcano plots of all DEGs in the training dataset. Blue and red spots represent downregulated and upregulated genes, respectively. A clear demarcation can be identified between upregulated genes and downregulated genes.

this study, we combined the strengths of machine learning techniques and mPS, not only to improve the statistical power of our predictive model, but also to transfer theoretical predictive gene panels for use in routine clinical practice. The main advantages of RF include its relatively good accuracy, robustness, and ease of use, allowing it to recognize the discriminative genes with the highest possible accuracy (24). High fault and failure tolerance, scalability, and consistent generalization ability are merits of ANN, making the model more stable and reliable (25). Therefore, our model is equipped with outstanding predictive power (AUC = 0.93) compared to another genetic model built by Bruke et al. (AUC = 0.87) (7). In addition, the mPS scoring system has proven to be simple, cost-effective, and excellent in recognizing heterogeneity among different subtypes (18). It converted complex gene expression values into simple clinical scores, so that the model can facilitate doctors in formulating a reasonable, personalized, and economical IFX regimen for UC patients.

Gene enrichment analysis of DEGs showed that most of the screened DEGs were involved in myeloid leukocyte activation and leukocyte chemotaxis. It has been reported that an increased abundance of leukocytes in non-responders promotes an increase in inflammatory macrophages, which secrete proinflammatory cytokines, including TNF-α (26). Therefore, vedolizumab, an inhibitor of α4β7 that blocks leukocyte traffic to the gut, can be used to treat non-responders as an alternative therapy. We

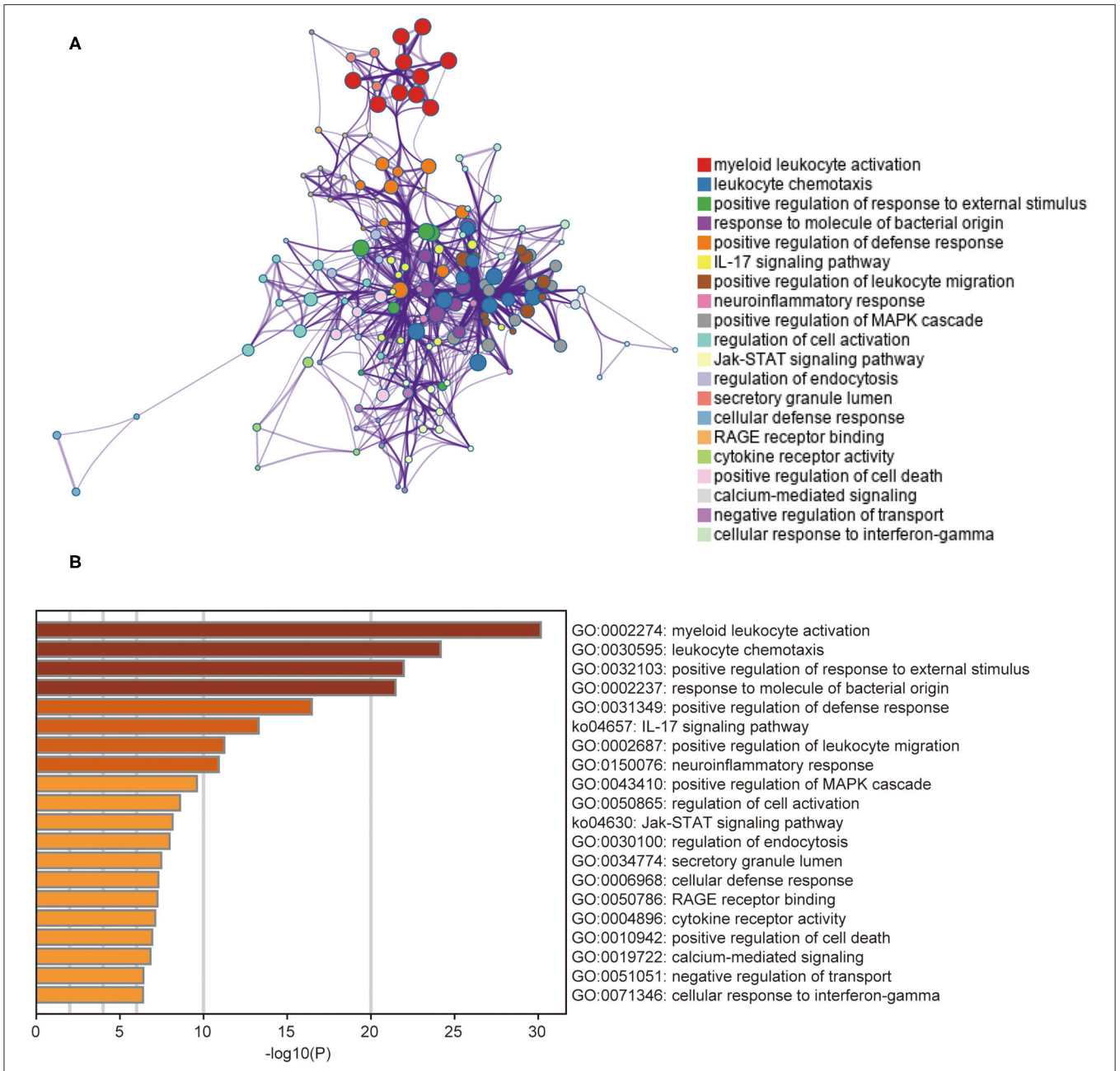

FIGURE 3 | The results of gene functional enrichment analysis. **(A)** The network of the top 20 clusters of enriched terms. Cluster identity is represented by color, the similarity score is represented by the thickness of the edge, and terms with a similarity score > 0.3 are linked by an edge. **(B)** Heat map of the top 20 clusters. Cluster identity is represented by color; the smaller the *P*-value the deeper the color.

also identified several pathways, such as the "IL-17 signaling pathway" and "JAK-STAT signaling pathway," which were significantly enriched in the non-response group. IL-17 is involved in the induction and persistence of IBD mucosal inflammation (27). The JAK-STAT pathway is the main signal mechanism for a variety of cytokines and growth factors. It transmits extracellular cytokine stimulation signals to the nucleus, coordinates appropriate cellular responses through target gene expression, and is closely related to human inflammatory diseases (28–30). Inhibitors of Janus kinases, such as Tofacitinib or Filgotinib, can thus be considered alternative treatment options for IFX non-responders. Although increased level of IL-17 was detected in intestinal mucosa of patients with Crohn's disease and ulcerative colitis, IL-17 might be a predictor or protective factor for intestinal inflammation rather than therapeutic target due to the ineffectiveness in clinical trials of Crohn's disease (31, 32). The correct interpretation of gene enrichment analysis not only contributes to understanding

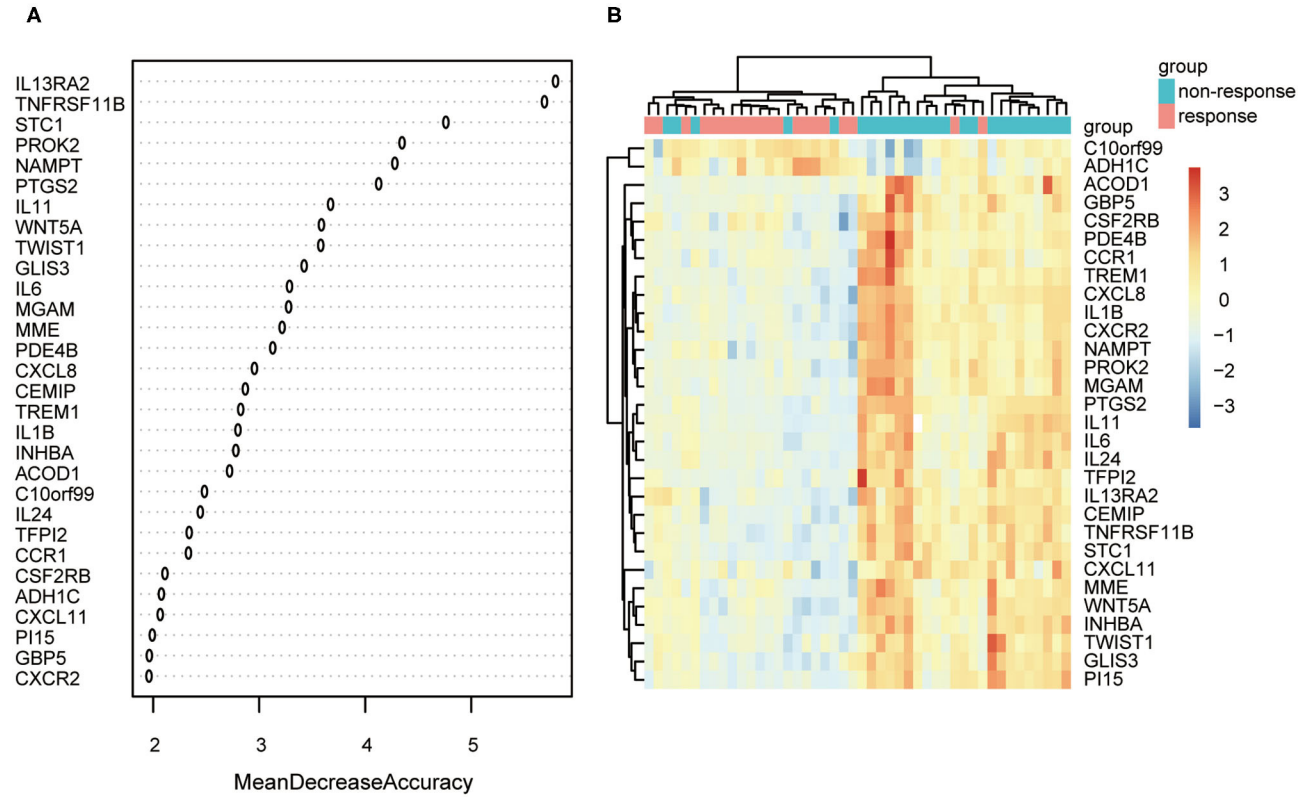

FIGURE 4 | The results of the top 30 genes screened by random forest. **(A)** The importance of the top 30 genes ranked by mean decrease of accuracy. **(B)** Heat map of the top 30 genes.

TABLE 2 | The "Gene Weight" of top 30 DEGs in the training dataset.

Gene symbol	weight	Gene symbol	weight
IL13RA2	0.3404	CEMIP	0.3602
TNFRSF11B	0.2082	TREM1	0.3959
STC1	0.3198	IL1B	0.3385
PROK2	0.3326	INHBA	0.2298
NAMPT	0.3178	ACOD1	0.4049
PTGS2	0.0912	C10orf99	0.3389
IL11	0.342	IL24	0.2098
WNT5A	0.3351	TFPI2	0.4268
TWIST1	0.3171	CCR1	0.3559
GLIS3	0.3627	CSF2RB	0.2966
IL6	0.4434	ADH1C	0.0852
MGAM	0.3457	CXCL11	0.4086
MME	0.4478	PI15	0.2851
PDE4B	0.2839	GBP5	0.1814
CXCL8	0.3087	CXCR2	0.1764

the molecular mechanism of PNR, but also provides a scientific basis for the research and development of new alternative drugs.

Moreover, the gene signatures identified in this study seem to be predictive of secondary loss of response (LOR) to IFX as well. Although secondary LOR is commonly attributed to the formation of anti-TNF antibody, it does share several common risk factors with PNR such as high inflammatory burden, male gender and so on (33). In addition, our previous work has confirmed the value of TNFRSF1B in the prediction of secondary

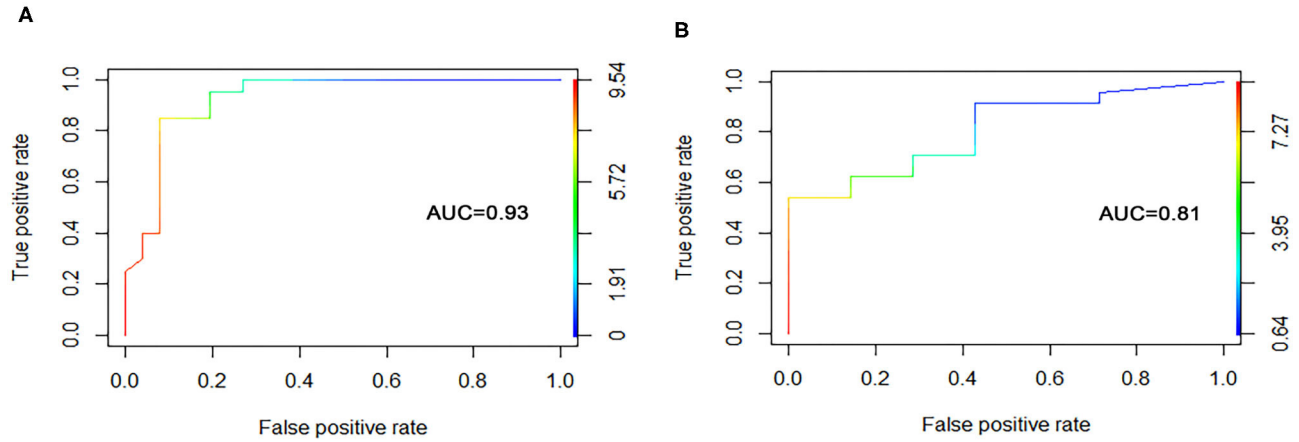

FIGURE 5 | The ROC curve of the predictive model. **(A)** Training dataset. **(B)** Validation dataset.

LOR to IFX in Crohn's disease, indicating that other predictive genes of PNR may be equally applicable to secondary LOR (34). In contrast to PNR, secondary LOR is generally accompanied by a lower serum level of IFX due to high levels of anti-TNF antibody stimulated by frequent infusion of IFX (35). Proactive monitoring drug concentration in patients with predictive gene signatures may be a good method to optimize therapy and prevent the occurrence of secondary LOR.

However, our study has several limitations. First, although our predictive model performed satisfactorily on the training and validation datasets, the sample size used to develop and validate the predictive model was relatively small. Second, the model was validated on a dataset from GEO. To make the model plausible, further bench experimental verification should be carried out. Third, the genetic model we built only applies to the identification of primary non-responders from responders in UC. Whether this model can be applied to predict the PNR of CD patient needs to be further vitrificated.

CONCLUSIONS

We established a predictive model based on machine learning techniques and an mPS scoring system that could be used to predict which UC patients will exhibit PNR to IFX, and validated this model with an independent cohort from the GEO database. Our study provides clinicians with a new treatment strategy that can improve therapeutic decision-making. The predictive genes and corresponding pathways identified in this model should be further studied to explore the molecular mechanisms underlying patient response to IFX.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

AUTHOR CONTRIBUTIONS

JF analyzed and interpreted the high throughput data, prepared, and wrote the manuscript. ZR and JS designed and drafted the work. YC helped analyse part of the data. YC and QF edited and revised manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. *Lancet.* (2017) 389:1756–70. doi: 10.1016/S0140-6736(16)32126-2
2. Feuerstein JD, Moss AC, Farraye FA. Ulcerative colitis. *Mayo Clin Proc.* (2019) 94:1357–73. doi: 10.1016/j.mayocp.2019.01.018
3. Neurath MF, Leppkes M. Resolution of ulcerative colitis. *Semin Immunopathol.* (2019) 41:747–56. doi: 10.1007/s00281-019-00751-6
4. Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* (2005) 353:2462–76. doi: 10.1056/NEJMoa050516
5. Ben-Horin S, Chowers Y. Tailoring anti-TNF therapy in IBD: drug levels and disease activity. *Nat Rev Gastroenterol Hepatol.* (2014) 11:243–55. doi: 10.1038/nrgastro.2013.253
6. Papamichael K, Rivals-Lerebours O, Billiet T, Vande CN, Gils A, Ferrante M, et al. Long-term outcome of patients with ulcerative colitis and

primary non-response to infliximab. *J Crohns Colitis.* (2016) 10:1015–23. doi: 10.1093/ecco-jcc/jjw067
7. Burke KE, Khalili H, Garber JJ, Haritunians T, McGovern D, Xavier RJ, et al. Genetic markers predict primary nonresponse and durable response to anti-tumor necrosis factor therapy in ulcerative colitis. *Inflamm Bowel Dis.* (2018) 24:1840–8. doi: 10.1093/ibd/izy083
8. Grobman WA, Stamilio DM. Methods of clinical prediction. *Am J Obstet Gynecol.* (2006) 194:888–94. doi: 10.1016/j.ajog.2005.09.002
9. Worachartcheewan A, Shoombuatong W, Pidetcha P, Nopnithipat W, Prachayasittikul V, Nantasenamat C. Predicting metabolic syndrome using the random forest method. *Sci World J.* (2015) 2015:581501. doi: 10.1155/2015/581501
10. Zafeiris D, Rutella S, Ball GR. An artificial neural network integrated pipeline for biomarker discovery using alzheimer's disease as a case study. *Comp Struct Biotechnol J.* (2018) 16:77–87. doi: 10.1016/j.csbj.2018.02.001
11. Bar-Yoseph H, Levhar N, Selinger L, Manor U, Yavzori M, Picard O, et al. Early drug and anti-infliximab antibody levels for prediction of primary nonresponse to infliximab therapy. *Aliment Pharmacol Ther.* (2018) 47:212–8. doi: 10.1111/apt.14410
12. Arijs I, Li K, Toedter G, Quintens R, Van Lommel L, Van Steen K, et al. Mucosal gene signatures to predict response to infliximab in patients with ulcerative colitis. *Gut.* (2009) 58:1612–9. doi: 10.1136/gut.2009.178665
13. Toedter G, Li K, Marano C, Ma K, Sague S, Huang CC, et al. Gene expression profiling and response signatures associated with differential responses to infliximab treatment in ulcerative colitis. *Am J Gastroenterol.* (2011) 106:1272–80. doi: 10.1038/ajg.2011.83
14. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun.* (2019) 10:1523. doi: 10.1038/s41467-019-09234-6
15. Barry G, Guennewig B, Fung S, Kaczorowski D, Weickert CS. Long non-coding RNA expression during aging in the human subependymal zone. *Front Neurol.* (2015) 6:45. doi: 10.3389/fneur.2015.00045
16. Xin W, Zhang L, Zhang W, Gao J, Yi J, Zhen X, et al. An integrated analysis of the rice transcriptome and metabolome reveals root growth regulation mechanisms in response to nitrogen availability. *Int J Mol Sci.* (2019) 20:5893. doi: 10.3390/ijms20235893
17. Li H, Lai L, Shen J. Development of a susceptibility gene based novel predictive model for the diagnosis of ulcerative colitis using random forest and artificial neural network. *Aging.* (2020) 12:20471–82. doi: 10.18632/aging.103861
18. Shimizu H, Nakayama KI. A 23 gene-based molecular prognostic score precisely predicts overall survival of breast cancer patients. *EBio Med.* (2019) 46:150–9. doi: 10.1016/j.ebiom.2019.07.046
19. Rice M, Harris G. Comparing effect sizes in follow-up studies: ROC area, Cohen's d, and r. *Law Human Behav.* (2005) 29:615–20. doi: 10.1007/s10979-005-6832-7
20. Arijs I, De Hertogh G, Lemaire K, Quintens R, Van Lommel L, Van Steen K, et al. Mucosal gene expression of antimicrobial peptides in inflammatory bowel disease before and after first infliximab treatment. *PLoS ONE.* (2009) 4:e7984. doi: 10.1371/journal.pone.0007984
21. Cury DB, Cury MDS, Elias GVH, Mizsputen SJ. Infliximab to treat severe ulcerative colitis. *World J Gastroenterol.* (2009) 15:1771–3. doi: 10.3748/wjg.15.1771
22. Dubinsky MC, Mei L, Friedman M, Dhere T, Haritunians T, Hakonarson H, et al. Genome wide association (GWA) predictors of anti-TNFα therapeutic responsiveness in pediatric inflammatory bowel disease. *Inflamm Bowel Dis.* (2010) 16:1357–66. doi: 10.1002/ibd.21174
23. Bank S, Andersen PS, Burisch J, Pedersen N, Roug S, Galsgaard J, et al. Associations between functional polymorphisms in the NFκB signaling pathway and response to anti-TNF treatment in Danish patients with inflammatory bowel disease. *Pharmacogenomics J.* (2014) 14:526–34. doi: 10.1038/tpj.2014.19
24. Zhong X, Liu Y, Liu H, Zhang Y, Wang L, Zhang H. Identification of potential prognostic genes for neuroblastoma. *Front Genet.* (2018) 9:589. doi: 10.3389/fgene.2018.00589
25. Chatzimichail E, Matthaios D, Bouros D, Karakitsos P, Romanidis K, Kakolyris S, et al. gamma -H2AX: a novel prognostic marker in a prognosis prediction model of patients with early operable non-small cell lung cancer. *Int J Genomics.* (2014) 2014:160236. doi: 10.1155/2014/160236
26. Gaujoux R, Starosvetsky E, Maimon N, Vallania F, Bar-Yoseph H, Pressman S, et al. Cell-centred meta-analysis reveals baseline predictors of anti-TNFα non-response in biopsy and blood of patients with IBD. *Gut.* (2019) 68:604–14. doi: 10.1136/gutjnl-2017-315494
27. Fujino S. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut.* (2003) 52:65–70. doi: 10.1136/gut.52.1.65
28. O'Shea JJ, Murray PJ. Cytokine signaling modules in inflammatory responses. *Immunity.* (2008) 28:477–87. doi: 10.1016/j.immuni.2008.03.002
29. O'Shea JJ, Plenge R. JAK and STAT signaling molecules in immunoregulation and immune-mediated disease. *Immunity.* (2012) 36:542–50. doi: 10.1016/j.immuni.2012.03.014
30. Stark GR, Darnell JJ. The JAK-STAT pathway at twenty. *Immunity.* (2012) 36:503–14. doi: 10.1016/j.immuni.2012.03.013
31. Petitpain N, D'Amico F, Yelehe-Okouma M, Jouzeau JY, Netter P, Peyrin-Biroulet L, et al. IL-17 inhibitors and inflammatory bowel diseases: a postmarketing study in vigibase. *Clin Pharmacol Ther.* (2021) 110:159–68. doi: 10.1002/cpt.2155
32. Akiyama S, Sakuraba A. Distinct roles of interleukin-17 and T helper 17 cells among autoimmune diseases. *J Transl Autoimmun.* (2021) 4:100104. doi: 10.1016/j.jtauto.2021.100104
33. Wong U, Cross RK. Primary and secondary nonresponse to infliximab: mechanisms and countermeasures. *Expert Opin Drug Metab Toxicol.* (2017) 13:1039–46. doi: 10.1080/17425255.2017.1377180
34. Xu L, Shen J, Zheng Q. Development of a clinical model to predict secondary non-response of infliximab treatment in Crohn's disease. *Int J Colorectal Dis.* (2020) 35:2019–26. doi: 10.1007/s00384-020-03679-8
35. Ordas I, Mould DR, Feagan BG, Sandborn WJ. Anti-TNF monoclonal antibodies in inflammatory bowel disease: pharmacokinetics-based dosing paradigms. *Clin Pharmacol Ther.* (2012) 91:635–46. doi: 10.1038/clpt.2011.328

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ORIGINAL RESEARCH
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A Novel Neutrophil-Based Biomarker to Monitor Disease Activity and Predict Response to Infliximab Therapy in Patients With Ulcerative Colitis

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Background: Ulcerative colitis (UC) is characterized by refractory and recurrent mucosal inflammation, leading to a substantial healthcare burden. Diagnostic biomarkers predicting disease activity and treatment response remain elusive. To evaluate the application value of a novel neutrophil-based index (the neutrophil-to-albumin ratio, NAR) as a novel diagnostic biomarker in patients with UC and a predictive marker for disease activity and response to infliximab (IFX) therapy.

Methods: Clinical characteristics and laboratory parameters of enrolled subjects (patients with UC and healthy controls) were retrieved from the electronic medical record database of our hospital. Serum cytokine and fecal calprotectin levels were measured by enzyme-linked immunosorbent assay (ELISA). Mucosal expression levels of inflammatory agents were measured by quantitative RT-PCR (qRT-PCR).

Results: We found that NAR, which had not yet been explored in UC, was significantly increased in patients with UC ($n = 146$) compared to that in controls ($n = 133$) (1.95 $\pm$ 0.41 vs. 1.41 $\pm$ 0.23, $p < 0.0001$). NAR showed a positive association with the disease activity and inflammatory load in patients with UC. Pre-treatment NAR was significantly lower in IFX responders than that in non-responders (2.18 $\pm$ 0.29 vs. 2.44 $\pm$ 0.21, $p = 0.0118$), showing a significant ability to discriminate initial responders from primary non-responders to IFX induction therapy (AUC = 0.7866, $p = 0.0076$). Moreover, pre-treatment NAR predicted postinduction serum IFX trough level.

Conclusion: Our study provides evidences to utilize NAR in the diagnosis, activity monitoring, and IFX response prediction in patients with UC.

Keywords: ulcerative colitis, biomarker, inflammatory bowel disease, neutrophil, albumin, neutrophil-to-albumin ratio, infliximab

INTRODUCTION

Inflammatory bowel disease (IBD) is characterized by chronic and relapsing inflammation in the gastrointestinal tract, including two eminent forms, ulcerative colitis (UC) and Crohn's disease (CD). The prevalence of IBD is rapidly growing worldwide and rising health expenditures (1–4), leading to a substantial healthcare burden (5). In addition, IBD is medically refractory and has become a great clinical challenge.

Endoscopic biopsies have been recognized as the diagnostic gold standard in IBD. However, endoscopy has drawbacks that limit its application particularly in long-term follow-up, such as invasiveness, high cost, and inter-observer variability (6). In addition, patients may not tolerate endoscopic examinations very well and those who undergo endoscopy may complained embarrassment, discomfort caused by bowel preparation, and increased abdominal pain (7, 8).

Besides endoscopy, a panel of adjunctive serological biomarkers, which are much less-invasive, have been applied at nearly every point in the management of IBD. They have shown practical values to distinguish IBD from gastrointestinal functional disease, differentiate active from inactive status, and predict therapeutic effect, recurrence, prognosis (9).

Blood tests including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fibrinogen, albumin, bilirubin, uric acid, auto-antibodies (e.g., anti-pTNP, ASCA antibodies) are widely utilized in IBD management (10–14), as well as fecal calprotectin and lactoferrin (15). Given the crucial role of neutrophils in the disease pathogenesis (16, 17), neutrophil-based indexes have been generated and applied in the IBD area (18–23). For example, the neutrophil-to-lymphocyte ratio (NLR) is easily accessible from routine blood tests for both inpatients and outpatients, and it has been strongly suggested as a valuable predictive marker to distinguish patients with IBD from non-IBD controls and differentiate disease activity in IBD (24).

Recently, the neutrophil-to-albumin ratio (NAR) has emerged as a sensitive index which indicates systemic inflammation and has been used in inflammatory, vascular diseases, and cancers (25–27). Since serum biochemical tests are also routine procedures to evaluate the nutritional status and screen renal/hepatobiliary dysfunctions in patients with IBD, NAR can be readily obtained for nearly any patient under care for IBD. However, to our best knowledge, this index has not yet been utilized in UC.

Therefore, we questioned whether NAR could be used as a reliable, non-invasive, and cost-effective biomarkers, regarding disease diagnosis, activity monitoring, or drug response prediction. In this study, we evaluated the application value of NAR as a diagnostic biomarker in patients with UC and a predictive marker for disease activity and response to infliximab (IFX) induction therapy.

Abbreviations: IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; NAR, neutrophil-to-albumin ratio; NLR, neutrophil-to-lymphocyte ratio; ROC, receiver operating characteristics; PCR, polymerase chain reaction; qRT-PCR, quantitative Real-time PCR; ELISA, enzyme-linked immunosorbent assay; IFX, infliximab; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; TNF, tumor necrosis factor; IFN, interferon; miR, microRNA; UCEIS, ulcerative colitis endoscopic index of severity; AUC, area under the curve.

METHODS

Participants

This prospective study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board for Clinical Research of Sichuan Provincial People's Hospital (No.201685, 2020204).

Patients with UC were consecutively recruited from the Department of Gastroenterology of Sichuan Provincial People's Hospital between January 2017 and December 2021. We also consecutively recruited non-IBD subjects, who underwent routine physical examinations in our hospital during the study. Participants enrolled in the current study were well-informed and signed an informed consent before participation. Patients with UC and controls were gender- and age-matched. Clinical characteristics and laboratory parameters of enrolled subjects were retrieved from the electronic medical record database of our hospital.

Exclusion criteria for both patients with UC and controls were as follows: smoking, excessive drinking, hematopoietic system disease, hepatobiliary disease, coagulation abnormalities, taking medications that can affect blood cell components or serum biochemistry profiles, hypertension, diabetes, infections, other systemic autoimmune diseases, other gastrointestinal diseases, and cancers. Demographics and clinical parameters of patients with UC ($n = 146$) and healthy controls ($n = 133$) were shown in **Table 1**.

As reported previously (28, 29), the diagnosis of UC was performed according to the comprehensive analysis of medical history, clinical manifestations, radiological, endoscopic, and histological examinations, as well as laboratory tests. The Mayo score system was utilized to determine the disease activity in patients with UC. To detailedly evaluate mucosal disease activity, the ulcerative colitis endoscopic index of severity (UCEIS) system was used. The disease extent of UC was identified based on the Montreal classification.

Infliximab (IFX) induction therapy was administered as reported previously (28). Briefly, patients with UC were infused with IFX (5 mg/kg) at weeks 0, 2 and 6 for induction. Short-term response to IFX were evaluated at 12–14 weeks post the initial infusion, when serum IFX trough levels were determined. Response (complete or partial) or primary non-response was defined by the physicians. To determine the predictive value of NAR in IFX response, patients were subjected to complete blood cell and serum biochemistry tests within 1 week before the first infusion of IFX, and pre-treatment NAR was calculated.

Mucosal Inflammatory Agent Assessment

Mucosal biopsy tissues were collected during endoscopic examinations, immediately frozen in liquid nitrogen, and then sent to our laboratory for extended storage at −80°C. Total RNA was extracted from mucosal tissues using TRIzol reagent (Thermo Scientific). Reverse transcription for mRNA and microRNA (miR) were performed, respectively. Relative

TABLE 1 | Demographics and clinical parameters of patients with UC and healthy controls.

	UC	Healthy controls	*p*-value
Number of subjects (*n*)	146	133	-
Age (year)	38.5 ± 9.8	37.2 ± 10.4	0.2834*
Gender (*n*)			
Female	52	50	0.7319**
Male	94	83	
Disease duration (months)	35.4 ± 16.9	-	-
Disease extent (*n*)***			
E1	27	-	
E2	48	-	
E3	70	-	
Blood neutrophil (%)	69.66 ± 11.04↑	59.38 ± 8.52	<0.0001*
Serum albumin (g/L)	36.80 ± 7.31 ↓	42.40 ± 2.57	<0.0001*
CRP (mg/L)	31.29 ± 23.28	-	-
ESR (mm/hour)	53.84 ± 36.96	-	-
NAR	1.95 ± 0.41 ↑	1.41 ± 0.23	<0.0001*

Data are presented as mean ± SD when applicable.
UC, ulcerative colitis; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; NAR, neutrophil-to-albumin ratio.
**Student's t-test (unpaired, two-tailed), $p < 0.05$ was considered statistically significant.*
***Chi-square test, $p < 0.05$ was considered statistically significant.*
****Phenotypes of UC were classified according to the Montreal classification system.*
↑and↓: increased and decreased compared with healthy controls, respectively.

expression of cytokines and miR-301a was determined by qRT-PCR using a SYBR Green real-time PCR system (Invitrogen, CA, USA). The GAPDH and U6 expression levels were employed to normalize the expression of mRNA and miR, respectively (28).

Measurement of Serum Cytokine and Fecal Calprotectin

Serum and fecal samples were collected, processed within 1 h, and stored at −80°C. Enzyme-linked immunosorbent assay (ELISA) was performed as described previously (28, 29). All ELISA kits (TNF-α, IFN-γ, calprotectin) were purchased from BioLegend (San Diego, CA, USA) and utilized according to the manufacturer's instructions.

Statistical Analysis

Data are presented as mean ± SD when applicable. Chi-square test was performed to examine the difference of gender between patients with UC and controls. Comparisons between UC and controls regarding age and clinical parameters were performed by unpaired Student's *t*-test (two-tailed). The difference of NAR between IFX responders and primary non-responders was also examined by unpaired Student's *t*-test (two-tailed). The discriminating performance of a biomarker in indicated scenarios was determined by receiver operator curves (ROC) analysis. Correlations between two parameters were analyzed using Pearson's correlation analysis. $p < 0.05$ was set as statistically significant. All statistical analysis was performed using a Prism software Version 8.4 (Graphpad Software, San Diego, California, USA).

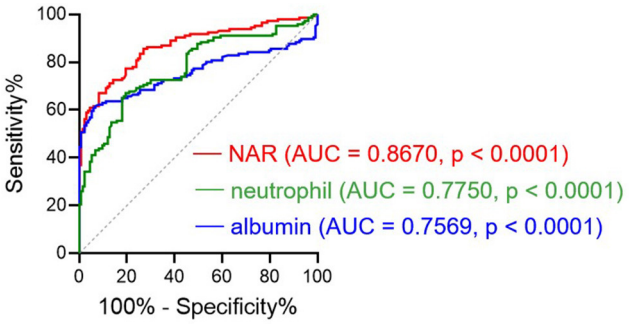

FIGURE 1 | Receiver operating characteristics (ROC) curve analysis. The performances of the neutrophil-to-albumin ratio (NAR), blood neutrophil percentages, and serum albumin levels to discriminate ulcerative colitis (UC) from controls were determined by ROC curve analysis. AUC, area under the ROC curve. $p < 0.05$ was considered significant. UC, $n = 146$; Controls, $n = 133$.

RESULTS

Demographics and Clinical Parameters of the Participants

In the current study, 146 patients with UC (female = 52, male = 94) and 133 healthy controls (female = 50, male = 83) were included (**Table 1**). The mean age of patients with UC and healthy control was 38.5 ± 9.8 and 37.2 ± 10.4 years old, respectively. The duration of disease in patients with UC was 35.4 ± 16.9 months. There were no significant differences between patients with UC and healthy control regarding the age ($p = 0.2834$) and gender ($p = 0.7319$). Disease extent of UC was identified according to the Montreal classification system.

Neutrophil percentages were determined by complete blood cell tests, and albumin levels were examined by serum biochemistry tests. In line with existing evidences (11), patients with UC, compared to healthy controls, displayed a significant increase in neutrophil percentages (69.66 ± 11.04 vs. 59.38 ± 8.52 %, $p < 0.0001$) and a decrease in serum albumin levels (36.80 ± 7.31 vs. 42.40 ± 2.57 g/L, $p < 0.0001$).

Next, we calculated NAR as the ratio of neutrophil percentages over serum albumin levels and found that NAR was up-regulated by 1.4-fold in patients with UC compared with in healthy controls (1.95 ± 0.41 vs. 1.41 ± 0.23, $p < 0.0001$).

In regard to the ability to discriminate UC from controls, we performed ROC analysis (**Figure 1**), showing that NAR had larger AUC (AUC = 0.8670) compared to neutrophil (AUC = 0.7750) or albumin (AUC = 0.7569) alone. Taken together, these data suggest that NAR could be a practical, rapid, and easily accessible biomarker for UC diagnosis.

NAR Reflects Disease Activity in UC

Since NAR could be used as a diagnostic biomarker for UC, we moved forward to explore whether this marker was able to distinguish UC activity. We looked at the potential association between NAR and Mayo scores, and patients with higher NAR

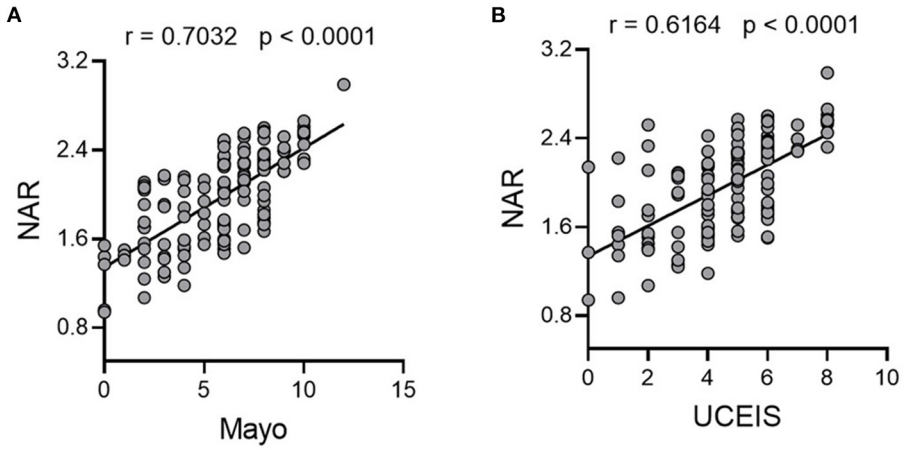

FIGURE 2 | NAR reflects disease activity in UC. All enrolled patients with UC ($n = 146$) were subjected to disease activity evaluation by the Mayo score and the ulcerative colitis endoscopic index of severity (UCEIS) system. Associations between NAR and **(A)** Mayo score, and **(B)** UCEIS were examined. The correlation analysis was performed using Pearson's correlation. $p < 0.05$ was considered significant.

exhibited more severe disease activity (**Figure 2A**, Pearson r = 0.7032, $p < 0.0001$).

Additionally, mucosal healing has been highlighted in the management of IBD and amounts of evidences have stressed that patients with mucosal healing display a lower rate of hospitalization and better prognosis (30–33). Therefore, we also employed UCEIS, which had a high interobserver agreement rate (34, 35), as an additional system to detailedly investigate the correlation between NAR and the endoscopic activity of patients with UC. As shown in **Figure 2B**, NAR showed a positive association with mucosal disease activity (Pearson r = 0.6164, $p < 0.0001$).

Associations Between NAR and Inflammatory Load in Patients With UC

Both mucosal and systemic inflammatory conditions are related to disease severity in patients with UC, and higher inflammatory responses tend to indicate more severe disease activity and poorer drug responses (36). To this end, we first looked at serum inflammatory factors. CRP and ESR are the most commonly used disease activity biomarkers of IBD and up-regulated serum pro-inflammatory cytokines such as TNF-α and IFN-γ are hallmarks of IBD. We found that NAR was positively correlated with CRP, ESR, serum TNF-α and IFN-γ (**Figures 3A–D**), suggesting that NAR can be utilized as a marker reflecting the systemic inflammatory load in patients with UC.

Subsequently, we examined associations between NAR and mucosal inflammatory responses. Since neutrophilic accumulation in the inflamed mucosa is intimately associated with the disease activity in UC, it has been suggested that detection of inflammatory proteins secreted by neutrophils in the feces may be a promising procedure for mucosal inflammation assessment (37). Fecal calprotectin is a neutrophil-derived protein that has been applied as a reliable screening tool for intestinal inflammation due to its sensitivity. As shown in **Figure 3E**, NAR correlated well with fecal calprotectin levels in UC.

Next, we analyzed mucosal expression of TNF- α and IFN-γ mRNA, both of which were also positively associated with NAR (**Figures 3F,G**). In addition, our previous studies have demonstrated that mucosal miR-301a is a useful biomarker for diagnosis and disease activity in UC (28, 29). Therefore, we employed mucosal expression of miR-301a as an inflammatory factor of patients with UC, which was positively associated with NAR (**Figure 3H**). Collectively, our findings demonstrate that NAR, which is more convenient and faster than cytokine and fecal protein measurement, might assist to evaluate the systemic and mucosal inflammatory load in patients with UC.

NAR Predicts Response to IFX in Patients With UC

The therapy for IBD has been revolutionized, thanks to biologics, especially anti-TNF agents (such as IFX), which are effective to induce and maintain remission in patients with both UC and CD. However, IFX does not work well in each patient, and about 65% of patients with UC respond to IFX induction therapy and the rate drops down to 45.5% during the maintenance therapy (38). Therefore, efforts have been made to identify reliable predictive biomarkers for both short- and long-term response to IFX.

To this end, we questioned whether NAR played as a predictor in this scenario. We enrolled 34 patients with UC, who were treated with IFX therapy (5 mg/kg at weeks 0, 2 and 6), including 23 responders (both complete or partial) and 11 with primary non-responders (evaluated at 12–14 weeks after the initial infusion). Patients were subjected to complete blood cell and serum biochemistry tests within 1 week before the first infusion of IFX, and pre-treatment NAR was calculated. As shown in **Figure 4A**, pre-treatment NAR was significantly lower in IFX responders than that in non-responders (2.18 ± 0.29 vs. 2.44 ± 0.21, $p = 0.0118$).

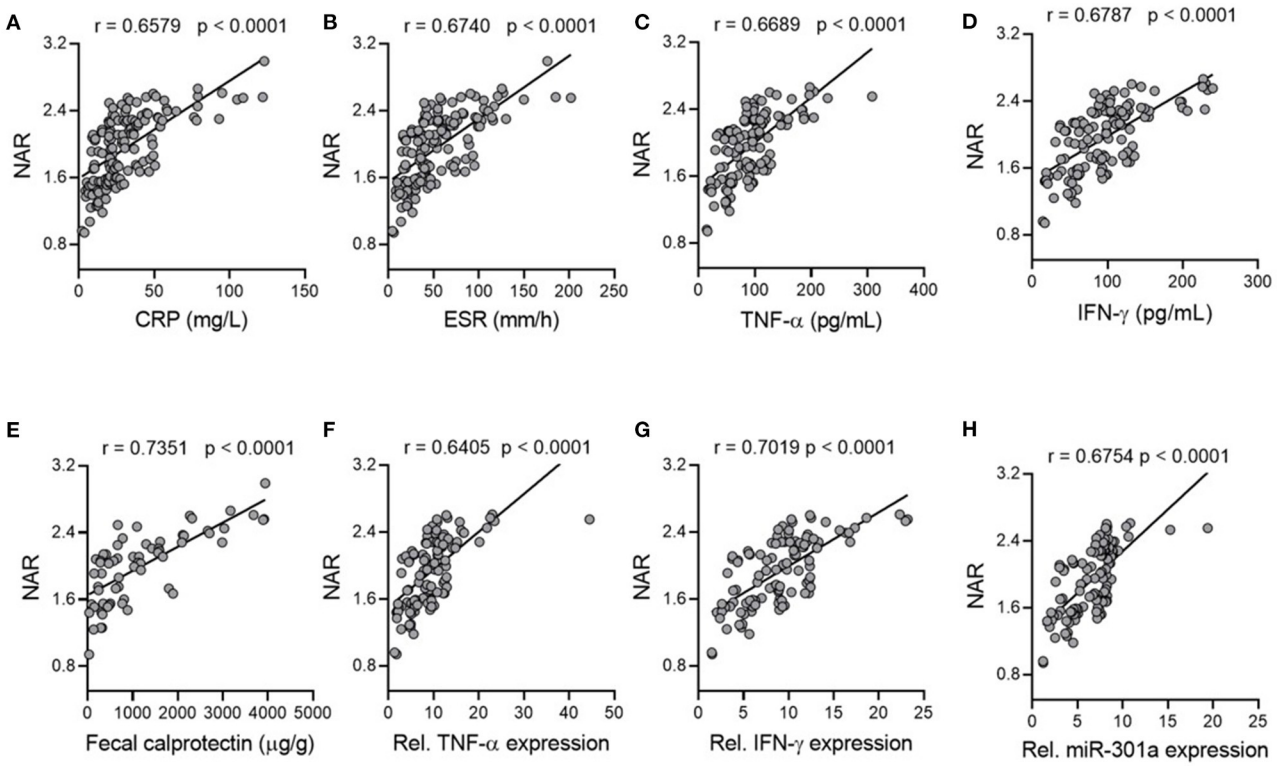

FIGURE 3 | Associations of NAR with inflammatory load in patients with UC. Correlation of NAR with **(A)** C-reactive protein (CRP) and **(B)** erythrocyte sedimentation rate (ESR). **(A,B)**, $n = 146$. Serum **(C)** TNF-α and **(D)** IFN-γ protein levels were positively associated with NAR. **(C,D)**, $n = 122$. **(E)** Fecal calprotectin levels ($n = 66$), mucosal **(F)** TNF-α, **(G)** IFN-γ, and **(H)** miR-301a relative expression levels positively correlated with NAR. **(F–H)**, UC, $n = 110$; Controls, $n = 75$ (for gene relative expression calculation).

Next, we performed the receiver operating characteristics (ROC) curve analysis to determine the discriminative performance of NAR as predictors for response to IFX induction. NAR exhibited a significant ability to discriminate between initial responders and primary non-responders to IFX induction therapy (**Figure 4B**, AUC = 0.7866, $p = 0.0076$).

NAR Predicts Serum IFX Trough Level (SIFX TL) in Patients With UC

Since one contributing factor of primary non-response is low serum trough levels in IFX-treated patients (39, 40), and IFX clearance is accelerated in patients with high inflammatory load, causing insufficient clinical response (41, 42). As presented above, we demonstrated that NAR was a reflective marker of systemic and mucosal inflammatory conditions and higher NAR indicated lower rate of IFX response. Therefore, we sought to explore whether NAR was associated with postinduction sIFX TL. As shown in **Figure 5**, all 34 patients who received IFX therapy were grouped by quartiles of NAR and patients in quartiles 1 ($\geq$ 1.67– $<$ 2.11) exhibited significantly higher sIFX TL than those in other 3 quartiles. The lowest sIFX TL was observed in quartiles 4 ($\geq$ 2.55), and no differences was found between quartiles 2 ($\geq$ 2.11– $<$ 2.31) and 3 ($\geq$ 2.31– $<$ 2.55). Collectively, these observations suggest that pre-treatment NAR might be a potential predictor for postinduction sIFX TL.

DISCUSSION

In the current study, we analyzed the alterations of a novel neutrophil- and albumin-based biomarker in patients with UC, demonstrating that: (1) NAR was significantly up-regulated in patients with UC compared with that in controls; (2) NAR showed significantly positive associations with the disease activity and inflammatory load in UC; (3) higher pre-treatment NAR indicated lower rate of short-term response to IFX therapy in patients with UC; (4) pre-treatment NAR predicted postinduction sIFX TL. These observations prompt us to speculate that NAR may be a promising biomarker in the diagnosis, activity monitoring, and IFX response prediction in patients with UC.

Although endoscopic examinations are the most powerful tool that can directly identify the lesion extent, location, and inflammation activity, several limitations are noteworthy such as high cost, inconvenience, and invasiveness, making this procedure unsuitable for frequent, long-term surveillance of UC. Given the advantage of non-invasiveness, serological and fecal biomarkers that can accurately reflect the gastrointestinal

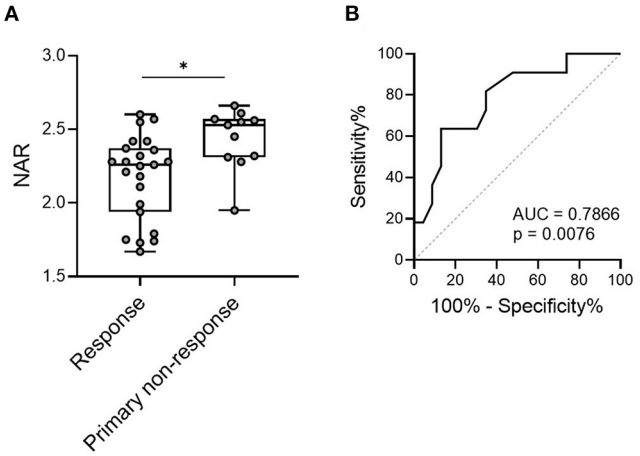

FIGURE 4 | NAR predicts short-term response to infliximab (IFX) in patients with UC. Patients with UC ($n = 34$) were infused with IFX (5 mg/kg) at weeks 0, 2 and 6 for induction. Short-term response to IFX were evaluated at 12–14 weeks post initial infusion. Patients were subjected to complete blood cell and serum biochemistry tests within 1 week before the first infusion of IFX, and pre-treatment NAR was calculated. **(A)** Comparison of pre-treatment NAR was performed between responders (complete and partial, $n = 23$) and primary non-responders ($n = 11$). *$p < 0.05$, unpaired Student's t-test (two-tailed). **(B)** The ability of NAR to discriminate IFX responders from primary non-responders were determined by ROC curve analysis. $p < 0.05$ was considered significant.

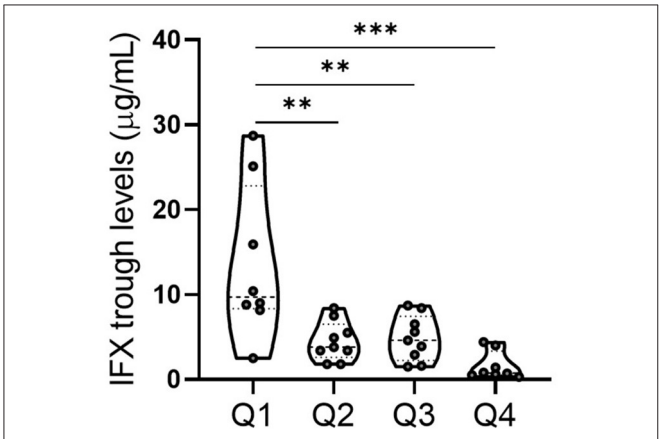

FIGURE 5 | Association between NAR and serum IFX trough level (sIFX TL) in patients with UC. All 34 patients who received IFX induction therapy were grouped by quartiles of NAR. Q1 (quartiles 1, $n = 8$): $1.67 \leq NAR < 2.11$; Q2 (quartiles 2, $n = 9$): $2.11 \leq NAR < 2.31$; Q3 (quartiles 3, $n = 9$): $2.31 \leq NAR < 2.55$; Q4 (quartiles 4, $n = 8$): $2.55 \leq NAR$. sIFX TL in each group were measured. **$p < 0.01$, ***$p < 0.001$. One-way ANOVA followed by Tukey's multiple comparisons test was performed and adjusted p-values were calculated.

mucosal inflammatory status have become a resurgent interest in the IBD area (10).

To date, a number of such biomarkers have been reported (6, 43). As the most commonly used biomarkers, both CPR and ESR are rapid, easily-measured, and sensitive in the assessment of IBD activity. However, they are not disease specific and rise fast under conditions other than IBD (tissue necrosis, infection, or other causes), which largely limits their practical significance (15).

Fecal calprotectin is now one of the most sensitive biological indicators for discriminating IBD from bowel dysfunction, and the endoscopically active from the inactive (37). However, for some patients, stool-based tests might not be their first choice, probably related to a reluctance to collect and process fecal samples or high cost. Therefore, rapid, convenient, inexpensive, standardized, and reproducible blood-based tests may be a preferable option for most patients who need long-term monitoring.

Complete blood cell and serum biochemical tests are two most common routine examinations for the management of both inpatients and out patients with IBD. In the current study, we employed a newly-identified index calculated as the ratio of neutrophil percentages (from complete blood cell tests) over serum albumin levels (from serum biochemical tests). Since excessive mucosal neutrophil infiltration is a remarkable feature of UC, which plays a crucial role in crypt abscesses and cryptitis, patients with mucosal histologic normalization display better clinical outcomes (44).

On the other hand, patients with IBD usually present nutritional deficiency, which leads to hypoalbuminemia. Moreover, albumin has been reported to have powerful antioxidant properties and could serve as serum biomarkers in oxidative stress/inflammation-associated disorders (45). It has been reported that patients with IBD have lower levels of serum albumin, and hypoproteinemia might reduce therapeutic efficiencies of biologics (46). In the current study, we identified a novel neutrophil- and albumin-based index NAR was significantly increased in patients with UC compared to that in healthy controls. More notably, NAR served as a reliable marker to reflect endoscopic activity and mucosal inflammatory responses in patients with UC.

Anti-tumor necrosis factor (TNF) therapies have revolutionized the treatment of patients with IBD. Particularly, IFX, as the most-investigated anti-TNF agent, is effective for induction and maintenance of clinical remission and mucosal healing in patients with moderate-to-severe UC, who have lower hospitalization and colectomy rates during follow-up compared to placebo-controls (38). However, about a third of IFX-treated patients with IBD fail to show significant clinical benefit after the induction therapy (14 weeks after the initiation infusion), which is called "primary non-response" (40). Furthermore, secondary non-response during the first year of maintenance therapy can be found in ∼40% of patients (47).

Since IFX does not work in all patients and has possibilities of major adverse effects, as well as high expense, it is important to develop predictive biomarkers for the loss of response in patients. Progress has been made in this research field and a panel of biomarkers have been identified, such as patient-related (age, weight, smoke, et al.) and disease-related (disease duration, CRP, albumin, et al.) (46). Regrettably, most markers have not shown practical application and many others remain controversial (46). Here, we found that NAR, which integrated neutrophils and albumin, showed significant abilities to discriminate IFX

responders from primary non-responders, providing a new approach for clinical practice.

A number of evidences have suggested that the loss of response to IFX correlates with low levels of serum IFX trough levels (41). In the current study, we observed that patients with higher pre-treatment NAR had lower serum IFX trough concentration. It has been reported that patients in high inflammatory status may have a higher rate of IFX clearance, leading to both insufficient clinical response and development of antibodies toward IFX (ATI) (42, 48, 49). We here demonstrated a role of NAR as an indicator for both systemic and mucosal inflammatory load and patients with more severe UC displayed higher NAR, which might promote IFX clearance and explain their loss of response to this agent.

In summary, we found a novel neutrophil- and albumin-based index NAR was significantly elevated in patients with UC, and showed satisfactory performance in the assessment of disease activity and inflammatory load. In note, NAR displayed predictive values of primary response to IFX induction therapy in patients with UC. Despite these findings, several issues need to be addressed in the following studies: studies with large sample sizes are needed to verify our current observations; whether NAR could be used in long-term follow-up to predict secondary non-response to IFX; the practical significance of NAR for other biologics (such as Vedolizumab and Ustekinumab) need to be investigated. Nevertheless, the current study provides evidences to utilize NAR in the diagnosis, activity monitoring, and IFX response prediction in patients with 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 the Institutional Review Board for Clinical Research of Sichuan Provincial People's Hospital (No.201685, 2020204). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CH and CG conceptualized, designed the study plan, and edited the manuscript. ZZ, YZ, and XY collected clinical information and samples from enrolled subjects. LL, YP, and CG diagnosed the patients. CH, CG, and YZ analyzed the data and prepare the original draft. ZZ and CH revised the manuscript. All authors discussed, revised the manuscript, and agreed to the published version of the manuscript.

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REFERENCES

1. Collaborators GBDIBD. The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol.* (2020) 5:17–30. doi: 10.1016/S2468-1253(19)30333-4
2. Kaplan GG. The global burden of IBD: from 2015 to 2025. *Nat Rev Gastroenterol Hepatol.* (2015) 12:720–7. doi: 10.1038/nrgastro.2015.150
3. Beard JA, Franco DL, Click BH. The burden of cost in inflammatory bowel disease: a medical economic perspective and the future of value-based care. *Curr Gastroenterol Rep.* (2020) 22:6. doi: 10.1007/s11894-020-0744-z
4. Park KT, Ehrlich OG, Allen JI, Meadows P, Szigethy EM, Henrichsen K, et al. The cost of inflammatory bowel disease: an initiative from the Crohn's & Colitis Foundation. *Inflamm Bowel Dis.* (2020) 26:1–10. doi: 10.1093/ibd/izz104
5. Rajbhandari R, Blakemore S, Gupta N, Adler AJ, Noble CA, Mannan S, et al. Crohn's disease in low and lower-middle income countries: a scoping review. *World J Gastroenterol.* (2020) 26:6891–908. doi: 10.3748/wjg.v26.i43.6891
6. Soubieres AA, Poullis A. Emerging biomarkers for the diagnosis and monitoring of inflammatory bowel diseases. *Inflamm Bowel Dis.* (2016) 22:2016–22. doi: 10.1097/MIB.0000000000000836
7. Navaneethan U, Parasa S, Venkatesh PG, Trikudanathan G, Shen B. Prevalence and risk factors for colonic perforation during colonoscopy in hospitalized inflammatory bowel disease patients. *J Crohns Colitis.* (2011) 5:189–95. doi: 10.1016/j.crohns.2010.12.005
8. Buisson A, Gonzalez F, Poullenot F, Nancey S, Sollellis E, Fumery M, et al. Comparative acceptability and perceived clinical utility of monitoring tools: a nationwide survey of patients with inflammatory bowel disease. *Inflamm Bowel Dis.* (2017) 23:1425–33. doi: 10.1097/MIB.0000000000001140
9. Lewis JD. The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease. *Gastroenterology.* (2011) 140:1817–26.e2. doi: 10.1053/j.gastro.2010.11.058
10. Sands BE. Biomarkers of Inflammation in Inflammatory Bowel Disease. *Gastroenterology.* (2015) 149:1275–85.e2. doi: 10.1053/j.gastro.2015.07.003
11. Su Q, Li X, Mo W, Yang Z. Low serum bilirubin, albumin, and uric acid levels in patients with Crohn's disease. *Medicine.* (2019) 98:e15664. doi: 10.1097/MD.0000000000015664
12. Chen XF, Zhao Y, Guo Y, Huang ZM, Huang XL. Predictive value of fibrinogen in identifying inflammatory bowel disease in active stage. *BMC Gastroenterol.* (2021) 21:472. doi: 10.1186/s12876-021-02040-9
13. Gao H, He Q, Xu C, Pang Z, Feng B, Chen T, et al. The development and validation of anti-paratuberculosis-nocardia polypeptide antibody [anti-pTNP] for the diagnosis of crohn's disease. *J Crohns Colitis.* (2022) 13:jjac008. doi: 10.1093/ecco-jcc/jjac008
14. Zholudev A, Zurakowski D, Young W, Leichtner A, Bousvaros A. Serologic testing with ANCA, ASCA, and anti-OmpC in children and young adults with Crohn's disease and ulcerative colitis: diagnostic value and correlation with disease phenotype. *Am J Gastroenterol.* (2004) 99:2235–41. doi: 10.1111/j.1572-0241.2004.40369.x
15. Mosli MH, Zou G, Garg SK, Feagan SG, MacDonald JK, Chande N, et al. C-Reactive protein, fecal calprotectin, and stool lactoferrin for detection of endoscopic activity in symptomatic inflammatory bowel disease patients: a systematic review and meta-analysis. *Am J Gastroenterol.* (2015) 110:802–19; quiz 20. doi: 10.1038/ajg.2015.120
16. Zhou GX, Liu ZJ. Potential roles of neutrophils in regulating intestinal mucosal inflammation of inflammatory bowel disease. *J Dig Dis.* (2017) 18:495–503. doi: 10.1111/1751-2980.12540

17. Magro F, Langner C, Driessen A, Ensari A, Geboes K, Mantzaris GJ, et al. European consensus on the histopathology of inflammatory bowel disease. *J Crohns Colitis.* (2013) 7:827–51. doi: 10.1016/j.crohns.2013.06.001
18. Fu W, Fu H, Ye W, Han Y, Liu X, Zhu S, et al. Peripheral blood neutrophil-to-lymphocyte ratio in inflammatory bowel disease and disease activity: a meta-analysis. *Int Immunopharmacol.* (2021) 101:108235. doi: 10.1016/j.intimp.2021.108235
19. Liu Z, Perry LA, Penny-Dimri JC, Raveendran D, Hu ML, Arslan J, et al. The association of neutrophil-lymphocyte ratio and platelet-lymphocyte ratio with retinal vein occlusion: a systematic review and meta-analysis. *Acta Ophthalmol.* (2021) 100:e635–e647. doi: 10.1111/aos.14955
20. Han SI, Cha KC, Roh YI, Hwang SO, Jung WJ, Kim TY. Association between novel marker (platelet-lymphocyte ratio, neutrophil-lymphocyte ratio, and delta neutrophil index) and outcomes in sudden cardiac arrest patients. *Emerg Med Int.* (2021) 2021:6650958. doi: 10.1155/2021/6650958
21. Ntalouka MP, Nana P, Kouvelos GN, Stamoulis K, Spanos K, Giannoukas A, et al. Association of neutrophil-lymphocyte and platelet-lymphocyte ratio with adverse events in endovascular repair for abdominal aortic aneurysm. *J Clin Med.* (2021) 10:1083. doi: 10.3390/jcm10051083
22. Gong P, Liu Y, Gong Y, Chen G, Zhang X, Wang S, et al. The association of neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, and lymphocyte to monocyte ratio with post-thrombolysis early neurological outcomes in patients with acute ischemic stroke. *J Neuroinflammation.* (2021) 18:51. doi: 10.1186/s12974-021-02090-6
23. Cupp MA, Cariolou M, Tzoulaki I, Aune D, Evangelou E, Berlanga-Taylor AJ. Neutrophil to lymphocyte ratio and cancer prognosis: an umbrella review of systematic reviews and meta-analyses of observational studies. *BMC Med.* (2020) 18:360. doi: 10.1186/s12916-020-01817-1
24. Torun S, Tunc BD, Suvak B, Yildiz H, Tas A, Sayilir A, et al. Assessment of neutrophil-lymphocyte ratio in ulcerative colitis: a promising marker in predicting disease severity. *Clin Res Hepatol Gastroenterol.* (2012) 36:491–7. doi: 10.1016/j.clinre.2012.06.004
25. Peng Y, Xue Y, Wang J, Xiang H, Ji K, Wang J, et al. Association between neutrophil-to-albumin ratio and mortality in patients with cardiogenic shock: a retrospective cohort study. *BMJ Open.* (2020) 10:e039860. doi: 10.1136/bmjopen-2020-039860
26. Li R, Sun Z, Song S, He X, Shi X, Li Z, et al. NARFIB: a novel prognostic score based on the neutrophil-to-albumin ratio and fibrinogen can predict the prognosis of gastrointestinal stromal tumors. *Cancer Manag Res.* (2020) 12:11183–90. doi: 10.2147/CMAR.S281375
27. Tawfik B, Mokdad AA, Patel PM, Li HC, Huerta S. The neutrophil to albumin ratio as a predictor of pathological complete response in rectal cancer patients following neoadjuvant chemoradiation. *Anticancer Drugs.* (2016) 27:879–83. doi: 10.1097/CAD.0000000000000411
28. He C, Shi Y, Wu R, Sun M, Fang L, Wu W, et al. miR-301a promotes intestinal mucosal inflammation through induction of IL-17A and TNF-alpha in IBD. *Gut.* (2016) 65:1938–50. doi: 10.1136/gutjnl-2015-309389
29. He C, Yu T, Shi Y, Ma C, Yang W, Fang L, et al. MicroRNA 301A promotes intestinal inflammation and colitis-associated cancer development by inhibiting BTG1. *Gastroenterology.* (2017) 152:1434–48.e15. doi: 10.1053/j.gastro.2017.01.049
30. Neurath MF, Travis SP. Mucosal healing in inflammatory bowel diseases: a systematic review. *Gut.* (2012) 61:1619–35. doi: 10.1136/gutjnl-2012-302830
31. Maaser C, Sturm A, Vavricka SR, Kucharzik T, Fiorino G, Annese V, et al. ECCO-ESGAR guideline for diagnostic assessment in IBD Part 1: initial diagnosis, monitoring of known IBD, detection of complications. *J Crohns Colitis.* (2019) 13:144–64. doi: 10.1093/ecco-jcc/jjy113
32. Rutter M, Saunders B, Wilkinson K, Rumbles S, Schofield G, Kamm M, et al. Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology.* (2004) 126:451–9. doi: 10.1053/j.gastro.2003.11.010
33. Rutgeerts P, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, et al. Comparison of scheduled and episodic treatment strategies of infliximab in Crohn's disease. *Gastroenterology.* (2004) 126:402–13. doi: 10.1053/j.gastro.2003.11.014
34. Travis SP, Schnell D, Krzeski P, Abreu MT, Altman DG, Colombel JF, et al. Reliability and initial validation of the ulcerative colitis endoscopic index of severity. *Gastroenterology.* (2013) 145:987–95. doi: 10.1053/j.gastro.2013.07.024
35. Samaan MA, Mosli MH, Sandborn WJ, Feagan BG, D'Haens GR, Dubcenco E, et al. A systematic review of the measurement of endoscopic healing in ulcerative colitis clinical trials: recommendations and implications for future research. *Inflamm Bowel Dis.* (2014) 20:1465–71. doi: 10.1097/MIB.0000000000000046
36. D'Haens G, Sandborn WJ, Feagan BG, Geboes K, Hanauer SB, Irvine EJ, et al. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology.* (2007) 132:763–86. doi: 10.1053/j.gastro.2006.12.038
37. D'Haens G, Ferrante M, Vermeire S, Baert F, Noman M, Moortgat L, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. *Inflamm Bowel Dis.* (2012) 18:2218–24. doi: 10.1002/ibd.22917
38. Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* (2005) 353:2462–76. doi: 10.1056/NEJMoa050516
39. Cornillie F, Hanauer SB, Diamond RH, Wang J, Tang KL, Xu Z, et al. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. *Gut.* (2014) 63:1721–7. doi: 10.1136/gutjnl-2012-304094
40. Steenholdt C, Bendtzen K, Brynskov J, Thomsen OO, Munck LK, Christensen LA, et al. Changes in serum trough levels of infliximab during treatment intensification but not in anti-infliximab antibody detection are associated with clinical outcomes after therapeutic failure in Crohn's disease. *J Crohns Colitis.* (2015) 9:238–45. doi: 10.1093/ecco-jcc/jjv004
41. Hemperly A, Vande Casteele N. Clinical pharmacokinetics and pharmacodynamics of infliximab in the treatment of inflammatory bowel disease. *Clin Pharmacokinet.* (2018) 57:929–42. doi: 10.1007/s40262-017-0627-0
42. Bendtzen K, Geborek P, Svenson M, Larsson L, Kapetanovic MC, Saxne T. Individualized monitoring of drug bioavailability and immunogenicity in rheumatoid arthritis patients treated with the tumor necrosis factor alpha inhibitor infliximab. *Arthritis Rheum.* (2006) 54:3782–9. doi: 10.1002/art.22214
43. Caire MT, Strauss AT, Kumar A, Brady P. Emerging biomarkers for the diagnosis and monitoring of inflammatory bowel diseases. *Inflamm Bowel Dis.* (2016) 22:E46. doi: 10.1097/MIB.0000000000000973
44. Christensen B, Hanauer SB, Erlich J, Kassim O, Gibson PR, Turner JR, et al. Histologic normalization occurs in ulcerative colitis and is associated with improved clinical outcomes. *Clin Gastroenterol Hepatol.* (2017) 15:1557–64.e1. doi: 10.1016/j.cgh.2017.02.016
45. Das S, Maras JS, Hussain MS, Sharma S, David P, Sukriti S, et al. Hyperoxidized albumin modulates neutrophils to induce oxidative stress and inflammation in severe alcoholic hepatitis. *Hepatology.* (2017) 65:631–46. doi: 10.1002/hep.28897
46. Gisbert JP, Chaparro M. Predictors of primary response to biologic treatment [Anti-TNF, Vedolizumab, and Ustekinumab] in patients with inflammatory bowel disease: from basic science to clinical practice. *J Crohns Colitis.* (2020) 14:694–709. doi: 10.1093/ecco-jcc/jjz195
47. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. *Aliment Pharmacol Ther.* (2011) 33:987–95. doi: 10.1111/j.1365-2036.2011.04612.x
48. Wolbink GJ, Voskuyl AE, Lems WF, de Groot E, Nurmohamed MT, Tak PP, et al. Relationship between serum trough infliximab levels, pretreatment C reactive protein levels, and clinical response to infliximab treatment in patients with rheumatoid arthritis. *Ann Rheum Dis.* (2005) 64:704–7. doi: 10.1136/ard.2004.030452
49. Ducourau E, Mulleman D, Paintaud G, Miow Lin DC, Lauferon F, Ternant D, et al. Antibodies toward infliximab are associated with low infliximab concentration at treatment initiation and poor infliximab maintenance

in rheumatic diseases. *Arthritis Res Ther.* (2011) 13:R105. doi: 10.1186/ar3386

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Predicting Histological Healing and Recurrence in Ulcerative Colitis by Assessing Mucosal Vascular Pattern Under Narrow-Band Imaging Endoscopy

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This study investigated the predictive value of narrow-band imaging (NBI) endoscopic staging of different mucosal vascular patterns (MVPs) in patients with ulcerative colitis (UC) for histological healing or clinical recurrence of patients with UC. A total of 124 patients with UC in clinical remission attending the First Affiliated Hospital of Weifang Medical College were included in the study and underwent NBI colonoscopy. Inflammatory activity was assessed in the intestine using the Mayo endoscopic score (MES) and the MVP. Mucosal inflammation was histologically graded using the Nancy index (NI). The colons of 124 patients with UC were staged according to NBI endoscopic MVP staging criteria. The differences between NBI colonoscopy MVP typing and white light endoscopic MES in assessing histological healing (HH) were statistically significant ($p < 0.001$), and there was a moderate correlation between MES and the degree of HH ($r = 0.471$, $p < 0.001$). In addition, there was a significant correlation between the severity of mucosal activity determined by white light endoscopy (WLE) and MVP staging ($r = 0.811$, $p < 0.001$). The differences between NBI endoscopic MVP staging and white light endoscopic MES in assessing UC recurrence were statistically significant ($p < 0.001$). Spearman's correlation analysis showed a moderate correlation between NBI endoscopic MVP staging and clinical recurrence. NBI endoscopic MVP staging can predict HH and clinical recurrence status better than WLE.

Keywords: high-definition endoscopy, ulcerative colitis, recurrence, narrow-band optical imaging, histological healing

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INTRODUCTION

Inflammatory bowel disease, such as Crohn's disease and ulcerative colitis (UC), is a chronic inflammatory disorder of the gastrointestinal tract. Multiple variables are thought to be implicated in the pathogenesis *via* the activation of mucosal immune responses in gut-associated lymphoid tissues, such as intestinal microbiota, dietary antigens, and other environmental elements (1). Long-term treatment goals have changed from symptom control to endoscopic mucosal healing with clinical remission in recent years (2). However, several reports have shown that mucosa that had seemingly healed still had a slight histologically defined inflammation and the possibility of relapse (3). Therefore, histological remission becomes the next therapy objective after

endoscopic mucosal healing and has given rise to the new concept of "disease clearance," which includes clinical, endoscopic, and microscopic remission (4).

Endoscopy is essential for evaluating disease activity and the efficacy of treatment interventions in UC. There are several different scoring systems for the endoscopic evaluation of UC severity. The Mayo scoring system is commonly used, and Mayo scores of both 0 and 1 are defined as mucosal healing (5). Narrow-band imaging (NBI) is an image-enhanced endoscope-based approach to enhance the fine structure of the mucosa without the use of dyes. In addition, NBI can visualize subtle changes in the mucosa and the distribution of abnormal blood vessels (6). Accordingly, NBI colonoscopy might be a valuable tool to assess mucosal angiogenesis according to the mucosal vascular pattern (MVP) in UC (7). However, there seem to be few investigations into the relationship between MVP based on NBI colonoscopy and histological assessment in UC.

This study aims to establish the performance of and relationship between NBI and white light endoscopy (WLE) assessments in patients with UC and their association with histological healing (HH). Furthermore, we wanted to evaluate whether endoscopic pictures of MVP obtained by NBI are a predictor of clinical outcomes.

MATERIALS AND METHODS

Patients

This study was conducted at the First Affiliated Hospital of Weifang Medical College between January 2018 and December 2020 in patients with an established diagnosis of UC. The extent of UC was determined by colonoscopy, and the UC disease activity was assessed according to the Mayo score. Patients were examined by NBI colonoscopy with biopsy and had signed informed consent for colonoscopy.

Instrument

Each patient underwent a colonoscopy with an endoscope (CF-H290AI; Olympus, Tokyo, Japan), using a prototype of the NBI system (Elvis CV-290; Olympus, Tokyo, Japan).

Study Procedure

Total colonoscopy in 124 patients with UC was performed with WLE and NBI to observe the mucosa of each segment of the colorectum. The colorectum was divided into six segments for assessment, defined as the cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum. From the same lesions observed by WLE and NBI, targeted biopsies of the most inflamed area assessed by colonoscopy were obtained and analyzed.

We first defined the NBI findings into three categories, as described below. The WLE and NBI findings were compared, with the white light images being divided into two groups: mucosal healing or not. We first assessed this to determine if the standard white light classification can predict NBI findings, which seemed only to be able to indicate mucosal healing. Then, the pathological results from all of the biopsy specimens were compared with the NBI findings to assess the relationship between imaging and pathological activity.

The relationships between the NBI findings at initial ileocolonoscopy and 1-year follow-up clinical outcomes were assessed to reveal the different prognoses for the NBI findings.

Patients were followed until the end of the study in 2020 or recurrence. Recurrence was defined as a Mayo endoscopic score (MES) $\geq$ 2, therapy to induce remission, hospitalization, or colectomy (8).

Endoscopic Assessment

All WLE and NBI images from all target areas were randomly assigned to two experienced endoscopists who were unfamiliar with the clinical data. If there was a disagreement, an agreement was reached through negotiation. The endoscopic activity under WLE was classified into four degrees of severity, from 0 to 3 (0: inactive, 1: mild, 2: moderate, and 3: severe) according to MES (9).

When NBI colonoscopy demonstrated clear intramucosal capillaries, the MVP was defined as clear. When NBI failed to reveal a clear vascular network or blurry image, it was regarded as obscure. In areas where the intramucosal vessels were invisible under NBI, the MVP was regarded as absent. Based on this protocol, the MVPs were classified into three types: clear, obscure, or absent (10) (**Figure 1**).

Histological Assessment

All histologic specimens from colonoscopy were reviewed by a single senior gastrointestinal pathologist who was blinded to the outcome of interest. The site with the most histologically active disease was scored according to the Nancy index (NI) (11). The individual components of the NI were recorded for each patient: no significant histological disease (Grade 0), chronic inflammatory infiltrate with no acute inflammatory infiltrate (Grade 1), mildly active disease (Grade 2), moderately active disease (Grade 3), or severely active disease (Grade 4). We defined histological inactivity for grades 0 and 1 and histological activity for grades 2–4 (11) (**Figure 2**). The worst score obtained was considered if different NI classifications were obtained from the different segments of the colon.

Outcomes

Clinical outcomes at follow-up were specified as (1) hospitalization as a result of UC relapse, (2) colectomy, or (3) initiation or changes in medical therapy, such as steroids, immunomodulators, and biologics, due to UC relapse. The recorded notes of all patients were reviewed to record these clinical outcome events, with telephone calls made to clarify any uncertainty, 12 months after the colonoscopy procedure.

Statistical Analysis

All statistical analyses were performed using the IBM SPSS Statistics 26 software package (IBM, New York, NY, USA). Continuous data are presented using the mean and SD, whereas proportions and percentages are used to describe discrete variables. Wilcoxon Mann–Whitney *U*-tests and Kruskal–Wallis tests were used for non-parametric values, and one-way analysis

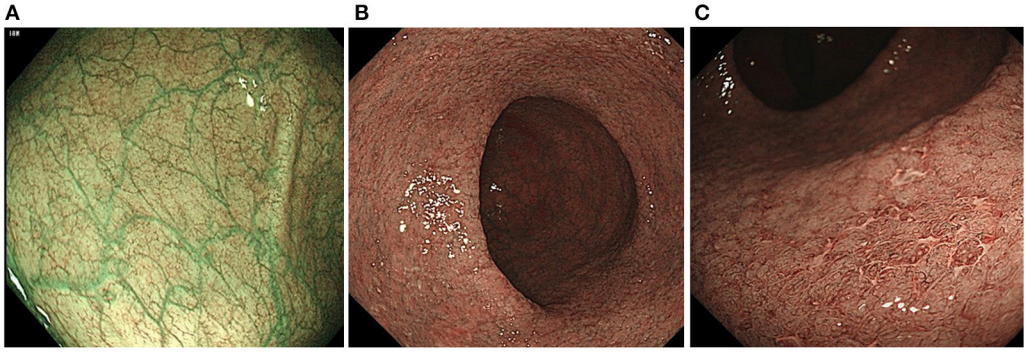

FIGURE 1 | Classification of the narrow-band imaging (NBI) findings: **(A)** clear; **(B)** obscure; and **(C)** absent.

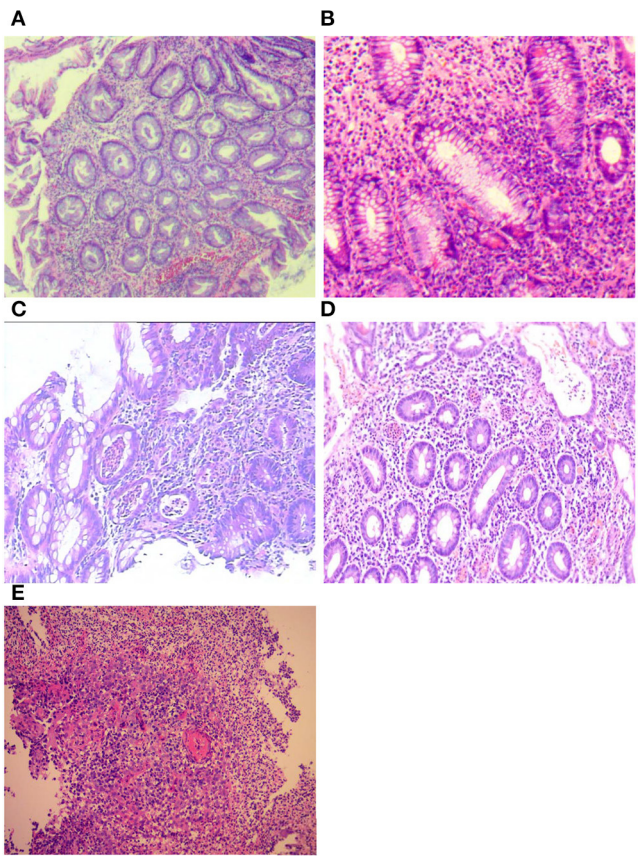

FIGURE 2 | Histological assessment of Nancy index (NI): **(A)** no significant histological disease (Grade 0); **(B)** chronic inflammatory infiltrate with no acute inflammatory infiltrate (Grade 1); **(C)** mildly active disease (Grade 2); **(D)** moderately active disease (Grade 3); and **(E)** severely active disease (Grade 4).

of variance (ANOVA) was used for the parametric values. For correlations, Spearman's rank correlation coefficient was used. The predictive power of the diagnostic model was evaluated using receiver operating characteristics (ROCs) analysis, and the cut-off value was obtained when the Youden index reached the maximum. Sensitivity, specificity, and area under the ROC curve (AUC) were used to assess the prediction accuracy. A two-tailed p-value < 0.05 was defined as statistically significant. A biomedical statistician reviewed the statistical methods of this study.

RESULTS

Clinical Characteristics of Patients

We obtained clinical data, endoscopic scores, and histological scores from 124 patients with UC (52 men [M]; 41.9%) who underwent colonoscopy and were recruited for this study. Clinical characteristics are shown in **Table 1**. Based on the colonoscopy and Mayo score, the extent and activity of the disease were determined.

NBI Colonoscopy Findings in UC

A total of 124 colorectal segments from 124 patients with UC were analyzed. Under NBI colonoscopy, 37 segments were determined as having a clear MVP, 57 segments were judged as having an obscure MVP, and 30 segments had an absent MVP (**Table 2**).

Correlation Between MVP and MES

The relationship between MVP and MES is depicted in **Table 3**. In the clear pattern group, 89.2% (33/37) of the segments were identified as having an MES of ≤ 1 and 10.8% (4/37) had an MES of 3. In the obscure pattern group, 3.5% (2/57) of the segments were identified as having an MES of ≤ 1, 94.7% (54/57) had an MES of 2, and 1.8% (1/57) had an MES of 3. In the absence of a pattern, 20% (6/30) of the segments were identified as having an MES of 2, and 80% (24/30) had an MES of 3. It was shown that the MVP correlated well with MES ($r = 0.811$, $p < 0.001$) (**Table 3**).

NBI Findings and Histology

The histological findings from the examined lesions are shown in **Table 4**. We defined HH as a NI ≤ 1 and histological activity as an NI > 1. Lesions classified as NBI Clear (29/37 showing HH: 78.4%) rarely showed activity on histology. On the contrary, those classified as NBI Obscure (14/57 showing HH: 24.7%) or NBI Absent (0/30 showing HH: 0%) indicated histological

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

Clinical characteristics	*N* (%)
Patients with UC	124
Sex	
Male	52 (41.9%)
Female	72 (58.1%)
Age (years), median (range)	48 (25–83)
Disease duration (years), median (range)	5 (1–30)
Maximum extent of UC	
Extensive colitis	26 (21.0%)
Left-sided colitis	54 (43.5%)
Proctitis	44 (35.5%)
Mayo endoscopic subscore	
MES ≤ 1	35 (28.2%)
MES = 2	60 (48.4%)
MES = 3	37 (23.4%)

UC, ulcerative colitis.

TABLE 2 | Colonoscopy findings in patients with UC.

Colonoscopy findings	*N* (%)
Colorectal segments	124
The MVP was assessed using NBI colonoscopy	
Clear	37 (29.8%)
Obscure	57 (46.0%)
Absent	30 (24.2%)

MVP, mucosal vascular pattern; NBI, narrow-band imaging.

TABLE 3 | Correlation between MVP and Mayo endoscopic score (MES).

MES/NBI	Clear ($n = 37$)	Obscure ($n = 57$)	Absent ($n = 30$)	*P*-value
≤ 1	33	2	0	0.000
2	0	54	6	
3	4	1	24	

TABLE 4 | Correlation between the degree of inflammation identified by Nancy index (NI) and endoscopy score.

Score	NI ≤ 1 ($n = 43$)	NI > 1 ($n = 81$)	*P*-value
MES			
0/1	26	9	0.000
2	13	47	
3	4	25	
NBI			
Clear	29	8	0.000
Obscure	14	43	
Absent	0	30	

NI, Nancy index; MES; Mayo endoscopic score.

TABLE 5 | Clinical relapse by endoscopic score.

Score	Relapse ($n = 75$)	No relapse ($n = 49$)	*P*-value
MES			
0/1	11	24	
2	40	20	0.000
3	24	5	
NBI			
Clear	10	27	
Obscure	37	20	0.000
Absent	28	2	

TABLE 6 | Area under the receiver operating characteristic (ROC) curve (AUC), sensitivity, specificity, and Youden index for defined endoscopic and histological healing (HH) in patients with UC.

	AUC	Youden index	Sensitivity	Specificity	95% Confidence interval
NBI	0.848	0.576	0.901	0.674	0.779–0.917
WLE	0.766	0.494	0.889	0.605	0.674–0.859

activity. There was a significant correlation between NBI findings and histological findings ($r = 0.614$, $p < 0.001$).

In the HH group, 60.5% (26/43) of the segments were identified as having an MES of ≤ 1, 30.2% (13/43) had an MES of 2, and 9.3% (4/43) had an MES of 3. In the histological activity group, 11.1% (9/81) of the segments were identified as having an MES of ≤ 1, 58.0% (47/81) had an MES of 2, and 30.9% (25/81) had an MES of 3. There was a significant correlation between WLE classification and histological findings ($r = 0.471$, $p < 0.001$) (**Table 4**).

Clinical Relapse in NBI and WLE

During the 12-month follow-up period, 75 patients (60.5%) experienced a clinical relapse. The association between endoscopic activity scores and clinical relapse during the follow-up period is shown in **Table 5**.

The time to relapse was significantly different between MES in our cohort. In patients with 1-year relapse, 11 patients (14.7%) had an MES of 0/1, 40 patients (53.3%) had an MES of 2, and 24 patients (32.0%) had an MES of 3. However, there was a low correlation between WLE and histological findings ($r = 0.383$, $p < 0.001$). Considering MVP, 10 (13.3%) patients with a clear pattern, 37 patients (49.3%) with an obscure pattern, and 28 patients (37.4%) with an absence of pattern suffered a relapse. There was a significant correlation between NBI and histological findings ($r = 0.5$, $p < 0.001$).

NBI and WLE and HH in UC

Narrow-band imaging has an AUC of 0.848, a sensitivity of 0.901, a specificity of 0.901, and a 95% confidence interval (*CI*) (0.779–0.971) to predict HH. WLE has an AUC of 0.766, a sensitivity of 0.889, a specificity of 0.605, and a 95% *CI* (0.674–0.859) to predict HH (**Table 6**, **Figure 3**). All AUC values were higher than 0.7,

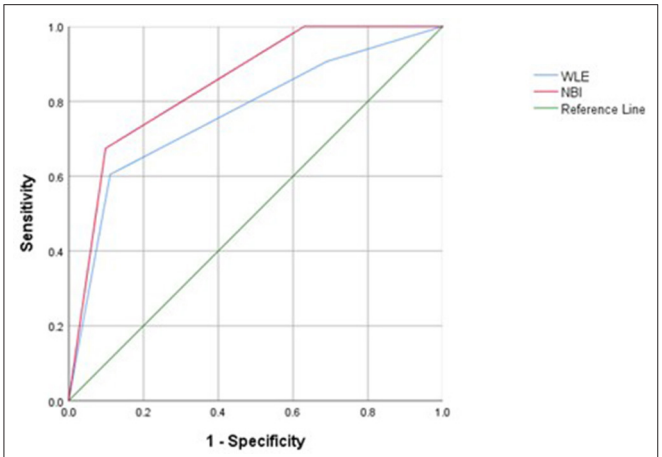

FIGURE 3 | Receiver operating characteristic (ROC) curves of histological healing (HH) assessed with white light endoscopy (WLE) and NBI in patients with UC.

TABLE 7 | Area under an ROC curve, sensitivity, specificity, and Youden index for defined endoscopic and clinical recurrence in patients with UC.

	AUC	Youden index	Sensitivity	Specificity	95% Confidence interval
NBI	0.775	0.418	0.867	0.551	0.692–0.858
WLE	0.710	0.343	0.853	0.490	0.616–0.803

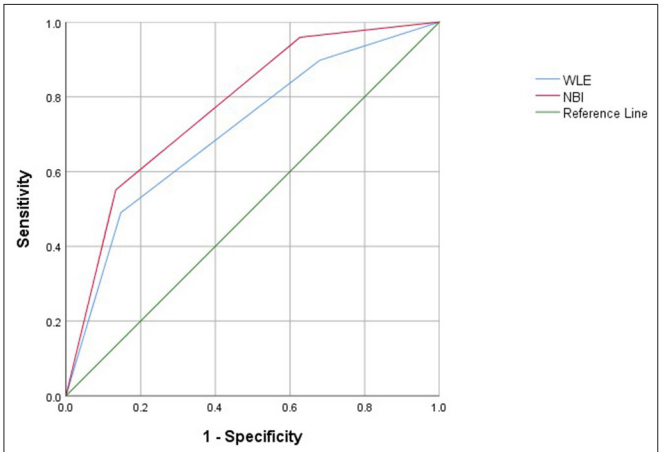

FIGURE 4 | Receiver operating characteristic curves of clinical recurrence assessed with WLE and NBI in patients with UC.

suggesting that NBI has a good predictive ability to distinguish the HH group from the other groups.

NBI and WLE and Clinical Recurrence in UC

Narrow-band imaging has an AUC of 0.775, a sensitivity of 0.867, a specificity of 0.551, and a 95% *CI* (0.692–0.858) to predict clinical recurrence. WLE has an AUC of 0.710, a sensitivity of 0.853, a specificity of 0.490, and a 95% *CI* (0.616–0.803) to predict HH (**Table 7**, **Figure 4**). All AUC values were higher than 0.7, suggesting that NBI has a good predictive ability to distinguish the clinical recurrence group from the other groups.

DISCUSSION

With the deepening of research and the emergence of various treatment measures, the treatment goal of UC has evolved from clinical remission to endoscopic remission, an absence of inflammatory activity (12). Histological disease activity is considered a predictor of early clinical relapse in patients with UC (13). Therefore, endoscopy plays an important role in determining the severity of inflammation in bowel wall tissue and is critical for monitoring and treating the disease. MES based on WLE assessment is widely used as an objective method to describe the extent of endoscopic activity in UC and has been shown to have a significant overall correlation with histologic severity (14). However, a recent study shows that the endoscopic NBI technique can effectively assess inflammatory activity in UC (15). In comparison with WLE colonoscopy, NBI colonoscopy may be more effective in evaluating locations of inflammation in UC due to greater visualization of the mucosal surface and vascular patterns. At present, it is still uncertain whether MVP based on NBI or MES based on WLE may predict mucosal inflammation and disease recurrence. In our study, we compared the capabilities of NBI colonoscopy with those of WLE for assessing histological activity and prognosis in UC, and the results indicate that NBI provides a better assessment of clinical prognosis and prediction of histology.

Narrow-band imaging is useful in the diagnosis of dysplastic lesions in patients with UC. However, its value for predicting clinical outcomes and HH remains unknown. Sasanuma et al. (16) reported that NBI findings of the colonic mucosa correlated well with histologic activity and discussed their potential to predict endoscopic relapse. In this study, we classified the MVPs of colorectal segments from patients with UC into three pattern types (clear, obscure, or absent) using NBI colonoscopy. The evidence from this study suggests that a clear pattern determined with NBI is a feature of non-recurrence. In patients with a clear pattern, 73.0% (27/37) had no recurrence. Other patterns have a high recurrence rate. In addition, Spearman's correlation analysis suggests a greater degree of correlation between NBI and HH compared with WLE. Because of the strong concordance between NBI and histopathological findings, the number of biopsies can be reduced to some extent, improving the accuracy of biopsies and reducing complications, such as bleeding and perforation.

Our data using NBI of the colonic mucosa suggest that UC relapse may be predicted by vascular changes and mucosal findings. We found that NBI is different from WLE in several respects. Our study showed that NBI is a better predictor of histological activity than WLE. NBI, with an AUC of 0.848 using ROC analysis, has a good capability to predict HH. The sensitivity and specificity of the diagnosing model were 90.1 and 67.4%, respectively, and our study showed that NBI is a better predictor of clinical recurrence than WLE. NBI, with an AUC of 0.775 using ROC analysis, has a good capability to predict clinical recurrence.

Therefore, we conclude that NBI correlates with HH and could be a better predictor of clinical recurrence than WLE. The vascular changes and mucosal findings may be due to active inflammation; NBI can better assess vascular change and mucosal findings.

This study has some limitations. First, the sample size is small and from a single center. Second, the follow-up period in patients with UC was relatively short. Further, the correlation between NBI and other endoscopy scores, such as Ulcerative Colitis Endoscopic Index of Severity (UCEIS), was not analyzed. Studies need to be carried out to validate NBI-predicted clinical recurrence in patients with an MES of 1 or 0. To further corroborate our results, prospective multicenter studies involving larger samples, lesions with dysplasia, and comparison of patients with UC are required.

In conclusion, the MVP based on NBI colonoscopy correlates well with the MES based on WLE in patients with UC. Moreover, MVP determined with NBI is a more useful method for predicting HH and clinical recurrence than MES determined with WLE in patients with UC. In the future, NBI will be performed even in patients with normal mucosa on endoscopy, especially in cases of vascular obscuration.

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 Weifang People Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

TH: data curation and writing-original draft preparation. LZ: conceptualization and methodology. PP: data sorting. SS: paper revision. HQ: funding acquisition. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Gajendran M, Loganathan P, Jimenez G, Catinella AP, Ng N, Umapathy C, et al. A comprehensive review and update on ulcerative colitis. *Disease-a-month: DM.* (2019) 65:100851. doi: 10.1016/j.disamonth.2019.02.004
2. Turner D, Ricciuto A, Lewis A, D'Amico F, Dhaliwal J, Griffiths AM, et al. International Organization for the Study of IBD. STRIDE-II: an update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) initiative of the International Organization for the Study of IBD (IOIBD): Determining therapeutic goals for treat-to-target strategies in IBD. *Gastroenterology.* (2021) 160:1570–1583. doi: 10.1053/j.gastro.2020.12.031
3. Snir Y, Iacucci, M. The role of narrowedv spectrum technologies and dye-based endoscopy in inflammatory bowel disease: new advances and opportunities. *TIGE.* (2021) 23:42–56. doi: 10.1016/j.tige.2020.10.005
4. Dal Buono A, Roda G, Argollo M, Zacharopoulou E, Peyrin-Biroulet L, Danese S. Treat to target or 'treat to clear' in inflammatory bowel diseases: One step further? *Expert Rev Gastroenterol Hepatol.* (2020) 14:807–17. doi: 10.1080/17474124.2020.1804361
5. Ikeya K, Hanai H, Sugimoto K, Osawa S, Kawasaki S, Iida T, et al. The ulcerative colitis endoscopic index of severity more accurately reflects clinical outcomes and long-term prognosis than the Mayo endoscopic score. *J Crohns Colitis.* (2016) 10:286–95. doi: 10.1093/ecco-jcc/jjv210
6. Sivanathan V, Tontini GE, Möhler M, Galle PR, Neumann H. Advanced endoscopic imaging for diagnosis of inflammatory bowel diseases: present and future perspectives. *Digestive Endoscopy.* (2018) 30:441–48. doi: 10.1111/den.13023
7. Guo T, Qian JM, Yang AM, Li Y, Zhou WX. Narrow-band imaging colonoscopy to assess mucosal angiogenesis in ulcerative colitis. *Gastroenterol Res Pract.* (2019) 2019:8312624. doi: 10.1155/2019/8312624
8. Lewis JD, Chuai S, Nessel L, Lichtenstein GR, Aberra FN, Ellenberg JH. Use of the noninvasive components of the Mayo score to assess clinical response in ulcerative colitis. *Inflamm Bowel Dis.* (2008) 14:1660–6. doi: 10.1002/ibd.20520
9. Mohammed Vashist N, Samaan M, Mosli MH, Parker CE, MacDonald JK, Nelson SA, et al. Endoscopic scoring indices for evaluation of disease activity in ulcerative colitis. *Cochrane Database Syst Rev.* (2018) 1:CD011450. doi: 10.1002/14651858.CD011450.pub2
10. Esaki M, Kubokura N, Kudo T, Matsumoto T. Endoscopic findings under narrow band imaging colonoscopy in ulcerative colitis. *Dig Endosc.* (2011) 23:140–2. doi: 10.1111/j.1443-1661.2011.01110.x
11. Magro F, Doherty G, Peyrin-Biroulet L, Svrcek M, Borralho P, Walsh AV, et al. ECCO Position paper: harmonization of the approach to ulcerative colitis histopathology. *J Crohns Colitis.* (2020) 14:1503–11. doi: 10.1093/ecco-jcc/jjaa110
12. Ungaro R, Colombel JF, Lissoos T, Peyrin-Biroulet L. A treat-to-target update in ulcerative colitis: a systematic review. *Am J Gastroenterol.* (2019) 114:874–83. doi: 10.14309/ajg.0000000000000183
13. Colombel JF, Keir ME, Scherl A, Zhao R, de Hertogh G, Faubion WA, et al. Discrepancies between patient-reported outcomes, and endoscopic and histological appearance in UC. *Gut.* (2017) 66:2063–8. doi: 10.1136/gutjnl-2016-312307
14. Silva JC, Fernandes C, Rodrigues J, Fernandes S, Ponte A, Rodrigues A, et al. Endoscopic and histologic activity assessment considering disease extent and prediction of treatment failure in ulcerative colitis. *Scand J Gastroenterol.* (2020) 55:1157–62. doi: 10.1080/00365521.2020.1803397
15. Guo T, Qian JM, Yang AM, Li Y, Zhou WX. Predicting mucosal proliferation in ulcerative colitis by assessing mucosal vascular pattern under narrow band imaging colonoscopy. *Turk J Gastroenterol.* (2021) 32:203–8. doi: 10.5152/tjg.2021.20256
16. Sasanuma S, Ohtsuka K, Kudo SE, Ogata N, Maeda Y, Misawa M, et al. Narrow band imaging efficiency in evaluation of mucosal healing/relapse of ulcerative colitis. *Endosc Int Open.* (2018) 6:E518–23. doi: 10.1055/s-0044-102297

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SYSTEMATIC REVIEW
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Diagnostic Utility of Non-invasive Tests for Inflammatory Bowel Disease: An Umbrella Review

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Background: This study aims to consolidate evidence from published systematic reviews and meta-analyses evaluating the diagnostic performances of non-invasive tests for inflammatory bowel disease (IBD) in various clinical conditions and age groups.

Methods: Two independent reviewers systematically identified and appraised systematic reviews and meta-analyses assessing the diagnostic utility of non-invasive tests for IBD. Each association was categorized as adults, children, and mixed population, based on the age ranges of patients included in the primary studies. We classified clinical scenarios into diagnosis, activity assessment, and predicting recurrence.

Results: In total, 106 assessments from 43 reviews were included, with 17 non-invasive tests. Fecal calprotectin (FC) and fecal lactoferrin (FL) were the most sensitive for distinguishing IBD from non-IBD. However, anti-neutrophil cytoplasmic antibodies (ANCA) and FL were the most specific for it. FC and FL were the most sensitive and specific tests, respectively, to distinguish IBD from irritable bowel syndrome (IBS). Anti-*Saccharomyces cerevisiae* antibodies (ASCA), IgA, were the best test to distinguish Crohn's disease (CD) from ulcerative colitis (UC). Interferon-γ release assay was the best test to distinguish CD from intestinal tuberculosis (ITB). Ultrasound (US) and magnetic resonance enterography (MRE) were both sensitive and specific for disease activity, along with the high sensitivity of FC. Small intestine contrast ultrasonography (SICUS) had the highest sensitivity, and FC had the highest specificity for operative CD recurrence.

Conclusion: In this umbrella review, we summarized the diagnostic performance of non-invasive tests for IBD in various clinical conditions and age groups. Clinicians can use the suggested non-invasive test depending on the appropriate clinical situation in IBD patients.

Keywords: inflammatory bowel disease, non-invasive tests, diagnostic performance, Crohn's disease, ulcerative colitis

INTRODUCTION

Inflammatory bowel diseases (IBD) [Crohn's disease (CD) and ulcerative colitis (UC)] are idiopathic disorders causing inflammation of the gastrointestinal tract. IBD is emerging as a globally important disease with increasing incidence. Although incidence has started to relatively stabilize in western countries, the disease burden remains high as prevalence surpasses 0.3% (1).

Gastrointestinal endoscopy has remained a reference standard but invasive test for the diagnosis, management, prognostics, and surveillance of IBD. However, endoscopy can be associated with considerable cost, risk, and burden to patients and healthcare systems, and it is the lowest acceptable tool for patients (2).

Accurate non-invasive tests such as biomarkers and radiological examinations would be ideal (3, 4). Several promising non-invasive tests that could fulfill this role, including fecal calprotectin (FC) (5) and ultrasound (US) (6), have been studied. Despite many studies assessing the diagnostic performance of non-invasive tests for IBD, to the best of our knowledge, there has been no systematic effort to summarize and critically appraise this body of evidence. Therefore, we performed an umbrella review of meta-analyses, based on different clinical conditions (including diagnosis, activity assessment, and recurrence) and age groups (children, adults, and mixed population), to provide a comprehensive synopsis of the diagnostic performance and validity of reported non-invasive tests for IBD.

METHODS

Search Strategy

Two reviewers (J-TS and Z-QW) independently searched PubMed, Embase, Web of Science, and Cochrane Library databases from inception to 16 April 2020. The search was limited to systematic reviews and meta-analyses without language restrictions. **Supplementary Appendix 1** provides a detailed search strategy.

Study Selection and Data Extraction

Systematic reviews or meta-analyses meeting the following criteria were included: it described the conduct of the systematic review in adequate detail, and an attempt was made to identify all of the relevant primary studies in at least one database with provided search strategy and quality appraisal of the primary studies (7). Guidelines, narrative reviews, literature reviews, genetic studies, protocol, conference abstracts, and reviews assessing scoring indices were excluded.

Two reviewers (J-TS and Z-QW) independently carried out the study selection and data extraction from the eligible articles. Extracted data included author, year of publication, number of participants, number and type of studies included, appraisal instrument used, reference standard, outcomes assessed, heterogeneity, and study findings.

Quality Assessment

The methodological quality of included reviews was assessed independently by J-TS and Z-QW using the online AMSTAR 2 (A Measurement Tool to Assess Systematic Reviews) checklist (8). AMSTAR 2 is a validated and reliable quality measurement tool for systematic reviews, with 16 domains. Seven of these domains are considered critical. Shortcomings in any of the critical domains could affect the overall validity of a review. It results in an assessment of the methodologic quality as 1 of 2 grades: high, moderate, low, or critically low (9).

Identification of Age Groups

Based on the age ranges of primary studies included, associations can be categorized as adults, children, and mixed population. We defined children as under the age of 18 years (10). If a systematic review purporting to assess the accuracy in adults included people younger than 18 years, it would be classified as a mixed population. **Supplementary Appendix 2** presents the process of identifying age groups.

Overlapping and Outdated Associations

Associations in two or more reviews overlapped if they evaluated the same test in the same clinical condition and same age group. Incorporating results of overlapping reviews could lead to double inclusion resulting in biased findings and estimates (11, 12). In addition, up to 50% of published systematic reviews were considered out of date after 5.5 years. Therefore, we categorized overlapping systematic reviews as outdated (published before October 2015) and contemporary (published after October 2015).

For contemporary reviews found to have overlapping assessments, we generated a graphical cross-tabulation (citation matrix) of the overlapping reviews (in columns) and the included primary studies (in rows) (13). Corrected covered area (CCA) was a validated method to quantify the degree of overlap between two or more reviews. We used a citation matrix to calculate CCA. According to CCA, the overlap can be categorized as very high (CCA > 15%), high (CCA 11–15%), moderate (CCA 6–10%), or slight (CCA 0–5%) (14).

In all the systematic reviews that met the inclusion criteria, all non-overlapping reviews were included. A rigorous management tool was used for the overlapping reviews. **Supplementary Appendix 3** shows the citation matrices for all overlapping studies. **Supplementary Appendix 4** presents the management of overlapping reviews.

Data Synthesis

Systematic reviews that met the inclusion criteria formed the unit of analysis. Only data available from systematic reviews were presented. Results from systematic reviews were synthesized with a narrative synthesis, with a tabular presentation of findings and forest plots for studies that performed a meta-analysis. Summary tables describing review characteristics and findings were also presented.

Update of Eligible Reviews

We used the framework recommended by Garner et al. (15) to determine whether an update was necessary. An existing review qualified for an update if all of the following were met:

- The review achieved at least a moderate rating with the AMSTAR 2 quality assessment tool (9).

TABLE 1 | Summary findings for each non-invasive tests and diagnostic performance (IBD).

Non-invasive tests		Diagnostic performance (95% CI)		
		Sensitivity	**Specificity**	**AUC**
Mixed				
Diagnosis- IBD vs. non-IBD				
FC	FC	0.99 (0.92–1.00) * 0.882 (0.827–0.921) †	0.65 (0.54–0.74) * 0.799 (0.693–0.875) †	NA
	Cut-off 50μg/g	0.850 (0.605–0.955)	0.847 (0.647–0.943)	NA
	Cut-off 100μg/g	0.72 (0.63–0.80)	0.82 (0.78–0.86)	NA
CRP		0.63 (0.51–0.73)	0.88 (0.80–0.93)	NA
ESR		0.66 (0.58–0.73)	0.84 (0.80–0.88)	NA
PLT		0.55 (0.36–0.73)	0.88 (0.81–0.93)	NA
Hb		0.37 (0.24–0.52)	0.90 (0.83–0.94)	NA
Alb		0.48 (0.31–0.66)	0.94 (0.86–0.98)	NA
ASCA	ASCA	0.397 (0.376–0.418)	0.925 (0.913–0.937)	0.783
	IgA	0.314 (0.285–0.345)	0.96 (0.943–0.973)	0.821
ANCA		0.328 (0.312–0.344)	0.971 (0.964–0.977)	0.872
FL		0.82 (0.72–0.89)	0.95 (0.88–0.98)	0.95 (0.93–0.97)
US		0.73 (0.65–0.80)	0.95 (0.91–0.97)	NA
CT- per segment		0.85 (0.81–0.88)	0.87 (0.84–0.90)	0.933
microRNA		0.80 (0.79–0.82)	0.84 (0.82–0.86)	0.89
Diagnosis- IBD vs. IBS				
FC	Cut-off 50μg/g	0.97 (0.91–0.99)	0.76 (0.66–0.84)	NA
	Cut-off 100μg/g	0.92 (0.85–0.96)	0.86 (0.82–0.89)	NA
FL		0.78 (0.75–0.82)	0.94 (0.91–0.96)	0.94
Activity				
CT-Per segment		0.856 (0.76–0.92)	0.855 (0.75–0.92)	NA
US-Per segment		0.864 (0.761–0.927) 0.82	0.883 (0.581–0.976) 0.9	NA 0.90 (0.75–1.00)
MRE	MRE	0.83 (0.75–0.89)	0.93 (0.90–0.95)	0.95 (0.93–0.97)
	Per-patient	0.86 (0.78–0.91)	0.91 (0.82–0.96)	NA
	Per-lesion	0.72 (0.55–0.84)	0.93 (0.90–0.95)	NA
	Per-segment	0.75	0.91	0.88 (0.82–0.93)
Scintigraphy	LS-per patient	0.91 (0.87–0.95)	0.85 (0.76–0.91)	NA
	LS-per segment	0.79 (0.76–0.82)	0.86 (0.82–0.89)	NA
FC	FC	0.85 (0.82–0.87)	0.75 (0.71–0.79)	NA
	Cut-off 50μg/g	0.92 (0.90–0.94)	0.60 (0.52–0.67)	NA
	Cut-off 100μg/g	0.84 (0.80–0.88)	0.66 (0.59–0.73)	NA
	Cut-off 250μg/g	0.80 (0.76–0.84)	0.82 (0.77–0.86)	NA
CRP		0.49 (0.34–0.64)	0.92 (0.72–0.98)	0.72 (0.68–0.76)
FL		0.82 (0.73–0.88)	0.79 (0.62–0.89)	0.87 (0.84–0.90)
Recurrence				
FC		0.78 (0.72–0.83)	0.73 (0.68–0.77)	0.83
Adults				
Diagnosis- IBD vs. non-IBD				
FC		0.825 (0.661–0.920)	0.900 (0.573–0.984)	NA
Diagnosis- IBD vs. FGID				
FC		0.88 (0.80–0.93)	0.72 (0.59–0.82)	0.89
Activity				
CT-Per segment		0.84 (0.78–0.90)	0.86 (0.81–0.90)	NA
US-Per segment		0.860 (0.745–0.928)	0.836 (0.533–0.958)	NA
Children				
Diagnosis-IBD vs. non-IBD				
FC	FC	NA	NA	0.95 (0.93–0.98)
	Cut-off 50μg/g	0.83 (0.73–0.90)	0.85 (0.77–0.91)	0.96

(Continued)

TABLE 1 | (Continued)

Non-invasive tests	Diagnostic performance (95% CI)		
	Sensitivity	**Specificity**	**AUC**
CRP	NA	NA	0.79 (0.73–0.85)
ESR	NA	NA	0.84 (0.82–0.87)
PLT	NA	NA	0.79 (0.75–0.83)
Hb	NA	NA	0.76 (0.71–0.80)
Alb	NA	NA	0.82 (0.73–0.90)
Activity			
US	0.876 (0.542–0.977)	1.0	NA
Scintigraphy-MAAS-per segment	0.45 (0.37–0.53)	0.94 (0.89–0.97)	NA

*NA, not available, *age range: 0.8–19.9, †: age range: 14–90. FC, fecal calprotectin; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PLT, platelet count; Hb, hemoglobin; Alb, albumin; ASCA, Anti-Saccharomyces cerevisiae antibodies; ANCA, anti-neutrophil cytoplasmic antibodies; FL, fecal lactoferrin; US, Ultrasound; CT, computed tomography; MRE, magnetic resonance imaging enterography.*

- A focused or abbreviated search of primary studies using the key search terms from the search strategy of an existing review to identify newly published studies that met the inclusion criteria of the review.
- The findings from newly published studies would change the conclusion or credibility of the review.

Supplementary Appendix 5 describes the search strategy used to identify newly published studies. YXZ and YHS initially screened the eligible newly published studies. Full-text screening and data extracting were accomplished by JTS and ZQW.

With findings from newly published studies, we relied on statistical methods using the bivariate model (16) to pool the data from newly published studies with the data from the original meta-analysis (17) (for meta-analyses) and discussion with senior authors (for reviews without meta-analyses) to determine whether a full update of the existing review was needed (18).

If an update was considered necessary, the original methods used in the conduct of the existing review were replicated. **Supplementary Appendix 6** summarizes the evaluation process for considering reviews for updates adapted from Ahmadzai et al. (19).

RESULTS

Literature Search

The search retrieved 1,897 articles. After removing duplicates and screening titles and abstracts, 113 articles qualified for full-text screening. Seven outdated reviews were further excluded. Finally, 46 reviews were included. **Supplementary Appendix 7** summarizes the study selection process with accurate numbers of studies. **Supplementary Appendix 8** provides the list of excluded studies with reasons for exclusion.

Methodological Quality

Twenty-two reviews (5, 6, 10, 20–38) were rated as moderate in quality, and twenty-three reviews (39–60) were rated as low, while one review (61) was rated as critically low in quality (**Supplementary Appendix 9**). In the seven critical domains, most low-quality reviews had not stated that the methods were established before conducting the study.

Overlapping and Non-overlapping Assessment

Seventeen reviews reported overlapping assessment (5, 6, 29, 32, 36, 37, 46, 49–52, 54, 58, 59, 61–63). **Supplementary Appendix 10** describes the general characteristics of overlapping reviews, including the decision to retain or exclude an assessment. **Supplementary Appendix 3** provides the citation matrices for overlapping reviews to assess the degree of overlap. **Supplementary Appendix 11** lists forty-six reviews with non-overlapping assessments that were included and one contemporary review that was excluded because of overlap.

Study Characteristics of Reviews With Non-overlapping Assessments

Non-invasive tests for IBD assessed in the included reviews were FC, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), platelet count (PLT), hemoglobin (Hb), albumin (Alb), ASCA, anti-neutrophil cytoplasmic antibodies (ANCA), fecal lactoferrin (FL), US, computed tomography (CT), magnetic resonance imaging enterography (MRE), scintigraphy, autoantibodies-to-glycoprotein-2 (AntiGP2), interferon-γ release assays (IGRA), fecal immunochemical (FIT), microRNA, and S100A12. Of the 46 reviews included, 43 conducted meta-analyses. **Supplementary Table 1** summarizes the general characteristics of the reviews and meta-analyses included in the umbrella review.

Summary Findings

Table 1 shows the diagnostic utility of non-invasive tests for IBD in different clinical scenarios and age groups. **Tables 2**, **3** show the diagnostic utility of non-invasive tests for CD and UC, respectively. The clinical scenarios include diagnosis (IBD vs. non-IBD), diagnosis (IBD vs. IBS), diagnosis (IBD vs.

TABLE 2 | Summary findings for each non-invasive tests and diagnostic performance (CD).

Non-invasive tests		Diagnostic performance (95% CI)		
		Sensitivity	**Specificity**	**AUC**
Mixed				
Diagnosis- IBD vs. non-IBD				
FC	Cut-off 50μg/g	0.95 (0.92–0.97)	0.84 (0.80–0.87)	NA
	SBCD	0.89 (0.68–0.97)	0.55 (0.36–0.73)	NA
FL		0.75 (0.65–0.84)	1.00 (0.50–1.00)	0.84 (0.81–0.87)
MRI-SBCD		0.84 (0.77–0.90)	0.97 (0.91–0.99)	0.95
AntiGP2	AntiGP2	0.24 (0.18–0.32)	0.96 (0.93–0.97)	0.72 (0.68–0.76)
	IgA	0.15 (0.12–0.18)	0.97 (median)	NA
	IgG	0.19 (0.14–0.25)	0.97 (0.94–0.98)	0.71 (0.67–0.75)
Diagnosis- CD vs. UC				
AntiGP2	AntiGP2	0.20 (0.04–0.35)	0.97 (median)	NA
	IgA	0.11 (0.03–0.20)	0.98 (median)	NA
	IgG	0.30 (0.24–0.36)	0.93 (median)	NA
ASCA	ASCA	0.533 (0.508–0.557)	0.892 (0.872–0.910)	0.836
	IgA	0.408 (0.381–0.435)	0.955 (0.938–0.967)	0.863
	IgG	0.457 (0.432–0.483)	0.935 (0.917–0.949)	0.85
Diagnosis- CD vs. ITB				
ASCA		0.33 (0.27–0.38)	0.83 (0.77–0.88)	0.58
IGRA		0.828 (0.784–0.855)	0.867 (0.832–0.896)	NA
Activity				
CT-SBCD-per patient		0.86 (0.79–0.91)	0.84 (0.75–0.90)	NA
FC	FC	0.824 (0.802–0.844)	0.721 (0.69–0.75)	0.84
	Cut-off 50μg/g	0.831 (0.740–0.895)	0.502 (0.359–0.644)	0.774
	Cut-off 100μg/g	0.725 (0.657–0.784)	0.728 (0.622–0.814)	0.763
	Cut-off 200μg/g	0.495 (0.361–0.629)	0.882 (0.738–0.952)	0.67
FL		0.82 (0.73–0.88)	0.71 (0.63–0.78)	0.84 (0.80–0.87)
MRI		0.9	0.89	NA
US	Per segment	0.725 (0.454–0.894)	0.977 (0.700–0.999)	NA
	CEUS	0.94 (0.87–0.97)	0.79 (0.67–0.88)	0.94
Recurrence				
FC	FC	0.75 (0.64–0.84)	0.71 (0.64–0.76)	0.79
	POR-ER	0.82 (0.73–0.89)	0.61 (0.51–0.71)	0.77 (0.74–0.81)
	POR-CR	0.59 (0.47–0.71)	0.88 (0.80–0.93)	0.97
	POR-Cut-off 50μg/g	0.90 (0.83–0.96)	0.36 (0.25–0.47)	0.72
	POR-Cut-off 100μg/g	0.81 (0.71–0.91)	0.57 (0.48–0.64)	0.67
	POR-Cut-off 150μg/g	0.70 (0.59–0.81)	0.69 (0.61–0.77)	0.73
	POR-Cut-off 200μg/g	0.55 (0.43–0.69)	0.71 (0.62–0.79)	0.69
US	POR	0.94 (0.86–0.97)	0.84 (0.62–0.94)	0.9
	POR-BS	0.82 (0.76–0.88)	0.88 (0.74–0.95)	0.875
	POR-SICUS	0.99 (0.99–1.00)	0.74 (0.73–0.74)	0.92
	POR-SBCD–SICUS	0.899 (0.817–0.953)	0.808 (0.606–0.934)	NA
MRI-POR		0.973 (0.891–0.998)	0.837 (0.616–0.959)	0.9767
Children				
Diagnosis- IBD vs. non-IBD				
FC	Cut-off 50μg/g	0.97 (0.86–1.00)	0.79 (0.69–0.87)	NA
	Cut-off 100μg/g	1.00 (0.93–1.00)	0.98 (0.93–1.00)	NA

FGID, functional gastrointestinal disorders), diagnosis (CD vs. ITB, intestinal tuberculosis), diagnosis (CD vs. UC), activity assessment, and recurrence. **Figure 1** presents the forest plots of sensitivity (Se) and specificity (Sp) of non-invasive tests for IBD. **Figures 2**, **3** present the forest plots for CD and UC, respectively. **Supplementary Tables 2, 3** show the findings of meta-analyses and narrative synthesis from systematic reviews.

TABLE 3 | Summary findings for each non-invasive tests and diagnostic performance (UC).

Non-invasive tests		Diagnostic performance (95% CI)		
		Sensitivity	Specificity	AUC
Mixed				
Diagnosis- IBD vs. non-IBD				
FC		0.78 (0.69–0.86)	0.78 (0.70–0.84)	NA
ANCA		0.522	0.99	NA
FL		0.82 (0.67–0.91)	1.00 (0.67–1.00)	0.94 (0.91–0.96)
Diagnosis- UC vs. CD				
ANCA		0.553 (0.530–0.576)	0.885 (0.871–0.898)	0.818
Activity				
US-per segment		0.886 (0.800–0.939)	0.819 (0.456–0.961)	NA
MRE	MRE	0.88 (0.86–0.91)	0.88 (0.84–0.91)	0.93
	DWI-per segment	0.929 (0.858–0.966)	0.910 (0.797–0.963)	NA
	LP	0.493 (0.410–0.578)	0.891 (0.813–0.944)	0.82
	SBCD-per patient	0.88 (0.82–0.92)	0.81 (0.72–0.88)	0.91
FC		0.873 (0.854–0.891) * 0.76 (0.71–0.81) †	0.771 (0.737–0.803) * 0.71 (0.62–0.78) †	0.91* 0.79 (0.75–0.82) †
FIT		0.72 (0.57–0.84)	0.80 (0.67–0.89)	NA
FL		0.81 (0.64–0.92)	0.82 (0.61–0.93)	0.88 (0.85–0.91)
Recurrence				
FC		0.75 (0.70–0.79)	0.77 (0.74–0.80)	0.82

*NA, not available; *: endoscopic activity as reference; †: histological activity as reference.*

Diagnosis: Inflammatory Bowel Disease vs. Non-inflammatory Bowel Disease

Mixed Population

For IBD, FC was the most sensitive test with a sensitivity of 0.99 (0.92–1.00) (46). ANCA showed the highest specificity 0.971 (0.964–0.977) (20). The sensitivity and specificity of CT, FL, and microRNA were both balanced (41, 44). The other tests performed well in specificity but poorly in sensitivity, including US, ESR, CRP, PLT, Alb, Hb, and ASCA (20, 46).

For UC, FL had both the best sensitivity (0.82; 0.67–0.91) and the best specificity (1.00; 0.67–1.00) (44). The other biomarkers were FC (Se, 0.78; 0.69–0.86/Sp, 0.78; 0.70–0.84) (21), and ANCA (Se, 0.522/Sp, 0.99) (64).

For CD, FC showed the highest sensitivity: 0.95 (0.92–0.97) (21). FL showed the highest specificity: 1.00 (0.50–1.00) (44). Also, the specificity of anti-GP2 was good (49).

Adults

For IBD, only FC was performed with Se of 0.825 (0.661–0.920) and Sp of 0.900 (0.573–0.984) (5). For UC, there was a review showing that the Se and Sp for ANCA IgG were 0.67 (0.54–0.79) and 0.85 (0.70–0.94), respectively (39).

Children

For IBD, FC with a cutoff of 50 μg/g showed the highest AUC of 0.96 (21). The AUCs of other biomarkers [FC, CRP, ESR, PLT, Hb, and Alb (30)] ranged from 0.76 to 0.95. One review presented results of US from three primary studies: sensitivity range from 0.39 to 0.55 and specificity range from 0.90 to 1.00 (35).

For CD, FC with a cutoff of 100 μg/g performed best with a sensitivity of 1.00 (0.93–1.00) and specificity of 0.98 (0.93–1.00) (21). MRE (Se, 0.84; 0.77–0.90/Sp, 0.97; 0.91–0.99) (22) also performed well in SBCD.

Diagnosis: Inflammatory Bowel Disease vs. Irritable Bowel Syndrome

Mixed Population

For IBD, FC with a cutoff of 50 μg/g was the most sensitive test with a sensitivity of 0.97 (0.91–0.99) (52). As for specificity, FL was the best: 0.94 (0.91–0.96) (24). One review presented the diagnostic performance of fecal S100A12 (Se, 0.86; 0.73–0.94/Sp, 0.96; 0.79–0.99) (39) (**Supplementary Table 3**).

Diagnosis: Inflammatory Bowel Disease vs. Functional Gastrointestinal Disorders

Adults

For IBD, there was only one test: FC (Se, 0.88; 0.80–0.93/Sp, 0.71; 0.59–0.82) (53).

Diagnosis: Crohn's Disease vs. Ulcerative Colitis

Mixed Population

To differentiate CD from UC, the sensitivity of tests is generally low, including anti-GP2, ASCA (20, 54). ASCA IgA showed the highest specificity of 0.955 (0.938–0.967) (20). To differentiate UC from CD, the only test included in our analysis was ANCA (Se, 0.553; 0.530–0.576/Sp, 0.885; 0.871–0.898) (20).

Diagnosis: Crohn's Disease vs. Intestinal Tuberculosis

Mixed Population

IGRA (Se, 0.828; 0.784–0.855/Sp, 1.00; 0.867–0.896) (48) had better diagnostic performance than ASCA (Se, 0.828; 0.784–0.855/Sp, 0.867; 0.832–0.896) (25).

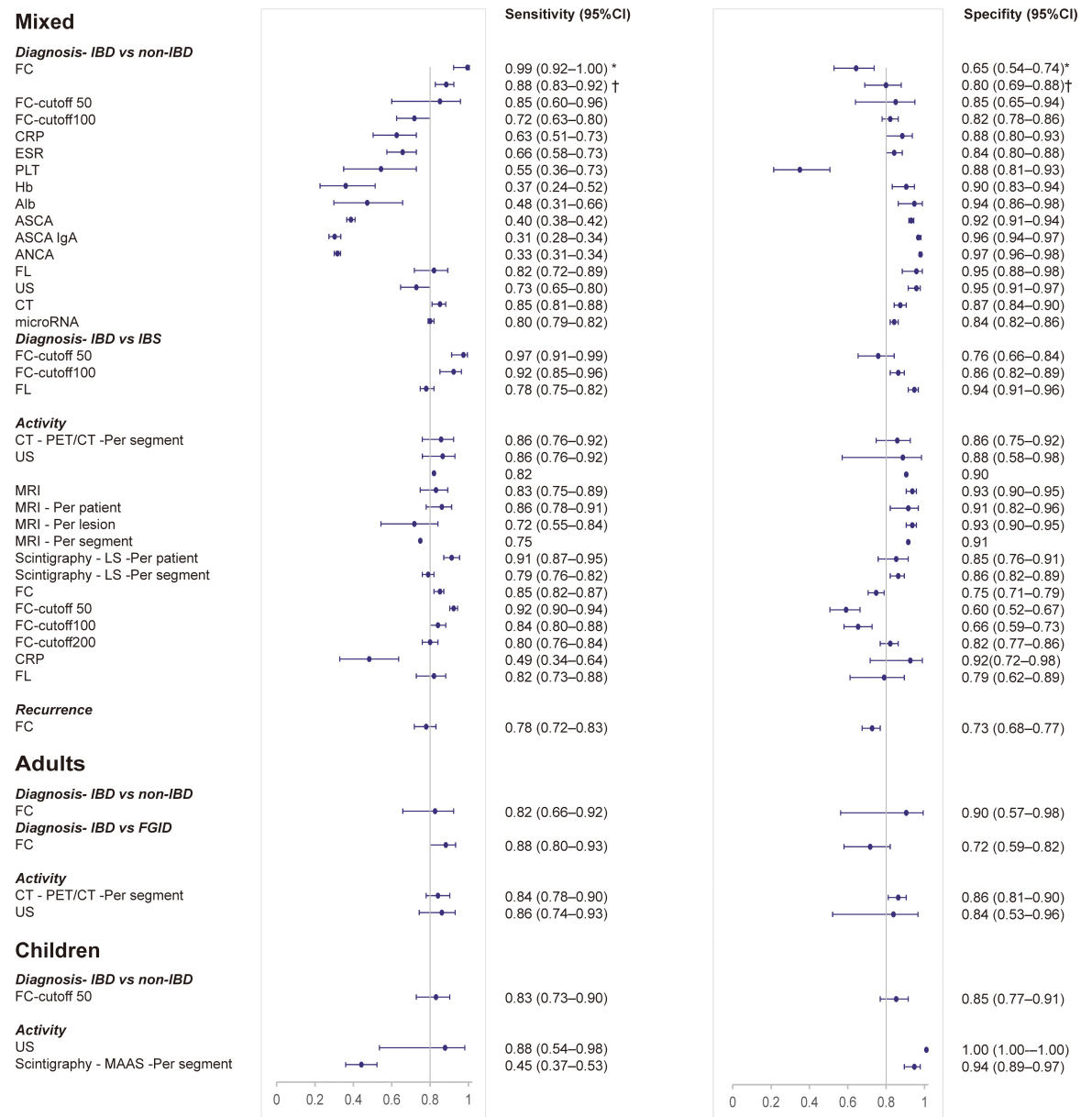

FIGURE 1 | Forest plots of sensitivity and specificity of non-invasive tests for inflammatory bowel disease from meta-analyses. CI, confidence interval. *Age range: 0.8–19.9, †age range: 14–90.

Activity

Mixed Population

For IBD, FC with a cutoff of 50 μg/g presented the highest sensitivity of 0.92 (0.90–0.94) (42), and MRE showed the highest specificity of 0.93 (0.90–0.95) (30). Besides, other radiological examinations [US, leukocyte scintigraphy (LS), and CT] all performed well with balanced sensitivity and specificity (6, 30, 43, 56, 59). However, other biomarkers (CRP and FL) were not as good as radiological examinations (27). One review suggested a sensitivity range of 0.64 to 0.93 and a specificity range of 0.71 to 1, showing that the diagnostic accuracy of TAUS (transabdominal US) remains inconclusive (**Supplementary Table 3**) (35).

For CD, US showed the best specificity of 0.977 (0.700–0.999) (6). Contrast-enhanced ultrasound (CEUS) was the most sensitive test with a sensitivity of 0.94 (0.87–0.97) (45). CT

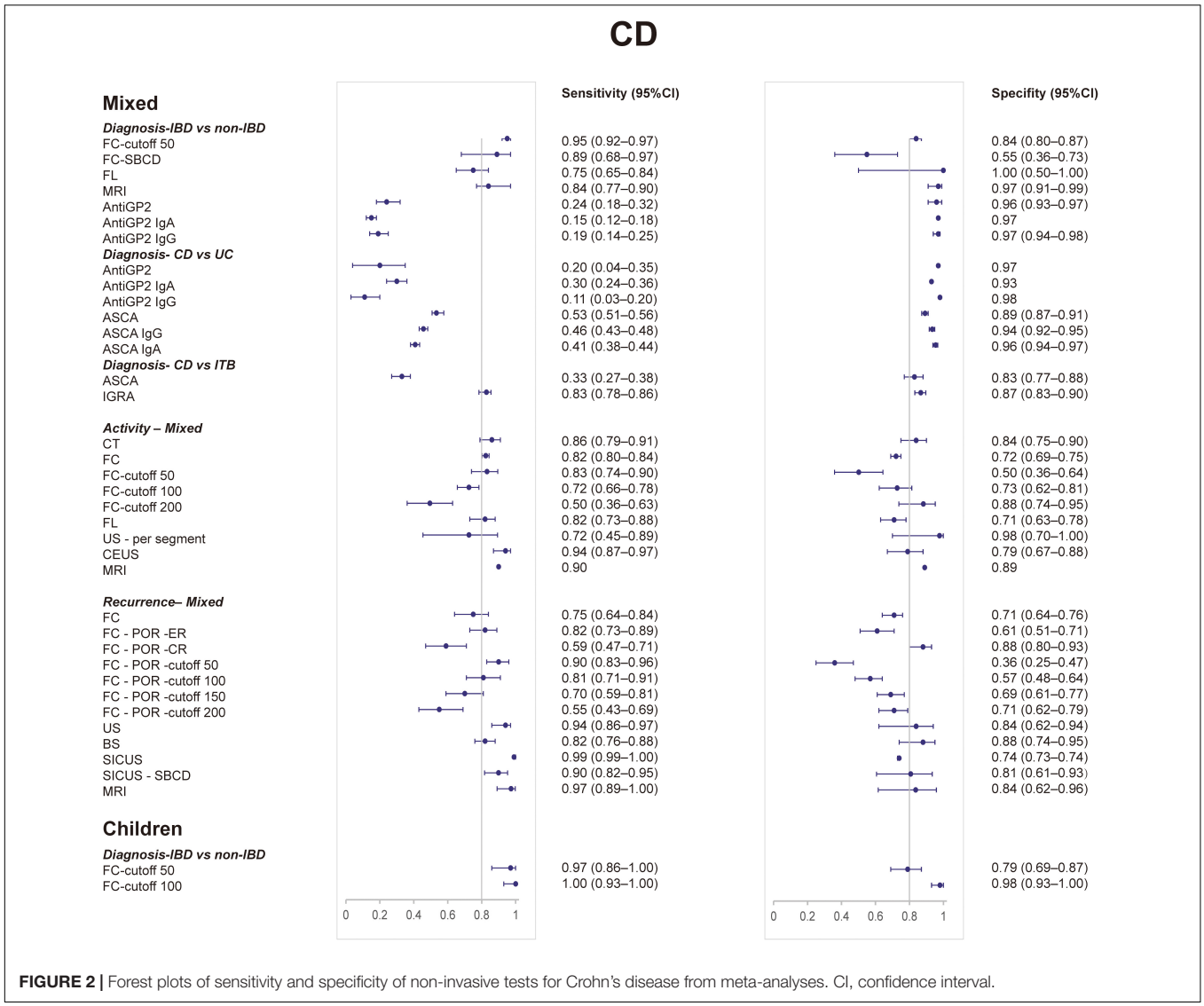

FIGURE 2 | Forest plots of sensitivity and specificity of non-invasive tests for Crohn's disease from meta-analyses. CI, confidence interval.

and MRE also performed well; however, the sensitivity of DWI-MRE was poor (23, 28, 34, 47). FC and FL performed slightly worse than CT and MRE (32, 55). For UC, US had both best sensitivity (0.886, 0.800–0.939) and specificity (0.819, 0.456–0.961) (6). Among other tests, the specificity of FIT and FL and the sensitivity of FC and FL were fair (32, 36).

Adults

For IBD, US and CT have similar diagnostic performance. The sensitivity of ultrasound was slightly higher (0.860; 0.745–0.928) (6), while the specificity of CT was slightly higher (0.86; 0.81–0.90) (43). Monoclonal anti-granulocyte antibody scintigraphy (MAAS) was sensitive (Se, 0.94; 0.89–0.97), but its specificity was not good (Sp, 0.45; 0.37–0.53) (43).

Children

For IBD, US had great performance: Se, 0.876 (0.542–0.977); Sp, 1.00 (6). One review reported the diagnostic accuracy of TAUS, but showed that it remained inconclusive (**Supplementary Table 3**) (35). The other review showed that the sensitivity of positron emission tomography/CT (PET/CT): 0.59 (0.36–0.79) (SBFT, small-bowel follow through, used as the reference standard); 0.86 (0.70–0.95) (colonoscopy used as the reference standard) and the specificity: 1.00 (0.77–1.00) and 0.50 (0.01–0.99), respectively (43).

Recurrence

Mixed Population

For IBD, the only test was FC (Se, 0.78; 0.72–0.83/Sp, 0.73; 0.68–0.77/AUC, 0.83) (40). For UC, the sensitivity and specificity of FC were 0.75 (0.70–0.79) and 0.77 (0.74–0.80), respectively (33). For CD, FC showed the sensitivity of 0.75 (0.64–0.84) and specificity of 0.71 (0.64–0.76) (40).

For postoperative CD, SICUS showed the highest sensitivity of 0.99 (0.99–1.00) (62). FC for clinical recurrence presented the highest specificity of 0.88 (0.80–0.93), while FC for endoscopic

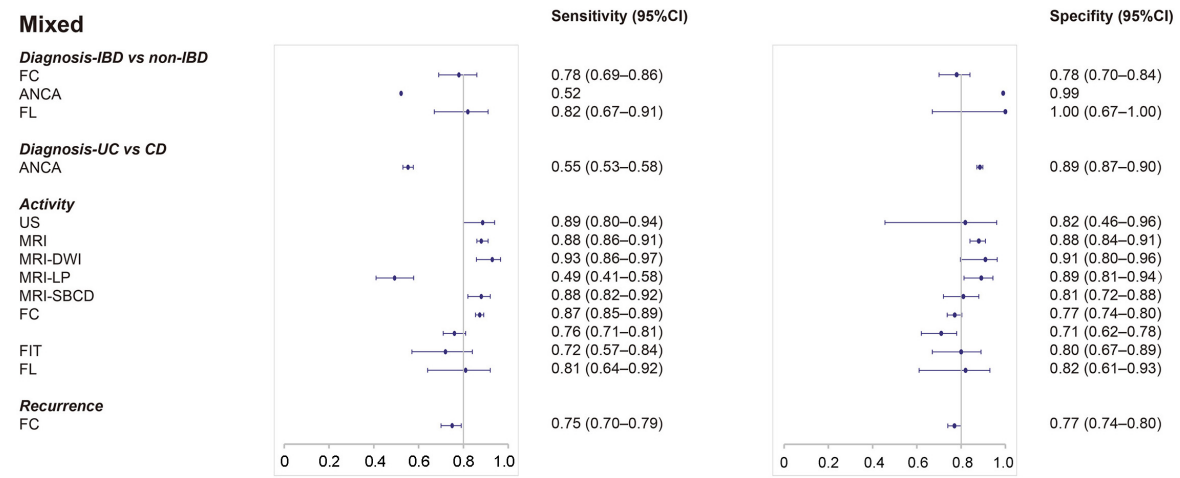

FIGURE 3 | Forest plots of sensitivity and specificity of non-invasive tests for ulcerative colitis from meta-analyses. CI, confidence interval.

recurrence presented with better sensitivity (26). Besides, MRE and other subtypes of US performed well in both sensitivity and specificity (50, 51, 57).

Reviews Eligible for Update

We searched for newly published studies for each moderate quality review (**Supplementary Appendix 6**). After screening, 8 reviews (20, 22, 26, 28–30, 32, 35) have eligible new published studies. However, after calculation, no reviews need to be updated. The overview of updating was presented in **Supplementary Appendix 12**.

DISCUSSION

Our detailed umbrella review synthesized existing systematic reviews and meta-analyses into one user-friendly document. A total of 106 associations, including 17 non-invasive tests, have been studied.

Main Findings

Evidence from the umbrella review suggests that FC (0.99) and FL (0.82) were the most sensitive markers for distinguishing IBD from non-IBD. Similarly, ANCA (0.971) and FL (0.95) were the most specific marker for this purpose. To distinguish IBD from IBS, the most sensitive one was FC (cutoff 50 μg/g, 0.97; cutoff 100 μg/g, 0.92) and the most specific marker was FL (0.94). To distinguish CD from UC, all tests had low sensitivity, with ASCA IgA (0.955) having the highest specificity. IGRA (Se, 0.828; Sp, 0.867) was the best test to distinguish CD from ITB. There is only one test to diagnose IBD from FGID and only one test to distinguish UC from CD, FC, and ANCA. As for assessing activity, US (Se, 0.864; Sp, 0.883) and MRE (Se, 0.83; Sp, 0.93) perform well. The sensitivity of FC (0.85) was also good. As for postoperative recurrence of CD, SICUS (0.99) had the highest sensitivity and FC (CR: 0.88) had the highest specificity. We concluded that biomarkers played a good role in diagnosis, while radiological examinations, especially MRE and US, were more prominent in assessing activity and predicting recurrence. **Supplementary Table 4** presents the characteristic of diagnostic performance and clinical use of each test.

Strengths and Limitations

Compared with other studies summarizing non-invasive tests for IBD (65, 66), our umbrella review provides the first systematic appraisal of the evidence using robust criteria. We used the AMSTAR 2 tool to assess the quality of reviews and used CCA to evaluate the degree of overlapping and report the highest quality and most current review. Besides, our umbrella review included both blood, stool biomarkers and radiological examinations. Furthermore, we rigorously classified the assessments into age groups based on the exact age range of the primary studies included and into several groups to discuss the diagnostic performance in a different clinical condition more rigorously and reasonably.

Several limitations are present in this review. Lack of data, including missing meta-data, hindered the reporting of some elements of the umbrella review and lack of reviews of children or adults alone. In addition, one review (20) could not undergo the normal updating process because it did not report the included studies of each assessment. Besides, some reviews were rated as low quality for the most common reason: lack of protocol. However, registering protocol has been rare, especially in the IBD field. What's more, since most articles do not report the value of AUC, we can't do a good comparison and analysis of AUC.

Implications for Practice and Future Research

This comprehensive umbrella review could help clinicians make better decisions about the appropriate tests prior to endoscopy. In

terms of diagnosis, we suggested that in patients with symptoms suggestive of IBD in whom the clinician considers endoscopy, FC could be a sensitive test for safely excluding IBD. For patients with a negative result, we recommend that they continue to be monitored rather than do endoscopic examination immediately, unless it is very urgent. In patients with a positive result, FL is a good choice because of their low false-positive rate and consequent reduction of unnecessary endoscopies if patients are willing to have a stool test; if not, ANCA is an alternative. Clinicians can use our results to select a specific marker based on the practical situation. If both tests are positive, the patient is highly likely to have IBD. Endoscopic examination can be followed to confirm the diagnosis and disease classification. Radiation examinations, especially US and MRE, performed well in the activity assessment and predicting relapse. For patients with CD, we recommend having FC or US tests regularly to monitor the disease activity. Specifically, US or MRE is recommended for patients requiring postoperative recurrence monitoring. For patients with UC, MRE is the best choice to assess activity and predict relapse.

Our results show that there are not many reviews for children, especially in activity assessment and predicting recurrence. However, the use of endoscopy, invasive and requiring general anesthesia, can lead to child disobedience and disapproval of parents. An attitude of "wait and see" may cause unnecessary concerns and loss of wellbeing in children with IBD. Therefore, high-quality prospective studies on non-invasive testing in children should be complemented.

CONCLUSION

In summary, this umbrella review summarized the diagnostic performance of non-invasive tests for IBD in different clinical conditions and age groups and offered our suggestions on how to use the non-invasive tests appropriately. Researchers and clinicians could choose a suitable test based on our results. Further studies on non-invasive tests in children are needed.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

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

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet.* (2017) 390:2769–78. doi: 10.1016/s0140-6736(17)32448-0
2. Buisson A, Gonzalez F, Poullenot F, Nancey S, Sollellis E, Fumery M, et al. Comparative acceptability and perceived clinical utility of monitoring tools: a nationwide survey of patients with inflammatory bowel disease. *Inflamm Bowel Dis.* (2017) 23:1425–33. doi: 10.1097/MIB.0000000000001140
3. Dulai PS, Peyrin-Biroulet L, Danese S, Sands BE, Dignass A, Turner D, et al. Approaches to integrating biomarkers into clinical trials and care pathways as targets for the treatment of inflammatory bowel diseases. *Gastroenterology.* (2019) 157:1032–43.e1. doi: 10.1053/j.gastro.2019.06.018
4. Allocca M, Danese S, Laurent V, Peyrin-Biroulet L. Use of cross-sectional imaging for tight monitoring of inflammatory bowel diseases. *Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc.* (2020) 18:1309–23.e4. doi: 10.1016/j.cgh.2019.11.052
5. Petryszyn P, Staniak A, Wolosianska A, Ekk-Cierniakowski P. Faecal calprotectin as a diagnostic marker of inflammatory bowel disease in patients with gastrointestinal symptoms: meta-analysis. *Eur J Gastroenterol Hepatol.* (2019) 31:1306–12. doi: 10.1097/meg.0000000000001509
6. Sagami S, Kobayashi T, Miyatani Y, Okabayashi S, Yamazaki H, Takada T, et al. Accuracy of ultrasound for evaluation of colorectal segments in patients with inflammatory bowel diseases: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc.* (2020) 19:908–21.e6. doi: 10.1016/j.cgh.2020.07.067
7. Haddaway NR, Land M, Macura B. "A little learning is a dangerous thing": a call for better understanding of the term 'systematic review'. *Environ Int.* (2017) 99:356–60. doi: 10.1016/j.envint.2016.12.020
8. AMSTAR. *Assessing the Methodological Quality of Systematic Reviews*. (2017). Available online at: https://amstar.ca/Amstar_Checklist.php (accessed 2021).
9. Shea BJ, Reeves BC, Wells G, Thuku M, Hamel C, Moran J, et al. AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. *BMJ (Clin Res Ed).* (2017) 358:j4008. doi: 10.1136/bmj.j4008
10. Holtman GA, Lisman-van Leeuwen Y, Day AS, Fagerberg UL, Henderson P, Leach ST, et al. Use of laboratory markers in addition to symptoms for diagnosis of inflammatory bowel disease in children a meta-analysis of individual patient data. *JAMA Pediatr.* (2017) 171:984–91. doi: 10.1001/jamapediatrics.2017.1736
11. Smith V, Devane D, Begley CM, Clarke M. Methodology in conducting a systematic review of systematic reviews of healthcare interventions. *BMC Med Res Methodol.* (2011) 11:15. doi: 10.1186/1471-2288-11-15

12. Senn SJ. Overstating the evidence: double counting in meta-analysis and related problems. *BMC Med Res Methodol.* (2009) 9:10. doi: 10.1186/1471-2288-9-10
13. Bougioukas KI, Liakos A, Tsapas A, Ntzani E, Haidich AB. Preferred reporting items for overviews of systematic reviews including harms checklist: a pilot tool to be used for balanced reporting of benefits and harms. *J Clin Epidemiol.* (2018) 93:9–24. doi: 10.1016/j.jclinepi.2017.10.002
14. Pieper D, Antoine SL, Mathes T, Neugebauer EA, Eikermann M. Systematic review finds overlapping reviews were not mentioned in every other overview. *J Clin Epidemiol.* (2014) 67:368–75. doi: 10.1016/j.jclinepi.2013.11.007
15. Garner P, Hopewell S, Chandler J, MacLehose H, Schünemann HJ, Akl EA, et al. When and how to update systematic reviews: consensus and checklist. *BMJ (Clin Res Ed).* (2016) 354:i3507. doi: 10.1136/bmj.i3507
16. Vogelgesang F, Schlattmann P, Dewey M. The evaluation of bivariate mixed models in meta-analyses of diagnostic accuracy studies with SAS, Stata and R. *Methods Inf Med.* (2018) 57:111–9. doi: 10.3414/ME17-01-0021
17. Okoth K, Chandan JS, Marshall T, Thangaratinam S, Thomas GN, Nirantharakumar K, et al. Association between the reproductive health of young women and cardiovascular disease in later life: umbrella review. *BMJ (Clin Res Ed).* (2020) 371:m3502. doi: 10.1136/bmj.m3502
18. Chung M, Newberry SJ, Ansari MT, Yu WW, Wu H, Lee J, et al. Two methods provide similar signals for the need to update systematic reviews. *J Clin Epidemiol.* (2012) 65:660–8. doi: 10.1016/j.jclinepi.2011.12.004
19. Ahmadzai N, Newberry SJ, Maglione MA, Tsertsvadze A, Ansari MT, Hempel S, et al. A surveillance system to assess the need for updating systematic reviews. *Syst Rev.* (2013) 2:104.
20. Reese GE, Constantinides VA, Simillis C, Darzi AW, Orchard TR, Fazio VW, et al. Diagnostic precision of anti-Saccharomyces cerevisiae antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. *Am J Gastroenterol.* (2006) 101:2410–22. doi: 10.1111/j.1572-0241.2006.00840.x
21. von Roon AC, Karamountzos L, Purkayastha S, Reese GE, Darzi AW, Teare JP, et al. Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. *Am J Gastroenterol.* (2007) 102:803–13. doi: 10.1111/j.1572-0241.2007.01126.x
22. Giles E, Barclay AR, Chippington S, Wilson DC. Systematic review: MRI enterography for assessment of small bowel involvement in paediatric Crohn's disease. *Alimentary Pharmacol Ther.* (2013) 37:1121–31. doi: 10.1111/apt.12323
23. Qiu Y, Mao R, Chen BL, Li XH, He Y, Zeng ZR, et al. Systematic review with meta-analysis: magnetic resonance enterography vs. computed tomography enterography for evaluating disease activity in small bowel Crohn's disease. *Alimentary Pharmacol Ther.* (2014) 40:134–46. doi: 10.1111/apt.12815
24. Zhou XL, Xu W, Tang XX, Luo LS, Tu JF, Zhang CJ, et al. Fecal lactoferrin in discriminating inflammatory bowel disease from irritable bowel syndrome: a diagnostic meta-analysis. *BMC Gastroenterol.* (2014) 14:121. doi: 10.1186/1471-230x-14-121
25. Ng SC, Hirai HW, Tsoi KK, Wong SH, Chan FK, Sung JJ, et al. Systematic review with meta-analysis: accuracy of interferon-gamma releasing assay and anti-Saccharomyces cerevisiae antibody in differentiating intestinal tuberculosis from Crohn's disease in Asians. *J Gastroenterol Hepatol.* (2014) 29:1664–70. doi: 10.1111/jgh.12645
26. Qiu Y, Mao R, Chen BL, He Y, Zeng ZR, Xue L, et al. Fecal calprotectin for evaluating postoperative recurrence of Crohn's disease: a meta-analysis of prospective studies. *Inflamm Bowel Dis.* (2015) 21:315–22. doi: 10.1097/mib.0000000000000262
27. Mosli MH, Zou G, Garg SK, Feagan SG, MacDonald JK, Chande N, et al. C-Reactive protein, fecal calprotectin, and stool lactoferrin for detection of endoscopic activity in symptomatic inflammatory bowel disease patients: a systematic review and meta-analysis. *Am J Gastroenterol.* (2015) 110:802–19, quiz 20. doi: 10.1038/ajg.2015.120
28. Ahmed O, Rodrigues DM, Nguyen GC. Magnetic resonance imaging of the small bowel in Crohn's disease: a systematic review and meta-analysis. *Can J Gastroenterol Hepatol.* (2016) 2016:7857352. doi: 10.1155/2016/7857352
29. Kopylov U, Yung DE, Engel T, Avni T, Battat R, Ben-Horin S, et al. Fecal calprotectin for the prediction of small-bowel Crohn's disease by capsule endoscopy: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol.* (2016) 28:1137–44. doi: 10.1097/meg.0000000000000692
30. Yoon HM, Suh CH, Kim JR, Lee JS, Jung AY, Kim KM, et al. Diagnostic performance of magnetic resonance enterography for detection of active inflammation in children and adolescents with inflammatory bowel disease: a systematic review and diagnostic meta-analysis. *JAMA Pediatr.* (2017) 171:1208–16. doi: 10.1001/jamapediatrics.2017.3400
31. Tham YS, Yung DE, Fay S, Yamamoto T, Ben-Horin S, Eliakim R, et al. Fecal calprotectin for detection of postoperative endoscopic recurrence in Crohn's disease: systematic review and meta-analysis. *Ther Adv Gastroenterol.* (2018) 11:1756284818785571. doi: 10.1177/1756284818785571
32. Rokkas T, Portincasa P, Koutroubakis IE. Fecal calprotectin in assessing inflammatory bowel disease endoscopic activity: a diagnostic accuracy meta-analysis. *J Gastrointestinal Liver Dis JGLD.* (2018) 27:299–306. doi: 10.15403/jgld.2014.1121.273.pti
33. Li J, Zhao X, Li X, Lu M, Zhang H. Systematic review with meta-analysis: fecal calprotectin as a surrogate marker for predicting relapse in adults with ulcerative colitis. *Mediators Inflamm.* (2019) 2019:2136501. doi: 10.1155/2019/2136501
34. Bellini D, Rivosecchi F, Panvini N, Rengo M, Caruso D, Carbone I, et al. Layered enhancement at magnetic resonance enterography in inflammatory bowel disease: a meta-analysis. *World J Gastroenterol.* (2019) 25:4555–66. doi: 10.3748/wjg.v25.i31.4555
35. van Wassenaer EA, de Voogd FAE, van Rijn RR, van der Lee JH, Tabbers MM, Van Etten-Jamaludin FS, et al. Diagnostic accuracy of transabdominal ultrasound in detecting intestinal inflammation in paediatric IBD patients-a systematic review. *J Crohn's Colitis.* (2019) 13:1501–9. doi: 10.1093/ecco-jcc/jjz085
36. Lee MW, Pourmorady JS, Laine L. Use of fecal occult blood testing as a diagnostic tool for clinical indications: a systematic review and meta-analysis. *Am J Gastroenterol.* (2020) 115:662–70. doi: 10.14309/ajg.0000000000000495
37. Jung ES, Lee SP, Kae SH, Kim JH, Kim HS, Jang HJ. Diagnostic accuracy of fecal calprotectin for the detection of small bowel Crohn's disease through capsule endoscopy: an updated meta-analysis and systematic review. *Gut Liver.* (2020) 15:732–41. doi: 10.5009/gnl20249
38. Ahmad R, Ajlan AM, Eskander AA, Alhazmi TA, Khashoggi K, Wazzan MA, et al. Magnetic resonance imaging in the management of Crohn's disease: a systematic review and meta-analysis. *Insights Imaging.* (2021) 12:118. doi: 10.1186/s13244-021-01064-9
39. Jellema P, van Tulder MW, van der Horst HE, Florie J, Mulder CJ, van der Windt DA. Inflammatory bowel disease: a systematic review on the value of diagnostic testing in primary care. *Colorectal Dis Off J Assoc Coloproctol Great Britain Ireland.* (2011) 13:239–54. doi: 10.1111/j.1463-1318.2009.02131.x
40. Mao R, Xiao YL, Gao X, Chen BL, He Y, Yang L, et al. Fecal calprotectin in predicting relapse of inflammatory bowel diseases: a meta-analysis of prospective studies. *Inflamm Bowel Dis.* (2012) 18:1894–9. doi: 10.1002/ibd.22861
41. Treglia G, Quartuccio N, Sadeghi R, Farchione A, Caldarella C, Bertagna F, et al. Diagnostic performance of Fluorine-18-Fluorodeoxyglucose positron emission tomography in patients with chronic inflammatory bowel disease: a systematic review and a meta-analysis. *J Crohn's Colitis.* (2013) 7:345–54. doi: 10.1016/j.crohns.2012.08.005
42. Lin JF, Chen JM, Zuo JH, Yu A, Xiao ZJ, Deng FH, et al. Meta-analysis: fecal calprotectin for assessment of inflammatory bowel disease activity. *Inflamm Bowel Dis.* (2014) 20:1407–15. doi: 10.1097/mib.0000000000000057
43. Zhang J, Li LF, Zhu YJ, Qiu H, Xu Q, Yang J, et al. Diagnostic performance of 18F-FDG-PET versus scintigraphy in patients with inflammatory bowel disease: a meta-analysis of prospective literature. *Nuclear Med Commun.* (2014) 35:1233–46. doi: 10.1097/mnm.0000000000000202
44. Wang Y, Pei F, Wang X, Sun Z, Hu C, Dou H. Diagnostic accuracy of fecal lactoferrin for inflammatory bowel disease: a meta-analysis. *Int J Clin Exp Pathol.* (2015) 8:12319–32.
45. Serafin Z, Białecki M, Białecka A, Sconfienza LM, Klopocka M. Contrast-enhanced ultrasound for detection of Crohn's disease activity: systematic review and meta-analysis. *J Crohn's Colitis.* (2016) 10:354–62. doi: 10.1093/ecco-jcc/jjv196

46. Holtman GA, Lisman-van Leeuwen Y, Reitsma JB, Berger MY. Noninvasive tests for inflammatory bowel disease: a meta-analysis. *Pediatrics.* (2016) 137:e20152126. doi: 10.1542/peds.2015-2126
47. Choi SH, Kim KW, Lee JY, Kim KJ, Park SH. Diffusion-weighted magnetic resonance enterography for evaluating bowel inflammation in Crohn's disease: a systematic review and meta-analysis. *Inflamm Bowel Dis.* (2016) 22:669–79. doi: 10.1097/mib.0000000000000607
48. Xu H, Li Y, Qian JM. [A Meta-analysis of the accuracy of interferon-γ release assays in differentiating intestinal tuberculosis from Crohn's disease in Asia]. *Zhonghua Nei Ke Za Zhi.* (2016) 55:535–40. doi: 10.3760/cma.j.issn.0578-1426.2016.07.010
49. Deng C, Li W, Li J, Zhang S, Li Y. Diagnostic value of the antiglycoprotein-2 antibody for Crohn's disease: a PRISMA-compliant systematic review and meta-analysis. *BMJ Open.* (2017) 7:e014843. doi: 10.1136/bmjopen-2016-014843
50. Yung DE, Har-Noy O, Tham YS, Ben-Horin S, Eliakim R, Koulaouzidis A, et al. Capsule endoscopy, magnetic resonance enterography, and small bowel ultrasound for evaluation of postoperative recurrence in Crohn's disease: systematic review and meta-analysis. *Inflamm Bowel Dis.* (2017) 24:93–100. doi: 10.1093/ibd/izx027
51. Rispo A, Imperatore N, Testa A, Nardone OM, Luglio G, Caporaso N, et al. Diagnostic accuracy of ultrasonography in the detection of postsurgical recurrence in Crohn's disease: a systematic review with meta-analysis. *Inflamm Bowel Dis.* (2018) 24:977–88. doi: 10.1093/ibd/izy012
52. Freeman K, Willis BH, Fraser H, Taylor-Phillips S, Clarke A. Faecal calprotectin to detect inflammatory bowel disease: a systematic review and exploratory meta-analysis of test accuracy. *BMJ Open.* (2019) 9:e027428. doi: 10.1136/bmjopen-2018-027428
53. An YK, Prince D, Gardiner F, Neeman T, Linedale EC, Andrews JM, et al. Faecal calprotectin testing for identifying patients with organic gastrointestinal disease: systematic review and meta-analysis. *Med J Australia.* (2019) 211:461–7. doi: 10.5694/mja2.50384
54. Gkiouras K, Grammatikopoulou MG, Theodoridis X, Pagkalidou E, Chatzikyriakou E, Apostolidou AG, et al. Diagnostic and clinical significance of antigen-specific pancreatic antibodies in inflammatory bowel diseases: a meta-analysis. *World J Gastroenterol.* (2020) 26:246–65. doi: 10.3748/wjg.v26.i2.246
55. Dai C, Jiang M, Sun MJ, Cao Q. Fecal lactoferrin for assessment of inflammatory bowel disease activity: a systematic review and meta-analysis. *J Clin Gastroenterol.* (2020) 54:545–53. doi: 10.1097/mcg.0000000000001212
56. Treglia G, Sadeghi R, Viccaro A, Muoio B, Giovanella L. Clinical role and accuracy of 18F-FDG PET in evaluating disease activity in patients with chronic inflammatory bowel disease: an updated systematic review and a bivariate meta-analysis. *Clin Transl Imaging.* (2017) 5:303–13. doi: 10.1007/s40336-017-0234-y
57. Bollegala N, Griller N, Bannerman H, Habal M, Nguyen GC. Ultrasound vs endoscopy, surgery, or pathology for the diagnosis of small bowel Crohn's disease and its complications. *Inflamm Bowel Dis.* (2019) 25:1313–38. doi: 10.1093/ibd/izy392
58. Ye X, Wang Y, Wang HHX, Feng R, Ye Z, Han J, et al. Can fecal calprotectin accurately identify histological activity of ulcerative colitis? A meta-analysis. *Ther Adv Gastroenterol.* (2021) 14:1756284821994741. doi: 10.1177/1756284821994741
59. Alshammari MT, Stevenson R, Abdul-Aema B, Zou G, Jairath V, Radford S, et al. Diagnostic accuracy of non-invasive imaging for detection of colonic inflammation in patients with inflammatory bowel disease: a systematic review and meta-analysis. *Diagnostics (Basel).* (2021) 11:1926. doi: 10.3390/diagnostics11101926
60. Sun L, Han Y, Wang H, Liu H, Liu S, Yang H, et al. MicroRNAs as potential biomarkers for the diagnosis of inflammatory bowel disease: a systematic review and meta-analysis. *J Int Med Res.* (2022) 50:3000605221089503. doi: 10.1177/03000605221089503
61. Dai C, Jiang M, Sun MJ, Cao Q. Fecal immunochemical test for predicting mucosal healing in ulcerative colitis patients: a systematic review and meta-analysis. *J Gastroenterol Hepatol.* (2018) 33:990–7. doi: 10.1111/jgh.14121
62. Zhu C, Ma X, Xue L, Xu J, Li Q, Wang Y, et al. Small intestine contrast ultrasonography for the detection and assessment of Crohn disease: a meta-analysis. *Medicine.* (2016) 95:e4235. doi: 10.1097/md.0000000000004235
63. Kopylov U, Yung DE, Engel T, Vijayan S, Har-Noy O, Katz L, et al. Diagnostic yield of capsule endoscopy versus magnetic resonance enterography and small bowel contrast ultrasound in the evaluation of small bowel Crohn's disease: systematic review and meta-analysis. *Digest Liver Dis Off J Italian Soc Gastroenterol Italian Assoc Study Liver.* (2017) 49:854–63. doi: 10.1016/j.dld.2017.04.013
64. Cui MH, Liu YL, Yu WD, Li XY. [Diagnostic value of antineutrophil cytoplasmic antibodies in patients with ulcerative colitis: a meta-analysis]. *Zhonghua Yi Xue Za Zhi.* (2008) 88:3116–9.
65. Ince MN, Elliott DE. Effective use of the laboratory in the management of patients with inflammatory bowel diseases. *Gastroenterol Clin North Am.* (2019) 48:237–58. doi: 10.1016/j.gtc.2019.02.006
66. Nielsen OH, Vainer B, Madsen SM, Seidelin JB, Heegaard NH. Established and emerging biological activity markers of inflammatory bowel disease. *Am J Gastroenterol.* (2000) 95:359–67.

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Urinary chemerin as a potential biomarker for inflammatory bowel disease

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Purpose: Systemic levels of the adipokine chemerin are elevated in different inflammatory conditions such as inflammatory bowel disease (IBD). In IBD, chemerin protein expression in colon mucosa is induced and serum chemerin levels are increased. Aim of this study was to identify chemerin protein in human feces and/or urine and to evaluate a possible association with IBD activity.

Materials and methods: Feces and urine of 40 patients with IBD and the respective sera of 34 patients were collected. Chemerin levels were analyzed by immunoblot in feces and urine samples. In addition, enzyme-linked immunosorbent assay (ELISA) was used to measure chemerin in all urine, feces and serum samples of the patients and in urine of 17 healthy controls.

Results: Chemerin was not detectable in 80% of the human feces samples by ELISA. Chemerin in human urine was detected by immunoblot and ELISA. Compared to serum levels, urinary concentration was about 6,000-fold lower. Urinary chemerin did not differ between patients with ulcerative colitis ($n = 15$) and Crohn's disease ($n = 25$). Urinary chemerin was not related to its serum levels, did not correlate with serum C-reactive protein level and negatively correlated with serum creatinine. Of note, urinary chemerin of patients with a fecal calprotectin > 500 μg/g was significantly higher compared to patients with lower calprotectin levels and compared to healthy controls. Serum creatinine did not differ between the patient groups.

Conclusion: Urinary chemerin might present a novel non-invasive biomarker for monitoring IBD severity and clinical course.

KEYWORDS

urine biomarker, feces, calprotectin, creatinine, C-reactive protein, Crohn's disease, ulcerative colitis, inflammatory bowel disease (IBD)

Introduction

Inflammatory bowel disease (IBD) with the two main entities Crohn's disease (CD) and ulcerative colitis (UC) is a chronic inflammatory disease with rising prevalence (1–4).

Chemerin is an adipokine and functions as an attractant for immune cells. Moreover, this protein is involved in glucose metabolism, blood pressure homeostasis, and carcinogenesis (5–10). Serum chemerin levels are increased in obesity and various studies proved an association of circulating chemerin with systemic markers of inflammation (11–17).

Chemerin exerts pro- and anti-inflammatory activities. Chemerin as well as chemerin derived C-terminal peptides function as pro-resolving factors (18–21). Chemokine-like receptor 1 (CMKLR1) is the best studied chemerin receptor, so far. G-protein receptor 1 is a further functional chemerin receptor whereas chemokine (C–C motif) receptor-like 2 (CCRL2) has no signaling activity (6, 22, 23). Chemerin and CMKLR1 are abundant in intestinal epithelial cells and their expression in the epithelial barrier is associated with disease severity of IBD (24, 25). CMKLR1 knockout could, however, neither prevent nor improve dextran sulfate sodium (DSS) colitis. Accordingly, intraperitoneal application of recombinant chemerin was without any effect (24). Contrary to this study, it has been demonstrated that exogenous chemerin suppressed the polarization of macrophages from M1 to M2 type and thereby aggravated DSS colitis (25).

Chemerin deficient mice and animals with lack of intestinal epithelial cell CMKLR1 were more sensitive to microbiota-driven colon inflammation. Loss of chemerin-CMKLR1 signaling reduced expression of lactoperoxidase, which is highly abundant in colonic epithelial cells. Lactoperoxidase exerts antimicrobial effects suggesting that chemerin protected from dysbiosis in IBD (26). Interestingly, an antimicrobial activity of chemerin as well as an internal twenty amino acid chemerin peptide in epidermis has also been described (27, 28).

Circulating chemerin was higher in experimental colitis and was increased in serum of patients with CD and UC in comparison to healthy controls (24, 29, 30).

Fecal biomarkers have emerged as tools in diagnosis and monitoring the therapeutic response of IBD. Fecal calprotectin is generally used to monitor intestinal inflammation and to anticipate disease relapse in clinical practice (31, 32). This biomarker is released by granulocytes, and therefore, associated with intestinal inflammation. Thus, it is not specific for IBD (33, 34). Extending fecal calprotectin to include additional biomarkers may improve IBD diagnosis. In consideration that serum and colonic chemerin are higher in IBD (24, 25, 29, 30), fecal chemerin may become a valuable non-invasive biomarker for IBD diagnosis.

Until now, to our knowledge, there are no studies that have examined whether chemerin can be detected in feces. Urine is a further biological fluid becoming increasingly important for biomarker studies and development (35). Serum chemerin is strongly induced in patients with renal dysfunction (36), and impaired renal excretion may contribute to higher circulating chemerin levels. Chemerin protein was indeed detected in urine of rats by enzyme-linked immunosorbent assay (ELISA) (37). Rat urine has a protein content of approximately 1 g/l (38) suggesting that there is about 10 pg chemerin/µl urine. Serum chemerin of the rats was about 40 ng/ml and was 4-fold higher than urinary levels (37).

The aim of the current investigation was to study whether fecal and/or urinary chemerin has the potential to become a diagnostic non-invasive biomarker for IBD.

Materials and methods

Patients

Patients with confirmed IBD diagnosis were recruited from the outpatient and inpatient clinic at the Department of Internal Medicine I at the University Hospital of Regensburg from 06.12.2021 to 23.06.2022. IBD was diagnosed based on accepted endoscopic, histologic, and clinical criteria (39, 40). Patients who were pregnant, had known coagulopathy, or were unable to give informed consent were excluded from the study. Moreover, urine of 17 healthy controls was collected. Controls were students, hospital staff and spouse of the patients. Details of the study groups are summarized in **Table 1**.

Enzyme-linked immunosorbent assay

ELISA to measure human chemerin was from R&D Systems (Wiesbaden, Nordenstadt, Germany; Cat # DY2324). Serum was diluted 1:250 fold for analysis. Urine was centrifuged for 5 min at 4,000 rpm and was used undiluted. Fecal protein was prepared as described below and was used undiluted.

Immunoblot analysis

Immunoblot was performed as described (41). Urine as well as fecal protein was used undiluted and 16 µl were loaded

TABLE 1 Characteristics of the patients and controls.

Characteristics	Patients	Controls
Number (females/males)	40 (16/24)	17 (10/7)
Age (years)	42 (19–67)	42 (23–78)
BMI (kg/m^2)	25.0 (16–44)	n.d.
CRP (mg/l)	2.7 (0.6–144.0)	n.d.
Creatinine (mg/dl)	0.83 (0.51–1.12)	n.d.
GFR (ml/min)	100 (72–136)	n.d.
First diagnosis (years)	10 (0–42)	n.a.

BMI, Body mass index; CRP, C-reactive protein; GFR, Glomerular filtration rate; n.a., Not applicable; n.d., Not documented.

per lane. Chemerin antibody was from R&D Systems (Cat # AF2324, RRID:AB_416577). Coomassie Brilliant Blue R-250 Staining Solution was from Bio-Rad Laboratories (Feldkirchen, Germany, Cat #1610436). ImageJ was used to quantify protein levels (42).

Isolation of fecal protein

Fecal homogenates were prepared as described (43). Feces (0.5 to 2.0 g) was homogenized in phosphate buffered saline (PBS) supplemented with protease inhibitor (Protease Inhibitor Cocktail cOmplete^{TM} EDTA-free, Roche Diagnostics, Penzberg, Germany; Cat # 11836170001) by the gentleMACS Dissociator using gentleMACs M-tubes (Miltenyi Biotec, Bergisch Gladbach, Germany; Cat # 130-093-236). One ml aliquots were dried overnight in a vacuum concentrator. The homogenate was dissolved in PBS with protease inhibitor to 2 mg dry weight/ml. Material was stored at −80°C until use.

Statistical analysis

Data are presented as boxplots. Statistical differences were analyzed by Mann Whitney *U*-test or Kruskal-Wallis Test and associations between two measures were analyzed by Spearman correlation (SPSS Statistics 25.0 program, IBM, Leibniz Rechenzentrum, München, Germany), and a value of $p < 0.05$ was regarded significant.

Results

Chemerin is rarely detectable in feces

Protein isolated from human feces was used for immunoblot analysis. Chemerin could not be detected in feces of six patients analyzed by this method (**Figure 1** and data not shown). ELISA was used to measure fecal chemerin of 40 patients. Fecal chemerin levels of 80% of the patients were below the detection limit of the assay with a range from 31.2 to 2,000 pg/ml chemerin. The eight patients with fecal chemerin levels above 0 had a low median level of 35.0 (8.8–156.7) pg/ml. Fecal chemerin was not related to fecal calprotectin levels ($p = 0.533$).

Chemerin is detectable in urinary samples

Human urine was used for immunoblot analysis and chemerin was detected in twenty-one of the twenty-four samples

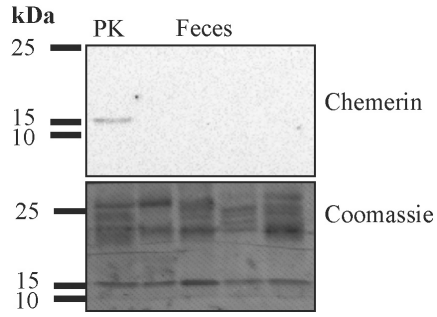

FIGURE 1
Immunoblot of feces for analysis of chemerin protein expression. After the experiment the membrane was stained with Coomassie Brilliant Blue R-250. The molecular weight size markers are given. Human liver lysate was used as positive control (PK).

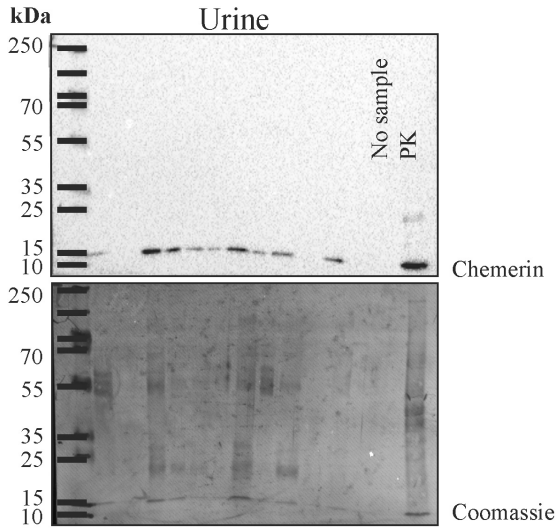

FIGURE 2
Immunoblot of urine for analysis of chemerin protein. After the experiment the membrane was stained with Coomassie Brilliant Blue R-250. The molecular weight size marker is shown and the respective molecular weights are given. Human liver lysate was used as positive control (PK).

(**Figure 2** and data not shown). Urinary chemerin had a median expression of 13.4 and ranged from 0 to 51.0.

After the immunoblot experiment, total urinary protein on the membrane was stained with Coomassie Brilliant Blue R-250 and quantified using ImageG (42; **Figure 2**). Urinary chemerin protein levels were not correlated with total urinary protein concentrations ($r = 0.262$, $p = 0.217$; **Figure 3A**). Urinary chemerin levels as determined by immunoblot did not correlate with serum chemerin measured by ELISA ($r = -0.336$, $p = 0.109$; **Figure 3B**).

Urinary chemerin concentrations in relation to serum chemerin, gender, BMI, and serum creatinine

Enzyme-linked immunosorbent assay (ELISA) was used to quantify urinary chemerin protein of 40 patients with IBD. Median chemerin protein in urine was 34 (20–1,470) pg/ml. Serum of 34 of these patients was available, and in serum chemerin was 190 (82–391) ng/ml. Thus, urinary chemerin expression was about 6,000-fold lower than serum levels. There was no correlation between urinary and serum chemerin levels ($r = -0.095$, $p = 0.593$).

Urinary chemerin levels did not differ between females and males ($p = 0.633$). Age ($r = 0.023$, $p = 0.887$) and body mass index (BMI; $r = -0.191$, $p = 0.250$) did not correlate with urinary chemerin concentration. When patients were stratified for BMI there were two patients with a BMI < 18.5 kg/m^2, 20 patients with a BMI between 18.5 and 24.9 kg/m^2, nine patients with a BMI between 25.0 and 29.9 kg/m^2, seven patients with a BMI between 30.0 and 34.9 kg/m^2, one patient with a BMI between 35.0 and 39.9 kg/m^2, and one patient with a BMI > 40 kg/m^2. Urinary chemerin expression did not significantly differ between these groups ($p = 0.330$) (**Figure 4A**).

Urinary chemerin expression negatively correlated with serum creatinine (**Figure 4B**) whereas the association with glomerular filtration rate was not significant ($r = 0.287$, $p = 0.073$).

Serum chemerin did neither correlate with serum creatinine ($r = 0.045$, $p = 0.799$) nor glomerular filtration rate ($r = 0.086$, $p = 0.627$).

Urinary chemerin in relation to fecal calprotectin and serum C-reactive protein

In the group of 40 IBD patients, 25 patients had been diagnosed with CD and 15 patients with CU. Urinary chemerin levels were comparable between the two groups (**Figure 5A**).

There was no correlation between urinary chemerin concentration and fecal calprotectin levels ($r = 0.117$, $p = 0.417$). Therefore, we analyzed patient cohorts divided according to their fecal calprotectin levels. Seventeen patients had calprotectin levels below 50 μg/g, 10 patients had calprotectin levels from 50 to 149 μg/g, 6 patients had calprotectin levels from 150 to 500 μg/g and seven patients calprotectin levels > 500 μ g/g.

Patients with fecal calprotectin < 50 μg/g were the oldest and had the lowest glomerular filtration rate (GFR). CRP was highest in patients with calprotectin > 500 μg/g (**Table 2**). Gender distribution, BMI, creatinine, and time since first diagnosis were similar between the groups (**Table 2**).

Patients with high fecal calprotectin > 500 μg/g had higher urinary chemerin levels in comparison to the three other groups with similar levels (**Figure 5B**). Urinary chemerin protein varied within the groups (11.0 to 18.4-fold in patients with fecal calprotectin < 500 μg/g) and the variation was 4 to 6-fold higher in patients with fecal calprotectin > 500 μg/g. The coefficient of variation (CV%) is a measure of relative variability and did not markedly differ between the groups (**Table 2**).

C-reactive protein (CRP) as a serum marker for inflammation correlated positively with fecal calprotectin in our cohort ($r = 0.516$, $p = 0.001$) but there was no significant association of urinary chemerin and serum CRP ($r = 0.284$, $p = 0.080$).

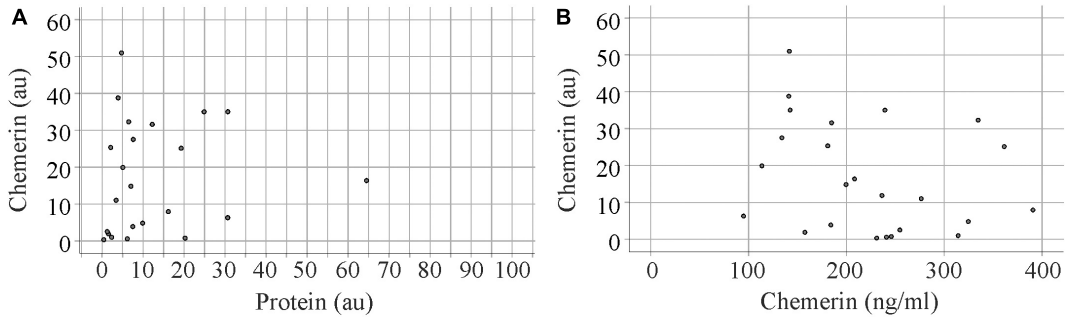

FIGURE 3
Correlation analysis of urinary chemerin protein with total protein levels and serum chemerin. **(A)** Correlation of urinary chemerin protein determined by immunoblot and total protein in urine. Data of one patient is not shown in the figure because of the high protein content of this urine sample. **(B)** Correlation of urinary chemerin protein determined by immunoblot and serum chemerin measured by enzyme-linked immunosorbent assay (ELISA).

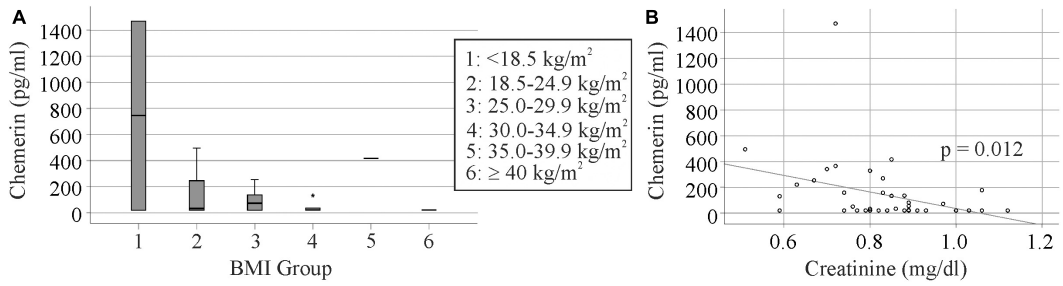

FIGURE 4
Urinary chemerin levels in relation to BMI and serum creatinine. **(A)** Urinary chemerin stratified for BMI. The asterisk marks an outlier (three box lengths from the median). **(B)** Correlation of urinary chemerin with serum creatinine.

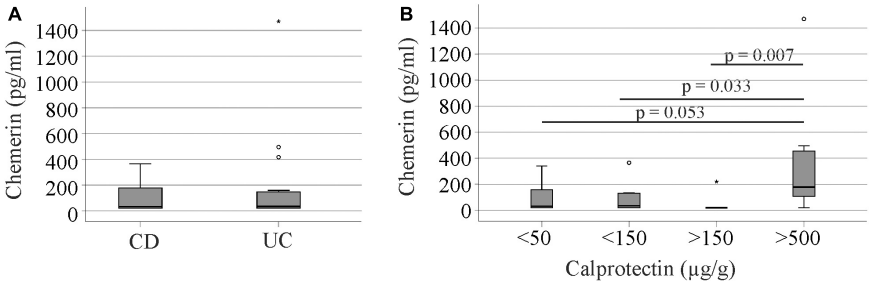

FIGURE 5
Urinary chemerin protein in Crohn's disease (CD) and ulcerative colitis (UC) patients and in relation to fecal calprotectin. **(A)** Urinary chemerin of CD and UC patients. **(B)** Urinary chemerin protein stratified for fecal calprotectin levels. Circles (1.5 box lengths from the median) and asterisks (three box lengths from the median) mark outliers.

TABLE 2 Characteristics of the patients stratified for fecal calprotectin levels.

Calprotectin µg/g	< 50	< 150	> 150	> 500	*P*-value
Number (females/males)	17 (8/9)	10 (5/5)	6 (2/4)	7 (1/6)	
Age (years)	53[a,b,c] (30–67)	34[b] (23–55)	29[c] (19–56)	28[a] (20–65)	0.038[a] 0.010[b] 0.013[c]
BMI (kg/m^2)	26 (20–44)	24 (17–35)	25 (22–32)	22 (16–40)	
CRP (mg/l)	1.9[a] (0.6–21.1)	1.2[b] (0.6–26.3)	6.7[c] (0.6–18.1)	25.0[a,b,c] (11.2–144.0)	0.001[a] 0.001[b] 0.025[c]
Creatinine (mg/dl)	0.84 (0.67–1.06)	0.78 (0.59–1.12)	0.86 (0.63–1.03)	0.80 (0.51–1.06)	
GFR (ml/min)	89[a,b,c] (72–119)	105[b] (91–120)	106[c] (95–131)	110[a] (97–136)	0.007[a] 0.020[b] 0.025[c]
First diagnosis (years)	14 (2–42)	10 (0–32)	6 (0–35)	3 (1–16)	
Chemerin pg/ml	32.9 (20.0–341.7)	35.2[a] (19.9–365.7)	20.1[b] (20.1–220.8)	178.3[a,b] (19.8–1470.3)	0.033[a] 0.007[b]
Chemerin CV%	113	127	153	126	

Median values and ranges are listed. Significant different measures were marked with identical uppercase letters (BMI, Body mass index; CV, Coefficient of variation; CRP, C-reactive protein; GFR, Glomerular filtration rate).

Serum chemerin levels positively correlated with CRP ($r = 0.321$, $p = 0.064$) though this association was not significant. Serum chemerin concentrations did not correlate with fecal calprotectin ($r = 0.124$, $p = 0.484$) and levels did not differ between patients stratified for fecal calprotectin levels ($p = 0.224$).

Histologic remission was documented for 32 patients and was achieved in nine patients. Urinary chemerin did not differ

between these groups ($p = 1.000$). When patients with fecal calprotectin > 500 μg/g were excluded, urinary chemerin levels were still similar between patients with and without histologic remission ($p = 0.215$).

Urinary chemerin concentrations of healthy controls

Urinary chemerin protein of 17 healthy controls was also measured. Gender distribution and age of controls and patients were comparable (Table 1). The 10 female and 7 male controls had similar levels of chemerin in urine ($p = 0.813$). Urinary chemerin did not correlate with age ($r = 0.130$, $p = 0.618$) Urinary chemerin protein did not differ between the controls, patients with CD or UC (Figure 6A). Controls had urinary chemerin levels similar to the patients stratified for calprotectin levels with the exception of the group with fecal calprotectin > 500 μg/g. Urinary chemerin protein of this group was significantly higher compared to the controls (Figure 6B).

Urinary chemerin in relation to complications and the current inflammatory bowel disease medication

There were 17 patients who suffered from extra-intestinal manifestations of IBD and seven patients with fistulas. Urinary chemerin did not differ in patients with and without these complications ($p = 0.812$ and $p = 0.707$, respectively).

Common medications used to treat IBD were anti-tumor necrosis factor monoclonal antibodies (10 patients), anti-p40 monoclonal antibodies (12 patients), mesalazine (15 patients) and corticosteroids (10 patients). Urinary chemerin of patients taking anti-tumor necrosis factor monoclonal antibodies ($p = 0.548$), anti-p40 monoclonal antibodies ($p = 0.079$), mesalazine ($p = 0.192$) and corticosteroids ($p = 0.914$) was similar to patients without these medications.

Discussion

This is to our knowledge the first study to detect chemerin protein in human urine samples. Urinary chemerin protein levels were higher in patients with IBD and fecal calprotectin > 500 μg/g compared to patients with lower levels and healthy controls. Thus, urinary chemerin levels may be discussed as a novel non-invasive marker for intestinal inflammation in patients with IBD.

It has been described that serum chemerin is elevated in patients with impaired renal clearance (36, 44). In the general population serum chemerin levels were inversely associated with renal function (45). We were able to detect chemerin protein in human urine samples and could show that urinary chemerin was inversely correlated with serum creatinine. This suggests that serum chemerin is partly cleared from the body by renal elimination. Urinary chemerin concentration is low and about 6,000-fold less than serum levels. A rough estimate for male rats was a 4-fold difference between serum and urine (37) suggesting that renal chemerin excretion greatly differs between humans and rats. Urinary volume of humans is about 2 l per day (46) and about 70 ng chemerin may be excreted by the kidneys. To get exact numbers it has to be clarified whether degraded chemerin, which is no longer detected by the ELISA, is also present in human urine.

Immunoblot analysis revealed that chemerin protein in urine has a molecular weight of about 15 kDa suggesting that at least part of the chemerin protein is not degraded. Chemerin in human serum is mostly inactive and C-terminal cleavage of a few amino acids produces biologic active variants (6,

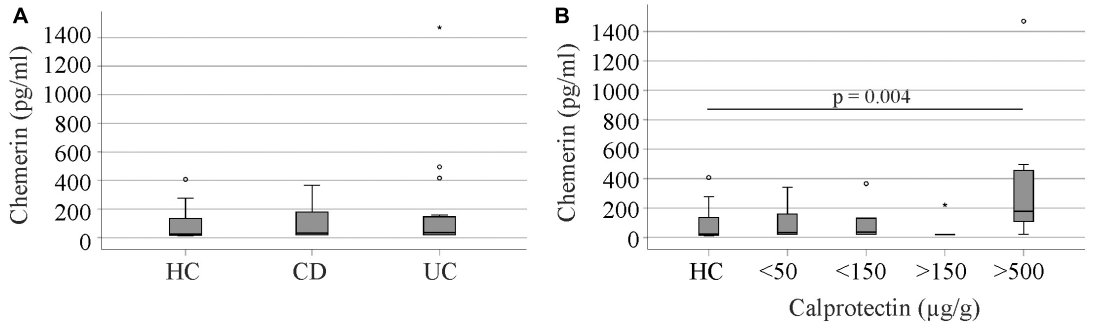

FIGURE 6
Urinary chemerin protein of healthy controls (HC), Crohn's disease (CD), and ulcerative colitis (UC) patients. **(A)** Urinary chemerin of HC, CD, and UC patients. **(B)** Urinary chemerin protein of HCs and patients stratified for fecal calprotectin levels. Circles (1.5 box lengths from the median) and asterisks (three box lengths from the median) mark outliers.

47). Activation of chemerin is achieved by several proteases including the inflammatory serine proteases tryptase, plasmin and elastase (48). So further studies should be designed to investigate which isoforms of chemerin are abundant in urine.

Also noteworthy, high inter-individual variations of urinary chemerin protein levels can be detected. Whereas serum chemerin of the patients studied varied about 5-fold (CV% 39), urinary chemerin varied about 70-fold in the whole cohort (CV% 176) and about 20-fold (CV% 123) when patients with calprotectin > 500 μg/g were excluded.

Urinary chemerin was not correlated to serum chemerin, total urinary protein content, gender, BMI or age. There was a negative correlation with serum creatinine with an about 2-fold variation, which thus cannot explain the wide range of urinary chemerin protein levels. Future studies are needed to characterize the mechanisms, which contribute to urinary chemerin concentrations.

It is important to note that fecal calprotectin ranged from 18 to 1,616 μg/g in the whole cohort and from 18 to 347 in patients with a calprotectin level < 500 μg/g. This corresponds to an about 19-fold variation in this cohort and an about 90-fold variation in the whole study group. Similar variations of fecal calprotectin in patients with IBD have been reported by others (49, 50). The CV% for calprotectin was 166% in our group and was reported to range from 48% up to 182% in other IBD cohorts (51, 52). Thus, the CV% of 176 for urinary chemerin in the patient cohort is comparable to CV% for fecal calprotectin.

Chemerin and CMKLR1 are expressed in intestinal epithelial cells and are related to local inflammation (24, 25). In contrast to this knowledge, chemerin protein was not detectable in human feces by immunoblot analysis, and was only measurable in stool of 20% of the patients by ELISA. This might be due to low biliary elimination of chemerin and/or degradation of chemerin during fecal excretion.

Interestingly, urinary chemerin was increased in IBD patients with high fecal calprotectin. The median urinary chemerin levels of patients with low fecal calprotectin was 33 pg/ml and of patients with levels > 500 μg/g calprotectin was 180 pg/ml. Serum creatinine did not differ between these two groups excluding it as a confounding factor. The CV% of urinary chemerin levels did not differ too much between the calprotectin groups.

Urinary chemerin did not increase in parallel with calprotectin levels and did not correlate with CRP suggesting that it is not simply a marker of inflammation. Although the cause for higher urinary chemerin is unknown it may serve as an additional disease activity biomarker in IBD.

Urinary chemerin was comparable between healthy controls and IBD patients with calprotectin levels below 500 μg/g. This illustrates that urinary chemerin of the patients does not increase until they develop severe IBD.

Disease activity in IBD was found associated with a higher urinary albumin (53). The etiology of microalbuminuria in patients with IBD remains unclear and is supposed to be a consequence of the acute phase response (53). Higher urinary chemerin in patients with severe IBD may thus reflect renal impairment. This suggests that urinary chemerin may be analyzed for its suitability as a biomarker for renal dysfunction in IBD.

The current study cohort was rather small and relation of urinary chemerin with disease severity, progression or remission has to be assessed in larger study groups. Whether urinary chemerin may be useful to discriminate active IBD from intestinal inflammation caused by infections, specific drugs, cancer, or diverticulitis needs further analysis.

Urinary chemerin did not differ between patients with CD or UC. It could not discriminate patients with low from patients with medium fecal calprotectin levels; nor patients with histologic remission from patients without histologic remission. Therefore, chemerin protein in urine is not of diagnostic value in this regard but may be useful as an additional biomarker to fecal calprotectin to monitor IBD disease activity. A novel urine biomarker for IBD is highly desirable for clinical application as a follow-up and disease-activity marker because it can be collected recurrently by non-invasive techniques.

Limitation of this work is the small number of patients analyzed, and thus possible differences of urinary chemerin between CD and UC patients with high fecal calprotectin could not be analyzed. Moreover, urinary chemerin was not determined during therapy to monitor response to treatment.

To summarize, present study detected chemerin in human urine and showed that urinary chemerin levels of IBD patients with high fecal calprotectin were increased. Urinary chemerin is a potential novel and easily accessible biomarker for the monitoring of IBD patients.

Data availability statement

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

Ethics statement

This study was approved by the Ethics Committee of the University Hospital of Regensburg (Protocol No. 19-1309-101, Approval date: 20.02.2019) and all participants gave written informed consent to the study. The study was performed according to the updated guidelines of good clinical practice and updated Declaration of Helsinki.

Author contributions

CB, AK, and HT: conceptualization. TF, SS, HT, TE, SG, and JL: resources. SG: investigation. CB: statistical analysis and writing—original draft preparation. All authors: writing—review and editing, critically revised the manuscript, approved the final version to be published, and agree to be accountable for all aspects of the work.

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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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References

1. Brown SJ, Mayer L. The immune response in inflammatory bowel disease. *Am J Gastroenterol.* (2007) 102:2058–69.

2. Cho JH. The genetics and immunopathogenesis of inflammatory bowel disease. *Nat Rev Immunol.* (2008) 8:458–66.

3. Ott C, Obermeier F, Thieler S, Kemptner D, Bauer A, Scholmerich J, et al. The incidence of inflammatory bowel disease in a rural region of Southern Germany: a prospective population-based study. *Eur J Gastroenterol Hepatol.* (2008) 20:917–23. doi: 10.1097/MEG.0b013e3282f97b33

4. Dahlhamer JM, Zammitti EP, Ward BW, Wheaton AG, Croft JB. Prevalence of inflammatory bowel disease among adults aged ≥18 years - United States, 2015. *MMWR Morb Mortal Wkly Rep.* (2016) 65:1166–9. doi: 10.15585/mmwr.mm6542a3

5. Bondue B, Wittamer V, Parmentier M. Chemerin and its receptors in leukocyte trafficking, inflammation and metabolism. *Cytokine Growth Factor Rev.* (2011) 22:331–8. doi: 10.1016/j.cytogfr.2011.11.004

6. Buechler C, Feder S, Haberl EM, Aslanidis C. Chemerin isoforms and activity in obesity. *Int J Mol Sci.* (2019) 20:1128. doi: 10.3390/ijms20051128

7. Fatima SS, Rehman R, Baig M, Khan TA. New roles of the multidimensional adipokine: chemerin. *Peptides.* (2014) 62:15–20. doi: 10.1016/j.peptides.2014.09.019

8. Ferland DJ, Mullick AE, Watts SW. Chemerin as a driver of hypertension: a consideration. *Am J Hypertens.* (2020) 33:975–86. doi: 10.1093/ajh/hpaa084

9. Goralski KB, Jackson AE, McKeown BT, Sinal CJ. More than an adipokine: the complex roles of chemerin signaling in cancer. *Int J Mol Sci.* (2019) 20:4778. doi: 10.3390/ijms20194778

10. Treeck O, Buechler C, Ortmann O. Chemerin and cancer. *Int J Mol Sci.* (2019) 20:3750. doi: 10.3390/ijms20153750

11. Catalan V, Gomez-Ambrosi J, Rodriguez A, Ramirez B, Rotellar F, Valenti V, et al. Increased levels of chemerin and its receptor, chemokine-like receptor-1, in obesity are related to inflammation: tumor necrosis factor-alpha stimulates mRNA levels of chemerin in visceral adipocytes from obese patients. *Surg Obes Relat Dis.* (2013) 9:306–14. doi: 10.1016/j.soard.2011.11.001

12. Ernst MC, Sinal CJ. Chemerin: at the crossroads of inflammation and obesity. *Trends Endocrinol Metab.* (2010) 21:660–7. doi: 10.1016/j.tem.2010.08.001

13. Fulop P, Seres I, Lorincz H, Harangi M, Somodi S, Paragh G. Association of chemerin with oxidative stress, inflammation and classical adipokines in non-diabetic obese patients. *J Cell Mol Med.* (2014) 18:1313–20. doi: 10.1111/jcmm.12282

14. Gu P, Jiang W, Lu B, Shi Z. Chemerin is associated with inflammatory markers and metabolic syndrome phenotypes in hypertension patients. *Clin Exp Hypertens.* (2014) 36:326–32. doi: 10.3109/10641963.2013.827697

15. Maghsoudi Z, Kelishadi R, Hosseinzadeh-Attar MJ. Association of chemerin levels with anthropometric indexes and C-reactive protein in obese and non-obese adolescents. *ARYA Atheroscler.* (2015) 11:102–8.

16. Weigert J, Neumeier M, Wanninger J, Filarsky M, Bauer S, Wiest R, et al. Systemic chemerin is related to inflammation rather than obesity in type 2 diabetes. *Clin Endocrinol (Oxf).* (2010) 72:342–8.

17. Zylla S, Pietzner M, Kuhn JP, Volzke H, Dorr M, Nauck M, et al. Serum chemerin is associated with inflammatory and metabolic parameters-results of a population-based study. *Obesity (Silver Spring).* (2017) 25:468–75. doi: 10.1002/oby.21735

18. Cash JL, Hart R, Russ A, Dixon JP, Colledge WH, Doran J, et al. Synthetic chemerin-derived peptides suppress inflammation through ChemR23. *J Exp Med.* (2008) 205:767–75. doi: 10.1084/jem.20071601

19. Cash JL, Norling LV, Perretti M. Resolution of inflammation: targeting GPCRs that interact with lipids and peptides. *Drug Discov Today.* (2014) 19:1186–92. doi: 10.1016/j.drudis.2014.06.023

20. Yoshimura T, Oppenheim JJ. Chemerin reveals its chimeric nature. *J Exp Med.* (2008) 205:2187–90. doi: 10.1084/jem.20081736

21. Mariani F, Roncucci L. Chemerin/chemR23 axis in inflammation onset and resolution. *Inflamm Res.* (2015) 64:85–95. doi: 10.1007/s00011-014-0792-7

22. De Henau O, Degroot GN, Imbault V, Robert V, De Poorter C, McHeik S, et al. Signaling properties of chemerin receptors CMKLR1, GPR1 and CCRL2. *PLoS One.* (2016) 11:e0164179. doi: 10.1371/journal.pone.0164179

23. Mattern A, Zellmann T, Beck-Sickinger AG. Processing, signaling, and physiological function of chemerin. *IUBMB Life.* (2014) 66:19–26. doi: 10.1002/iub.1242

24. Dranse HJ, Rourke JL, Stadnyk AW, Sinal CJ. Local chemerin levels are positively associated with DSS-induced colitis but constitutive loss of CMKLR1 does not protect against development of colitis. *Physiol Rep.* (2015) 3:e12497. doi: 10.14814/phy2.12497

25. Lin YYX, Yue W, Xu X, Li B, Zou L, He R. Chemerin aggravates DSS-induced colitis by suppressing M2 macrophage polarization. *Cell Mol Immunol.* (2014) 11:355–66. doi: 10.1038/cmi.2014.15

26. Lin Y, Cai Q, Luo Y, Li B, Chen Y, Yang X, et al. Epithelial chemerin-CMKLR1 signaling restricts microbiota-driven colonic neutrophilia and tumorigenesis by up-regulating lactoperoxidase. *Proc Natl Acad Sci USA.* (2022) 119:e2205574119. doi: 10.1073/pnas.2205574119

27. Banas M, Zabieglo K, Kasetty G, Kapinska-Mrowiecka M, Borowczyk J, Drukala J, et al. Chemerin is an antimicrobial agent in human epidermis. *PLoS One.* (2013) 8:e58709. doi: 10.1371/journal.pone.0058709

28. Godlewska U, Bilska B, Zegar A, Brzoza P, Borek A, Murzyn K, et al. The antimicrobial activity of chemerin-derived peptide p4 requires oxidative conditions. *J Biol Chem.* (2019) 294:1267–78. doi: 10.1074/jbc.RA118.005495

29. Terzoudis S, Malliaraki N, Damilakis J, Dimitriadou DA, Zavos C, Koutroubakis IE. Chemerin, visfatin, and vaspin serum levels in relation to bone mineral density in patients with inflammatory bowel disease. *Eur J Gastroenterol Hepatol.* (2016) 28:814–9. doi: 10.1097/MEG.0000000000000617

30. Weigert J, Obermeier F, Neumeier M, Wanninger J, Filarsky M, Bauer S, et al. Circulating levels of chemerin and adiponectin are higher in ulcerative colitis and chemerin is elevated in Crohn's disease. *Inflamm Bowel Dis.* (2010) 16:630–7. doi: 10.1002/ibd.21091

31. Shi JT, Zhang Y, She Y, Goyal H, Wu ZQ, Xu HG. Diagnostic utility of non-invasive tests for inflammatory bowel disease: an umbrella review. *Front Med (Lausanne).* (2022) 9:920732. doi: 10.3389/fmed.2022.920732

32. Mao R, Xiao YL, Gao X, Chen BL, He Y, Yang L, et al. Fecal calprotectin in predicting relapse of inflammatory bowel diseases: a meta-analysis of prospective studies. *Inflamm Bowel Dis.* (2012) 18:1894–9. doi: 10.1002/ibd.22861

33. Guo X, Huang C, Xu J, Xu H, Liu L, Zhao H, et al. Gut microbiota is a potential biomarker in inflammatory Bowel disease. *Front Nutr.* (2021) 8:818902. doi: 10.3389/fnut.2021.818902

34. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ.* (2010) 341:c3369. doi: 10.1136/bmj.c3369

35. Harpole M, Davis J, Espina V. Current state of the art for enhancing urine biomarker discovery. *Expert Rev Proteomics.* (2016) 13:609–26. doi: 10.1080/14789450.2016.1190651

36. Bonomini M, Pandolfi A. Chemerin in renal dysfunction and cardiovascular disease. *Vascul Pharmacol.* (2016) 77:28–34. doi: 10.1016/j.vph.2015.10.007

37. Abais-Battad JM, Lund H, Fehrenbach DJ, Dasinger JH, Alsheikh AJ, Mattson DL. Parental dietary protein source and the role of CMKLR1 in determining the severity of dahl salt-sensitive hypertension. *Hypertension.* (2019) 73:440–8. doi: 10.1161/HYPERTENSIONAHA.118.11994

38. Evans GO, Parsons CE. Potential errors in the measurement of total protein in male rat urine using test strips. *Lab Anim.* (1986) 20:27–31. doi: 10.1258/002367786781062089

39. Kucharzik T, Dignass A, Siegmund BZ. Aktualisierung der S3-leitlinie colitis ulcerosa 2019. *Gastroenterology.* (2019) 57:1279–80. doi: 10.1055/a-1015-7048

40. Sturm A, Maaser C, Calabrese E, Annese V, Fiorino G, Kucharzik T, et al. ECCO-ESGAR guideline for diagnostic assessment in IBD part 2: IBD scores and general principles and technical aspects. *J Crohns Colitis.* (2019) 13:273–84. doi: 10.1093/ecco-jcc/jjy114

41. Bauer S, Wanninger J, Schmidhofer S, Weigert J, Neumeier M, Dorn C, et al. Sterol regulatory element-binding protein 2 (SREBP2) activation after excess triglyceride storage induces Chemerin in hypertrophic adipocytes. *Endocrinology.* (2011) 152:26–35. doi: 10.1210/en.2010-1157

42. Schneider CA, Rasband WS, Eliceiri KW. NIH image to imageJ: 25 years of image analysis. *Nat Methods.* (2012) 9:671–5.

43. Schott HF, Krautbauer S, Horing M, Liebisch G, Matysik SA. Validated, fast method for quantification of sterols and gut microbiome derived 5alpha/beta-stanols in human feces by isotope dilution LC-high-resolution MS. *Anal Chem.* (2018) 90:8487–94. doi: 10.1021/acs.analchem.8b01278

44. Lin S, Teng J, Li J, Sun F, Yuan D, Chang J. Association of chemerin and vascular endothelial growth factor (VEGF) with diabetic nephropathy. *Med Sci Monit.* (2016) 22:3209–14. doi: 10.12659/msm.896781

45. Zylla S, Rettig R, Volzke H, Endlich K, Nauck M, Friedrich N. Serum chemerin levels are inversely associated with renal function in a general population. *Clin Endocrinol (Oxf).* (2018) 88:146–53. doi: 10.1111/cen.13449

46. Bernard J, Song L, Henderson B, Tasian GE. Association between daily water intake and 24-hour urine volume among adolescents with kidney stones. *Urology.* (2020) 140:150–4. doi: 10.1016/j.urology.2020.01.024

47. Rourke JL, Dranse HJ, Sinal CJ. Towards an integrative approach to understanding the role of chemerin in human health and disease. *Obes Rev.* (2013) 14:245–62. doi: 10.1111/obr.12009

48. Ferland DJ, Watts SW. Chemerin: a comprehensive review elucidating the need for cardiovascular research. *Pharmacol Res.* (2015) 99:351–61. doi: 10.1016/j.phrs.2015.07.018

49. Vitali R, Palone F, Armuzzi A, Fulci V, Negroni A, Carissimi C, et al. Proteomic analysis identifies three reliable biomarkers of intestinal inflammation in the stools of patients with Inflammatory Bowel disease. *J Crohns Colitis.* (2022) 30:jjac110. doi: 10.1093/ecco-jcc/jjac110 [Epub ahead of print].

50. Swaminathan A, Borichevsky GM, Edwards TS, Hirschfeld E, Mules TC, Frampton CM, et al. Faecal myeloperoxidase as a biomarker of endoscopic activity in inflammatory Bowel disease. *J Crohns Colitis.* (2022) 8:jjac098. doi: 10.1093/ecco-jcc/jjac098 [Epub ahead of print].

51. Han X, Ordouie AA, Schmelz R, Hummel T. Chemosensory functions in patients with inflammatory bowel disease and their association with clinical disease activity. *Nutrients.* (2022) 14:3543. doi: 10.3390/nu14173543

52. Moein S, Qujeq D, Vaghari Tabari M, Kashifard M, Hajian-Tilaki K. Diagnostic accuracy of fecal calprotectin in assessing the severity of inflammatory bowel disease: from laboratory to clinic. *Caspian J Intern Med.* (2017) 8:178–82. doi: 10.22088/cjim.8.3.178

53. Mahmud N, Stinson J, O'Connell MA, Mantle TJ, Keeling PW, Feely J, et al. Microalbuminuria in inflammatory Bowel disease. *Gut.* (1994) 35:1599–604. doi: 10.1136/gut.35.11.1599

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Constructing a prediction model of inflammatory bowel disease recurrence based on factors affecting the quality of life

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Aim: This study aimed to determine the factors affecting the quality of life of patients with inflammatory bowel disease (IBD) and to construct a disease recurrence prediction model based on these influencing factors.

Methods: A prospective, single-center study in China was conducted between October 2020 and March 2021. The quality of life of patients was assessed using the Inflammatory Bowel Disease Questionnaire (IBDQ). Multiple stepwise regression analysis was used to analyze the factors influencing the quality of life of patients with IBD. The chi-square test and the point-biserial correlation analysis were performed to identify factors associated with clinical recurrence. A binary logistic regression model was constructed to predict the recurrence. The receiver operating characteristic curve was used to evaluate the prediction model. Patients with IBD from April 2021 to June 2021 were randomly included for model verification to evaluate the disease recurrence prediction model.

Results: The average IBDQ score of patients with IBD was 172.2 ± 35.0 (decreased by 23.2%). The scores of all dimensions of the IBDQ were decreased, especially emotional function and systemic symptoms. Disease activity, age, extraintestinal manifestations (EIMs), and annual household income were important factors influencing the IBDQ scores of patients with ulcerative colitis, and these accounted for ~57.0% of the factors affecting the quality of life. Disease activity, EIMs, and occupational stress were important factors influencing the IBDQ scores of patients with Crohn's disease, and they accounted for approximately 75.1% of the factors affecting the quality of life. Annual household income, occupational stress, and IBDQ scores were independent risk factors for recurrence. The area under the curve of the recurrence prediction model was 81.1%. The sensitivity and specificity were 81.7 and 71.7%, respectively. The Youden index of the model was 0.534. The established recurrence prediction model has good discriminant validity in the validation cohort.

Conclusion: The quality of life of patients with IBD was generally poor. The use of factors affecting the quality of life to predict disease recurrence has high predictive value and can support the management of IBD by selecting patients at a higher risk for relapse.

KEYWORDS

inflammatory bowel disease, quality of life, influencing factors, recurrence, prediction model

Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract that is believed to be caused by an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host. The most common forms of IBD include ulcerative colitis (UC) and Crohn's disease (CD) (1). IBD is a global disease, and its incidence and prevalence are still increasing worldwide (2, 3). The incidence of IBD in China is generally lower than that in developed countries, such as Western Europe and North America, but epidemiological studies show a significant increase in the incidence of IBD in China (4). The most common clinical symptoms of IBD are abdominal pain, diarrhea, and bloody stools, often accompanied by extraintestinal manifestations (EIMs). Some patients also suffer from complications such as abscesses, fistulas, and stenosis (5).

Due to its chronic, relapsing nature and therapeutic complexity, IBD requires long-term medical management and imposes significant costs on individuals and society. Several studies have reported that patients with IBD have lower employment rates and a higher percentage of work disability than the general population (6). With long-term medical care and productivity loss, the economic burden of patients with IBD is increasing (7). In addition, IBD has been shown to have a psychological impact on daily life, with a significant increase in the incidence of psychological disorders (8). This psychological load adds to the physical burden of the disease and is associated with direct and indirect costs (9). In short, the physical, economic, and psychological burden, as well as the progression of the disease, all affect the quality of life of patients (10, 11). A previous study revealed that one-third of patients with UC relapse within the first year of diagnosis and 70–82% of the patients relapse within 5 years. Additionally, 85% of patients with CD relapse at least one time within 5 years from diagnosis (10). Since patients with severe relapse need intensive treatment, which may accrue high costs and increase the risk of adverse events, detection of relapse and early therapeutic interventions before the severe progression of activity are desirable (12). The current treatment strategies for patients with IBD not only alleviate symptoms and reduce complications but also improve the quality of life and reduce recurrence rates (13).

In the past few years, more attention has been paid to the role of age, C-reactive protein (CRP), fecal calprotectin (FC), erythrocyte sedimentation rate (ESR), endoscopy, EIMs, diet, and pathological scores in the prediction of recurrence (14–17). However, the predictive values of these different parameters in identifying patients at risk of recurrence have been disappointing. Some studies indicated that standard laboratory parameters (e.g., CRP) did not prove to be useful predictors of clinical relapse in IBD as a whole (18, 19). In those studies, the predictive value of FC in patients with UC with clinical remission for relapse was not so prominent (AUC = 0.60–0.70) (20). The predictability of recurrence in CD has also been reported, and the ability to predict recurrence at 1 year was slightly higher than that in UC (AUC = 0.75–0.79) (21, 22). However, due to its relatively high cost, FC is not so frequently measured in patients. In addition, endoscopy for this purpose is sometimes invasive and burdensome for patients (12).

Therefore, prediction tools for disease recurrence need to be developed. The construction of a prediction model of recurrence based on the factors affecting the quality of life of patients with IBD is a new exploration. Based on data from an IBD center in Southwest China, we reported factors influencing the quality of life of patients with IBD and constructed a model to predict disease recurrence, which is of great value for understanding the characteristics of patients with IBD in China and improving their quality of life and outcomes.

Methods

Patients

This study was performed in the First Affiliated Hospital of Kunming Medical University, which is a tertiary hospital and an IBD center in Southwest China. We recruited patients with IBD who were hospitalized between October 2020 and March 2021 as a training cohort. Patients with IBD diagnosed from April 2021 to June 2021 were selected to establish a validation cohort. The inclusion criteria were as follows: (1) the diagnosis was confirmed according to the ECCO guidelines (23, 24) and (2) patients gave informed consent. The exclusion criteria were as follows: (1) patients with severe cognitive and mental disorders and (2) patients with other chronic serious diseases, such as heart, kidney, or liver failure, stroke, and serious lung disease. We followed the participants for 1 year and identified recurrence through telephone appointments and outpatient visits. The process for patient inclusion and exclusion is presented in Figure 1.

Data collection

Demographics, including age, gender, nature of occupation, occupational stress, education level, marital status, family size, annual family income, and type of medical insurance, were obtained by a general information questionnaire. Clinical information, including diagnosis, disease activity, disease localization (the Montreal classification of IBD) (25), disease duration, EIMs (e.g., peripheral and axial arthritis, pyoderma gangrenosum, erythema nodosum, Sweet syndrome, aphthous stomatitis, primary sclerosing cholangitis and episcleritis, anterior uveitis, and iritis), complications (e.g., intestinal obstruction or perforation, massive hemorrhage of the gastrointestinal tract, colon cancer, or toxic megacolon), intestinal surgery, and biologics (e.g., infliximab, vedolizumab, adalimumab, and ustekinumab) were collected from medical records. The disease activity of UC was assessed using the Mayo scores (a Mayo score of ≤ 2 with no single item score of >1 was classified as clinical remission, ranging between 3 and 5 as mild activity, ranging between 6 and 10 as moderate activity, and ranging between 11 and 12 as severe activity) (26). Clinical relapse was defined as the occurrence of symptoms accompanied by an increase in the partial Mayo score of 2 or more, which required a change in therapy, such as the escalation of ongoing therapy with the introduction of steroids and/or immunosuppressive biological drugs (27). The Crohn's disease activity index (CDAI) was used to assess disease activity

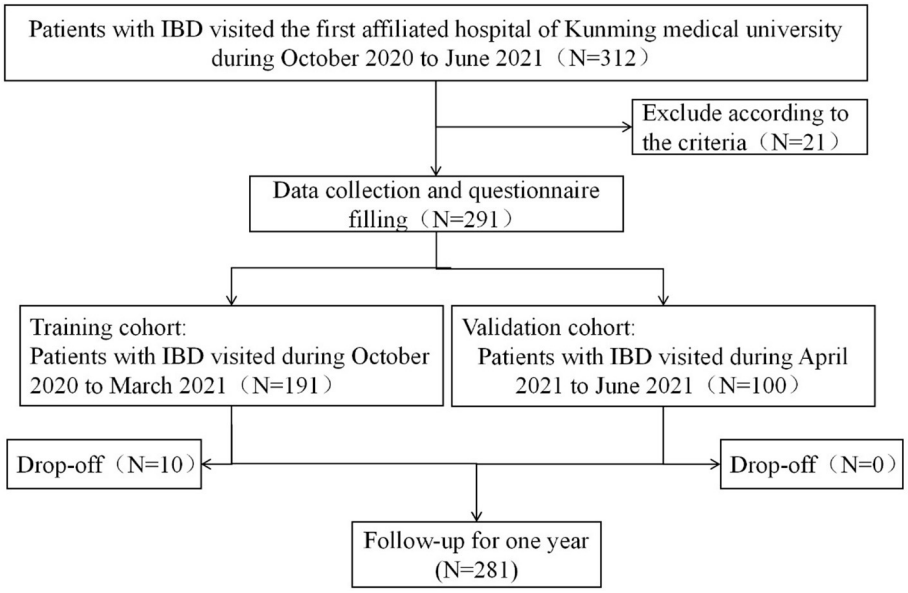

FIGURE 1
Flowchart summarizing the patients' selection process.

in CD (CDAI < 150 was classified as remission, 150 to 220 as mild activity, 221 to 450 as moderate activity, and >450 as severe activity) (28). Recurrence was defined as a CDAI of more than 220, an increase of at least 70 from the baseline between 150 and 220, or the need for a surgical procedure (29).

The quality of life of the patients was assessed using the Chinese translation of an Italian version of the Inflammatory Bowel Disease Questionnaire (IBDQ) (30), which is a widely recognized disease-specific questionnaire used to measure the quality of life. The IBDQ consists of 32 questions and four subscales, namely, bowel symptoms (e.g., bloody stools, abdominal pain), systemic symptoms (e.g., fatigue, sleep problems), emotional function (e.g., anxiety, anger, and depression), and social function (e.g., limited social activities, school, or work attendance). The score of each question ranges from 1 point (worst condition) to 7 points (best condition), with a total score ranging from 32 to 224, with a higher score indicating a better quality of life. All patients were asked to complete the IBDQ.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences, version 26.0 for Mac (IBM SPSS Statistics 26). Continuous variables were presented as the mean ± standard deviation (SD), and the counting data were expressed as frequencies and percentages. The Mann–Whitney U-tests were used to compare the mean values between the two groups. The mean values of multiple groups were compared using a one-way analysis of variance. Factors affecting the quality of life were analyzed by multiple linear stepwise regression. The chi-square test and point-biserial correlation analysis were used to screen relapse-related factors, and binary logistic regression analysis was used to construct predictive models of relapse. To assess the performance of the resulting predictions, receiver operating characteristic (ROC) curves were plotted to calculate the area under the curve (AUC). A *P*-value of <0.05 was considered statistically significant.

Results

Demographic and clinical characteristics of patients with IBD

A total of 212 questionnaires were distributed in the training cohort. Questionnaires were considered invalid if any question was unanswered. Finally, a total of 191 valid questionnaires were recovered, yielding a response rate of 90.1% (191/212). UC (132, 69.1%) was the most common type, followed by CD (55, 28.8%) and IBD-U (4, 2.1%). We only analyzed the data of patients with UC and CD to explore factors affecting the quality of life and recurrence. The demographic and clinical characteristics of patients are shown in Table 1. Among the patients with UC, 80 of them (60.6%) were men. The average age was 43.7 ± 12.8 years. The median duration of the disease was 6 years. EIMs occurred in 58 (43.9%) patients and 17 (12.9%) patients had complications. Among the patients with CD, 38 of them (69.1%) were men. The average age was 36.0 ± 13.5 years. The median duration of the disease was 7 years. A total of 24 (43.6%) patients had EIMs. Complications occurred in 24 (43.6%) patients.

General characteristics of patients with IBD

In the training cohort (Table 2), 41 (31.1%) patients with UC were engaged in manual labor and 19

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TABLE 1 Demographic and clinical characteristics of patients with inflammatory bowel disease.

Items		Training cohort			Verification cohort		
		IBD ($n = 191$)	UC ($n = 132$)	CD ($n = 55$)	IBD ($n = 100$)	UC ($n = 61$)	CD ($n = 39$)
Sex, *n* (%)	Male	118 (61.8)	80 (60.6)	38 (69.1)	63 (63.0)	38 (62.3)	25 (64.1)
	Female	73 (38.2)	52 (39.4)	17 (30.9)	37 (37.0)	23 (37.7)	14 (35.9)
Age, *n* (%)							
	0–18	5 (2.6)	1 (0.8)	4 (7.3)	4 (4.0)	0 (0.0)	4 (10.3)
	18–35	66 (34.6)	39 (29.5)	26 (47.3)	41 (41.0)	20 (32.8)	21 (53.8)
	36–60	102 (53.4)	79 (59.8)	22 (40.0)	41 (41.0)	29 (47.5)	12 (30.8)
	>60	18 (9.4)	13 (9.8)	3 (5.5)	14 (14.0)	12 (19.7)	2 (5.1)
Disease course (year), *n* (%)							
	<5	85 (44.5)	62 (47.0)	20 (36.4)	58 (58.0)	37 (60.7)	21 (53.8)
	5–10	54 (28.3)	38 (28.8)	15 (27.3)	25 (25.0)	14 (23.0)	11 (28.2)
	>10	52 (27.2)	32 (24.2)	20 (36.4)	17 (17.0)	10 (16.4)	7 (17.9)
Disease activity, *n* (%)							
	Remission	17 (8.9)	7 (5.3)	9 (16.4)	5 (5.0)	0 (0.0)	5 (12.8)
	Mild	38 (19.9)	31 (23.5)	7 (12.7)	30 (30.0)	17 (27.9)	13 (33.3)
	Moderate	80 (41.9)	59 (44.7)	18 (32.7)	40 (40.0)	26 (42.6)	14 (35.9)
	Severe	56 (29.3)	35 (26.5)	21 (38.2)	25 (25.0)	18 (29.5)	7 (17.9)
Bowel resection, *n* (%)		30 (15.7)	1 (0.8)	28 (50.9)	33 (33.0)	10 (16.4)	23 (59.0)
Extraintestinal manifestations, *n* (%)		84 (44.0)	58 (43.9)	24 (43.6)	61 (61.0)	39 (63.9)	22 (56.4)
Complications, *n* (%)		41 (21.5)	17 (12.9)	24 (43.6)	57 (57.0)	34 (55.7)	23 (59.0)
Biological agents, *n* (%)		24 (12.6)	9 (6.8)	15 (27.3)	42 (42.0)	18 (29.5)	24 (61.5)
Disease location, *n* (%)							
(Montreal classification)	E1		29 (22.0)			14 (23.0)	
	E2		39 (29.5)			11 (18.0)	
	E3		64 (48.5)			36 (59.0)	
	L1			35 (63.6)			21 (53.8)
	L2			11 (20.0)			10 (25.6)
	L3			8 (14.5)			8 (20.5)
	L4			1 (1.8)			0 (0.0)
	B1			45 (81.8)			34 (87.2)
	B2			7 (12.7)			4 (10.3)
	B3			3 (5.5)			1 (2.6)
	Perianal lesions			10 (18.2)			12 (30.8)

IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; and EIMS, extraintestinal manifestations.

(14.4%) patients with UC felt that their occupation was stressful. The annual household income of 25 patients (18.9%) was <RMB¥ 10,000. Among the patients with CD, the occupation of 19 (34.5%) patients was manual labor, and 11 (20.0%) patients felt that their occupation was stressful. The annual household income of 11 patients (20.0%) with CD was <RMB¥ 10,000.

Quality of life scores in patients with IBD

We used the IBDQ to investigate the quality of life of patients with IBD (Table 3). The results showed that all dimensions of the IBDQ were decreased, with the total scores reduced by 23.2%. The score of systemic symptoms decreased the most. There were no significant differences in the score of intestinal symptoms, systemic symptoms, emotional functioning, and social functioning between

TABLE 2 General characteristics of patients with inflammatory bowel disease.

Items	Training cohort			Verification cohort		
	IBD ($n = 191$)	UC ($n = 132$)	CD ($n = 55$)	IBD ($n = 100$)	UC ($n = 61$)	CD ($n = 39$)
Family size, *n* (%)						
0–3	91 (47.6)	68 (51.5)	23 (41.8)	40 (40.0)	21 (34.4)	19 (48.7)
4–5	80 (41.9)	53 (40.2)	23 (41.8)	41 (41.0)	26 (42.6)	15 (38.5)
>5	20 (10.5)	11 (8.3)	9 (16.4)	19 (19.0)	14 (23.0)	5 (12.8)
Marital status, *n* (%)						
Unmarried	34 (17.8)	15 (11.4)	19 (34.5)	31 (31.0)	10 (16.4)	21 (53.8)
Married	146 (76.4)	108 (81.8)	35 (63.6)	66 (66.0)	48 (78.7)	18 (46.2)
Divorced	8 (4.2)	7 (5.3)	1 (1.8)	2 (2.0)	2 (3.3)	0 (0.0)
Death of a spouse	3 (1.6)	2 (1.5)	0 (0.0)	1 (1.0)	1 (1.6)	0 (0.0)
Education background, *n* (%)						
Primary school or below	27 (14.1)	12 (9.1)	14 (25.5)	13 (13.0)	13 (21.3)	0 (0.0)
Middle school and high school	65 (34.0)	50 (37.9)	13 (23.6)	38 (38.0)	23 (37.7)	15 (38.5)
College degree	92 (48.2)	67 (50.8)	24 (43.6)	43 (43.0)	22 (36.1)	21 (53.8)
Graduate degree	6 (3.1)	3 (2.3)	3 (5.5)	5 (5.0)	2 (3.3)	3 (7.7)
Uneducated	1 (0.5)	0 (0.0)	1 (1.8)	1 (1.0)	1 (1.6)	0 (0.0)
Medical insurance, *n* (%)						
Worker's health insurance	90 (47.1)	68 (51.5)	21 (38.2)	41 (41.0)	22 (36.1)	19 (48.7)
Medical insurance for urban residents	20 (10.5)	14 (10.6)	6 (10.9)	8 (8.0)	4 (6.6)	4 (10.3)
Commercial insurance	1 (0.5)	1 (0.8)	0 (0.0)	1 (1.0)	1 (1.6)	0 (0.0)
Student health insurance	4 (2.1)	2 (1.5)	2 (3.6)	2 (2.0)	1 (1.6)	1 (2.6)
Rural health care	72 (37.7)	43 (32.6)	26 (47.3)	48 (48.0)	33 (54.1)	15 (38.5)
Without health insurance	4 (2.1)	4 (3.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Profession, *n* (%)						
Manual labor	63 (33.0)	41 (31.1)	19 (34.5)	32 (32.0)	24 (39.3)	8 (20.5)
Mental work	66 (34.6)	49 (37.1)	16 (29.1)	31 (31.0)	15 (24.6)	16 (41.0)
Both manual labor and mental work	62 (32.5)	42 (31.8)	20 (36.4)	37 (37.0)	22 (36.1)	15 (38.5)
Occupational stress, *n* (%)						
Relaxed	39 (20.4)	25 (18.9)	11 (20.0)	19 (19.0)	12 (19.7)	7 (17.9)
Standard	122 (63.9)	88 (66.7)	33 (60.0)	58 (58.0)	35 (57.4)	23 (59.0)
Stressful	30 (15.7)	19 (14.4)	11 (20.0)	23 (23.0)	14 (23.0)	9 (23.1)
Annual household income, *n* (%)						
RMB¥ 10,000 or less	37 (19.4)	25 (18.9)	11 (20.0)	25 (25.0)	16 (26.2)	9 (23.1)
RMB¥ 10,000–50,000	67 (35.1)	46 (34.8)	21 (38.2)	36 (36.0)	25 (41.0)	11 (28.2)
>RMB¥ 50,000	87 (45.5)	61 (46.2)	23 (41.8)	39 (39.0)	20 (32.8)	19 (48.7)

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

TABLE 3 Quality of life scores of patients with inflammatory bowel disease.

Dimensions	Total scores	Scores (decline rate %)			P^a
		IBD	UC	CD	
Bowel symptoms	10–70	55.8 ± 11.3 (20.2)	55.2 ± 11.1 (21.1)	56.7 ± 11.8 (18.6)	0.336
Systemic symptoms	5–35	24.9 ± 6.4 (28.9)	24.7 ± 6.2 (29.4)	25.0 ± 6.8 (28.6)	0.783
Emotional functioning	12–84	63.5 ± 12.5 (24.4)	63.3 ± 12.3 (24.7)	63.4 ± 13.2 (24.6)	0.967
Social functioning	5–35	27.9 ± 7.3 (20.3)	28.0 ± 7.2 (20.0)	27.4 ± 7.7 (21.6)	0.649
IBDQ total scores	32–224	172.2 ± 35.0 (23.2)	172.2 ± 34.4 (23.1)	172.8 ± 36.9 (22.9)	0.781

IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; and IBDQ, Inflammatory Bowel Disease Questionnaire. [a]The difference between ulcerative colitis and Crohn's disease; A P-value of <0.05 was considered statistically significant.

UC and CD patients. It showed that the quality of life of patients with IBD was decreased but was not affected by the type of disease.

Univariate and multivariate analyses of factors affecting the quality of life of patients with UC and CD

This study analyzed the factors influencing the quality of life of patients with UC and CD (Table 4). The related factors with a P-value of < 0.1 in univariate analysis were included in the multiple regression analyses. Disease activity, age, EIMs, and annual household income were the factors that affected the total score of the IBDQ in patients with UC. Several factors affect intestinal symptoms, systemic symptoms, emotional function, and social function in patients with UC to varying degrees. Disease activity, EIMs, and occupational stress were the factors affecting the total scores and scores of all four dimensions of the IBDQ in patients with CD.

Factors associated with the recurrence in patients with IBD

To analyze the factors associated with the recurrence of IBD in patients and construct a prediction model for disease recurrence, 191 patients with IBD in the training cohort were followed up for 1 year. In this period, these participants included 82 patients with recurrence, 99 patients without recurrence, and 10 (5.2%) patients lost to follow-up. The chi-square test and point-biserial correlation analysis were used to screen relapse-related factors. We included age, EIMs, profession, biological agents, complications, disease activity, annual household income, occupational stress, and IBDQ scores for analysis. Finally, several potential predictors for relapse were identified in our cohort. Disease activity, annual household income, occupational stress, and IBDQ scores were found to be associated with short-term recurrence in patients with IBD (Table 5).

The development of a model to predict recurrence

The relapse-related factors were included in the binary logistic regression analysis. The Hosmer–Lemeshow test was used to test the model fit and goodness, and the results showed that the model fit and goodness were good (P = 0.529). The linear predictor was 5.036 + (-0.026) × IBDQ + 1.976 × annual household income (RMB¥ 10,000 or less) + 0.898 × annual household income (RMB¥ 10,000 50,000) + (-2.067) × occupational stress (relaxed) + (-1.609) × occupational stress (standard) (Table 6). Predicted probabilities were calculated using the linear predictor in the formula: $1/(1+e^{-\text{linear predictor}})$. When the P-value was >0.5, the patient was predicted to relapse. In this study, the model correctly predicted 72.9% of patient outcomes. The results revealed that 79.8% of the patients without recurrence predicted by the model did not have a recurrence and that 64.6% of the patients with recurrence predicted by the model had a recurrence.

The ability to predict recurrence in patients with IBD

The discriminant ability was evaluated according to the AUC (Figure 2; Table 7). The results indicated that the predictive effect of the model was better than that of the independent predictors, and the predictive effect of the IBDQ scores on recurrence was better than that of annual household income and occupational stress. The AUC of the prediction model of this study was 81.1%, and the 95% confidence interval (CI) was 74.8–87.5%. The maximum value of the Youden index (a measure of the authenticity of a screening test, also known as the correctness index) was the best critical value of the prediction model. The Youden index of this study was 0.534. The sensitivity and specificity were 81.7 and 71.7%, respectively. The model could be considered to have a good discriminant effect.

Verification of the model

Another 100 patients with IBD diagnosed in the hospital from April 2021 to June 2021 were selected to establish a validation group. The demographic, clinical, and general characteristics of the patients in the validation cohort are shown in Tables 1, 2. Among these, 38 patients had recurrence within 1 year of follow-up and 62 patients had no recurrence. The successful modeling formula from the first stage was used to predict recurrence, with a sensitivity of 81.6%, a specificity of 83.9%, and an accuracy of 85.0%. The positive predictive value was 81.6%. The negative predictive value was 87.1%. The false-negative rate was 13.7%, and the false-positive rate was 20.5%. The ROC curve was drawn by the same method.

TABLE 4 Univariate analysis and multivariate stepwise regression analysis of factors affecting the quality of life of patients with ulcerative colitis and Crohn's disease.

Dimensions	Ulcerative colitis					Crohn's disease				
	Univariate analysis[a]	Multivariate analysis[b]				Univariate analysis[a]	Multivariate analysis[b]			
	P	R^2	β	t	*P*	*P*	R^2	β	t	*P*
IBDQ total scores		0.570					0.751			
Disease activity	<0.001		−0.656	−10.978	<0.001	<0.001		−0.674	−9.119	<0.001
Age	0.002		0.186	3.143	0.002	0.526				
Extraintestinal manifestations	0.022		0.135	2.228	0.024	0.001		0.253	3.440	0.001
Annual household income	0.064		0.124	2.118	0.036	0.016		0.067	0.904	0.370
Occupational stress	0.131					0.035		−0.251	−3.541	0.001
Profession	0.344					0.011		−0.101	−1.285	0.205
Education background	0.259					0.044		−0.045	−0.632	0.530
Complications	0.701					0.022		−0.029	−0.371	0.712
Bowel symptoms		0.473					0.607			
Disease activity	<0.001		−0.628	−9.714	<0.001	<0.001		−0.588	−6.339	<0.001
Age	0.001		0.203	3.149	0.002	0.360				
Extraintestinal manifestations	0.061		0.117	1.835	0.069	0.002		0.253	2.744	0.008
Occupational stress	0.335					0.064		−0.227	−2.540	0.014
Complications	0.721					0.046		−0.044	−0.451	0.654
Annual household income	0.150					0.008		0.108	1.175	0.246
Profession	0.252					0.022		−0.111	−1.115	0.270
Systemic symptoms		0.445					0.700			
Disease activity	<0.001		−0.667	−10.202	<0.001	<0.001		−0.641	−7.912	<0.001
Age	0.017		0.081	1.234	0.220	0.198				
Extraintestinal manifestations	0.043		0.117	1.790	0.076	0.001		0.259	3.213	0.002
Occupational stress	0.084		−0.097	−1.470	0.144	0.003		−0.244	−3.132	0.003
Complications	0.092		−0.109	−1.680	0.095	0.029		−0.018	−0.211	0.834
Profession	0.406					0.019		−0.046	−0.525	0.602
Annual household income	0.111					0.033		0.029	0.355	0.724
Education background	0.268					0.085		−0.038	−0.478	0.635
Emotional functioning		0.557					0.668			
Disease activity	<0.001		−0.668	−11.134	<0.001	<0.001		−0.654	−7.673	<0.001
Age	0.001		0.202	3.392	0.001	0.792				
Extraintestinal manifestations	0.033		0.118	1.983	0.050	0.005		0.213	2.509	0.015
Occupational stress	0.088		−0.090	−1.498	0.137	0.062		−0.229	−2.795	0.007
Complications	0.698					0.074		−0.017	−0.187	0.852
Profession	0.206					0.018		−0.157	−1.754	0.085
Annual household income	0.143					0.041		0.061	0.716	0.477
Education background	0.356					0.016		- 0.093	- 1.133	0.262
Social functioning		0.468					0.684			
Disease activity	<0.001		−0.531	−7.948	<0.001	< 0.001		−0.643	−7.721	<0.001
Annual household income	0.034		0.177	2.706	0.008	0.064		0.024	0.282	0.779

(Continued)

TABLE 4 (Continued)

Dimensions	Ulcerative colitis					Crohn's disease				
	Univariate analysis[a]	Multivariate analysis[b]				Univariate analysis[a]	Multivariate analysis[b]			
	P	R^2	β	t	*P*	*P*	R^2	β	t	*P*
Extraintestinal manifestations	0.018		0.162	2.467	0.015	0.002		0.231	2.794	0.007
Age	0.040		0.165	2.422	0.017	0.538				
Biological agents	0.050		0.206	3.062	0.003	0.601				
Occupational stress	0.212					0.043		−0.251	−3.140	0.003
Profession	0.648					0.004		−0.005	−0.060	0.952
Complications	0.870					0.013		−0.026	−0.298	0.767

[a]Univariate analysis was performed using Mann–Whitney U-tests and one-way analysis of variance. [b]Multivariate analysis included variables with $P < 0.1$ in the univariate analysis for multiple stepwise regression analysis. A *P*-value of <0.05 was considered statistically significant.

TABLE 5 Factors associated with recurrence.

Variables	X^2/r	*P*
Disease activity	18.195	<0.001
Annual household income	21.708	<0.001
Occupational stress	16.488	<0.001
IBDQ total scores	−0.411	<0.001
Age	4.052	0.256
Extraintestinal manifestations	2.383	0.123
Complications	3.910	0.059
Profession	4.693	0.096
Biological agents	1.023	0.312

IBDQ, Inflammatory Bowel Disease Questionnaire.

The results revealed that the AUC was 87.4%, and the 95% CI was 79.9–94.8%. The Youden index was 0.655 (Figure 3). This indicated that the established recurrence prediction model also had good discriminant validity in the validation cohort.

Discussion

Inflammatory bowel disease (IBD) is comprehensively affected by environmental factors, individual genotypes, intestinal microecology, autoimmunity, and other factors. Prognosis and recurrence prediction are of great significance for disease control. In recent years, the quality of life and social psychology of patients with IBD has drawn extensive concern. Due to the differences in clinical manifestations, treatment options, economic situation, cultural backgrounds, and lifestyles of patients with IBD, studies from other countries cannot reflect the quality of life and short-term recurrence of patients with IBD in China. In this study, patients with IBD in a tertiary hospital in Southwest China were selected as the research subjects. The results showed that the quality of life of patients with IBD was generally poor. Moreover, a prediction model of disease recurrence based on factors affecting the quality of life had a high predictive value. This study is of great significance for understanding the characteristics and quality of life of patients with IBD in China. The model established in this study can effectively guide treatment monitoring and follow-up.

The chronic progressive course, the side effects associated with medications, and the increasing financial burden reduce the quality of life of patients with IBD. A recent systematic review found that the quality of life of patients with IBD is worse than that of healthy people (31). We also found that patients with IBD had decreased quality of life in all dimensions, especially emotional functioning and systemic symptoms. This suggests that the therapeutic goals of IBD are not only mucosal healing but also an improved quality of life. Traditionally, patients with CD were believed to have a poorer quality of life than patients with UC due to worse disease behavior (32–34). Our study did not show a statistical difference between the two conditions. This may be because frequent diarrhea and bloody stool in patients with UC lead to lower intestinal symptom scores, and these symptoms are more likely to affect the physical health and social activities of the patients. In addition, patients with CD had a higher proportion of biologics than patients with UC in our study. Although our data revealed no significant association between biologics and the quality of life, Zhang et al. (35) found that the use of biologics had a positive effect on the quality of life of patients with CD. The differences may be due to the heterogeneity of patients and studies. Most patients treated with biologics have severe or complex conditions, and their emotional experiences may be completely different in the early stages of treatment and the period after disease control. This suggests that both UC and CD seriously affect the quality of life. Multivariate analysis showed that the independent factors influencing the quality of life of patients with UC were disease activity, annual household income, EIMs, and age, which explained ∼57.0% of the quality of life score. However, disease activity, occupational stress, and EIMs were independent factors influencing the quality of life of patients with CD, accounting for ∼75.1%.

In our study, disease activity affecting all dimensions of the IBDQ was an independent predictor of poor IBDQ scores, and the amount of explanation reached 50.7% (UC) and 62.3% (CD), respectively. This finding was consistent with other studies (36, 37). Zhao et al. (38) found that high disease activity scores were associated with the early recurrence of IBD after fecal bacteria

TABLE 6 Associations between influencing factors and relapse of inflammatory bowel disease using binary logistic regression[a].

Variables	B value	SE	*P*	OR	95%cCI
Occupational stress (Relaxed)[b]	−2.067	0.674	0.001	0.13	0.04–0.45
Occupational stress (Standard)[c]	−1.609	0.536	0.003	0.20	0.07–0.57
Annual household income (10,000 or less CNY)[d]	1.976	0.513	< 0.001	7.22	2.64–19.74
Annual household income (10,000–50,000 CNY)[e]	0.898	0.408	0.028	2.46	1.10–5.46
IBDQ total scores	−0.026	0.01	0.006	0.97	0.96–0.99
Constant	5.036	1.46	0.001	153.91	

B-value, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval; IBDQ, Inflammatory Bowel Disease Questionnaire; [a]Multivariable analysis included variables with a *P*-value of <0.1 in the univariate analyses; [b]occupational stress (relaxed) vs. occupational stress (stressful); [c]occupational stress (standard) vs. occupational stress (stressful); [d]annual household income (10,000 or less CNY) vs. annual household income (>50,000 CNY); and [e]annual household income (10,000–50,000 CNY) vs. annual household income (>50,000 CNY).

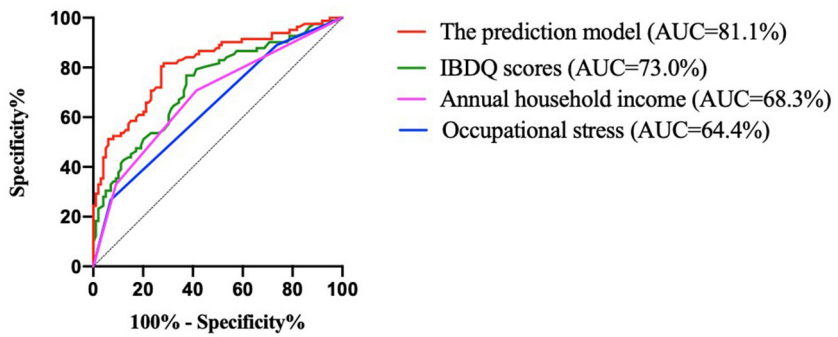

FIGURE 2
ROC curves for the developed model for predicting recurrence and variables. IBDQ, Inflammatory Bowel Disease Questionnaire; and AUC, area under the curve.

TABLE 7 Predictive power of each variable and prediction model for recurrence.

Variables	AUC	*P*	95% CI	Youden index	Sensitivity	Specificity
Occupational stress	64.4%	0.001	0.563–0.724	0.197	0.268	0.929
Annual household income	68.3%	<0.001	0.604–0.761	0.293	0.707	0.586
IBDQ scores	73.0%	<0.001	0.656–0.804	0.394	0.768	0.626
Model	81.1%	<0.001	0.748–0.875	0.534	0.817	0.717

AUC, area under the curve; CI, confidence interval; and IBDQ, Inflammatory Bowel Disease Questionnaire.

transplantation. Our data also confirmed that disease activity was associated with the recurrence of IBD. Patients in remission or relapse experience different levels of anxiety, depression, sleep problems, and stress (39). Effective treatment and psychological intervention can reduce disability and improve the quality of life of patients with IBD (40). The reported prevalence of EIMs ranges from 6 to 47% (41). These EIMs regularly result in significant morbidity in patients with IBD, even more so than the intestinal disease itself (42). We found that EIMs simultaneously affected the four dimensions of the quality of life of patients with CD and affected the total IBDQ scores by reducing the social functioning scores in patients with UC. Ott et al. (43) also found that EIMs significantly influence the quality of life. Multidisciplinary management of IBD and EIMs can simultaneously improve outcomes and the quality of life (44). Therefore, disease activity and EIMs should be assessed in patients with IBD on a regular basis as prevention and/or specific treatment can have a major benefit on patients' quality of life and outcomes. Although it was recognized that elderly patients have poorer physiological conditions and higher treatment risks, univariate analysis reported that age could affect several dimensions of the IBDQ in patients with UC, and it was confirmed that age could affect the total IBDQ score in multivariate analysis. Advanced age did positively impact the quality of life of patients with UC, but it was not evident in CD. Perera et al. also confirmed that advanced age did not have a negative impact on the health-related quality of life of patients with IBD (45). The elderly population may receive more social and family support to cope with the condition. We found that low annual household income reduced total IBDQ scores in patients with UC. Two studies, by Liu et al. (46) and Yoon et al. (47), found that annual income was an independent predictor of reduced quality of life of patients with IBD. In addition, low annual household income was found to be a risk factor for disease recurrence in our predictive model. This may be because

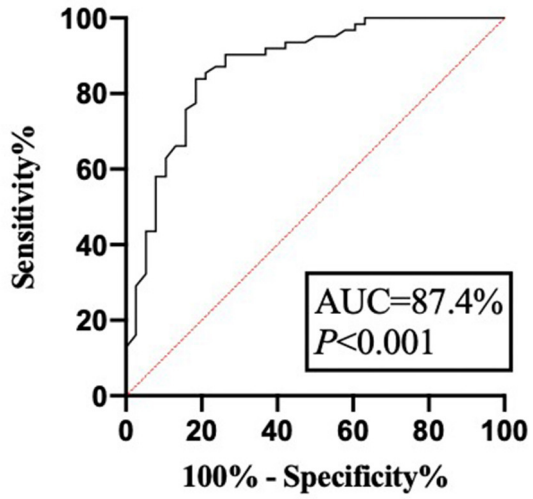

FIGURE 3
The ROC curve of model verification. AUC, area under the curve.

patients with low income experienced more stress and had fewer treatment options available. Patients with IBD are often impaired in their ability to be employed due to their morbidity. A study from Germany showed that more than half of the patients with IBD had a negative subjective prognosis for employment and experienced daily work-related problems, including reduced work ability, fear of not being fully productive, and work stress (48). Occupational stress was associated with the total IBDQ scores reflecting a decrease in all dimensions in patients with CD based on our results. Occupational stress was an independent risk factor for disease recurrence. Compared to patients with high occupational stress, patients with the standard [OR 0.20 (0.07–0.57)] and relaxed [OR 0.13 (0.04–0.45)] occupational stress had a lower risk of recurrence. For this complex condition, rehabilitation programs and support services that meet patients' needs should be implemented for work-related problems.

At least ∼10–50% of patients with CD undergo one or more surgical procedures during their lifetime and ∼5–10% of patients with UC require surgery within 5 years (10, 49). Studies suggest that surgery and the use of biologics were associated with the quality of life of patients with IBD (47, 50). Another study found that lower education levels and socioeconomic levels were related factors for the poor quality of life of patients with IBD (51). Our multivariate analysis did not show that intestinal surgery or biologics were independent predictors of the quality of life. This can be explained by the low rate of surgery and the use of biologics. In univariate analysis, we found that complications, profession, and educational background could affect several dimensions of scores of the IBDQ, but there was no statistical significance in multivariate analysis. Several studies have reported a significant correlation between the quality of life and the duration of disease, indicating better quality of life with a greater duration of IBD (50, 52, 53). Knowles et al. argued that, although symptoms persisted, patients no longer reacted negatively to them, viewing this condition as the new normal (54). This change reflects a process of self-adaptation to the chronic condition (55). Our results did not indicate a relationship between the duration of diseases and the quality of life. This may be because the time of our study was concentrated, and long-term longitudinal changes in patients were not visible.

Previous studies rarely considered the impact of disease burden on the recurrence of IBD. In this study, binary logistic regression analysis indicated that the quality of life, occupational stress, and annual household income were independent risk factors for recurrence, among which the quality of life was the most significant factor affecting recurrence. The higher the IBDQ scores, the lower the recurrence probability. Previous studies suggested that relapse-prone patients had a lower quality of life than patients in long-term remission (56, 57). This study found that patients with poor quality of life were more likely to relapse in the short term. These psychological factors will not trigger IBD, but they may have a negative effect on the progression and recurrence of the disease (58, 59). Patients with IBD with lower quality of life and higher occupational stress may be more likely to experience more anxiety, depression, sleep problems, and stress, and thus be more likely to relapse. Studies revealed that the medication compliance of patients with IBD is generally poor, ranging from 25.0 to 40.9%. Poor treatment compliance was closely related to recurrence (60, 61). Low annual household income may be a key factor affecting the treatment compliance of patients, resulting in a relatively high recurrence rate in the short term. The prediction model established by the risk factors affecting the quality of life had a good discriminative effect and could effectively screen for a population at high risk of recurrence. Our study analyzed the four dimensions of the IBDQ and found that they were affected by different factors. Compared with CRP, FC, ESR, endoscopy, or pathology scores, the model established based on the factors influencing the quality of life in this study is more practical, non-invasive, and cost-effective. Clinicians should pay more attention to the quality of life, occupational stress, and low income of patients with IBD. Early detection of patients at high risk of recurrence, as well as enhanced education and follow-up, will have a positive effect on disease control, show an improvement in the quality of life, and lead to a reduction of recurrence in patients with IBD.

This study has several limitations. The long-term quality of life and recurrence rate were not available during the observation period of this study. Due to the differences in socioeconomic factors, the cutoff values of factors affecting the quality of life, such as income, need to be adjusted. In addition, questionnaire participants in this study were mainly young and middle-aged, with the elderly and children being underrepresented. To reduce the selection bias, the study randomly investigated hospitalized patients with IBD at an IBD center and a tertiary hospital in Southwest China. Therefore, the results of the study are still reliable.

In conclusion, quality of life and recurrence rate are prognostic indicators of patients with IBD. The prediction model of recurrence based on factors affecting the quality of life can support the management of IBD by selecting patients at a higher risk of relapse. Interventions targeting the four dimensions of quality of life can be beneficial in reducing relapse and improving the quality of life of patients with IBD. Subsequently, longitudinal studies on the quality of life throughout treatment and after interventions can provide effective social support approaches and optimal management strategies for patients with IBD.

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 study involving human participants was approved by the Ethics Committee of Kunming Medical University, Kunming, Yunnan province, China (the approval number: KMMU20192032). Informed consent was obtained from all the included patients.

Author contributions

JN, YM, ML, and YT contributed to the study design. ML, YT, YS, JW, FZ, YW, MG, JY, HL, and XB wrote and revised the manuscript. JN and YM reviewed the manuscript. All the authors have read 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.

Publisher's note

References

1. Park J, Park S, Lee SA, Park SJ, Cheon JH. Improving the care of inflammatory bowel disease (IBD) patients: perspectives and strategies for IBD center management. *Korean J Intern Med.* (2021) 36:1040–8. doi: 10.3904/kjim.2021.114

2. Coward S, Clement F, Benchimol EI, Bernstein CN, Avina-Zubieta JA, Bitton A, et al. Past and future burden of inflammatory bowel diseases based on modeling of population-based data. *Gastroenterology.* (2019) 156:1345–53.e4. doi: 10.1053/j.gastro.2019.01.002

3. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet.* (2017) 390:2769–78. doi: 10.1016/S0140-6736(17)32448-0

4. Cui G, Yuan A. A systematic review of epidemiology and risk factors associated with Chinese inflammatory bowel disease. *Front Med.* (2018) 5:183. doi: 10.3389/fmed.2018.00183

5. Guo BJ, Bian ZX, Qiu HC, Wang YT, Wang Y. Biological and clinical implications of herbal medicine and natural products for the treatment of inflammatory bowel disease. *Ann Ny Acad Sci.* (2017) 1401:37–48. doi: 10.1111/nyas.13414

6. Netjes JE, Rijken M. Labor participation among patients with inflammatory bowel disease. *Inflamm Bowel Dis.* (2013) 19:81–91. doi: 10.1002/ibd.22921

7. Kawalec P. Indirect costs of inflammatory bowel diseases: crohn's disease and ulcerative colitis. a systematic review. *Arch Med Sci.* (2016) 12:295–302. doi: 10.5114/aoms.2016.59254

8. Lo B, Julsgaard M, Vester-Andersen MK, Vind I, Burisch J. Disease activity, steroid use and extraintestinal manifestation are associated with increased disability in patients with inflammatory bowel disease using the inflammatory bowel disease disability index: a cross-sectional multicentre cohort study. *Eur J Gastroen Hepat.* (2018) 30:1130–6. doi: 10.1097/MEG.0000000000001199

9. Marrie RA, Graff LA, Fisk JD, Patten SB, Bernstein CN. The relationship between symptoms of depression and anxiety and disease activity in IBD over time. *Inflamm Bowel Dis.* (2021) 27:1285–93. doi: 10.1093/ibd/izaa349

10. Zhao M, Gönczi L, Lakatos PL, Burisch J. The burden of inflammatory bowel disease in Europe in 2020. *J Crohns Colitis.* (2021) 15:1573–87. doi: 10.1093/ecco-jcc/jjab029

11. Aladraj H, Abdulla M, Guraya SY, Guraya SS. Health-related quality of life of patients treated with biological agents and new small-molecule drugs for moderate to severe crohn's disease: a systematic review. *J Clin Med.* (2022) 11:3743. doi: 10.3390/jcm11133743

12. Kato J, Yoshida T, Hiraoka S. Prediction of treatment outcome and relapse in inflammatory bowel disease. *Expert Rev Clin Immu.* (2019) 15:667–77. doi: 10.1080/1744666X.2019.1593140

13. Turner D, Ricciuto A, Lewis A, D'Amico F, Dhaliwal J, Griffiths AM, et al. STRIDE-II: an update on the selecting therapeutic targets in inflammatory bowel disease (STRIDE) initiative of the international organization for the study of IBD (IOIBD): determining therapeutic goals for treat-to-target strategies in IBD. *Gastroenterology.* (2021) 160:1570–83. doi: 10.1053/j.gastro.2020.12.031

14. Fukuda T, Naganuma M, Sugimoto S, Nanki K, Mizuno S, Mutaguchi M, et al. The risk factor of clinical relapse in ulcerative colitis patients with low dose 5-aminosalicylic acid as maintenance therapy: a report from the IBD registry. *PLoS ONE.* (2017) 12:e0187737. doi: 10.1371/journal.pone.0187737

15. Bots SJ, Kuin S, Ponsioen CY, Gecse KB, Duijvestein M, D'Haens GR, et al. Relapse rates and predictors for relapse in a real-life cohort of IBD patients after discontinuation of anti-TNF therapy. *Scand J Gastroentero.* (2019) 54:281–8. doi: 10.1080/00365521.2019.1582693

16. Connelly TM, Messaris E. Predictors of recurrence of Crohn's disease after ileocolectomy: a review. *World J Gastroentero.* (2014) 20:14393–406. doi: 10.3748/wjg.v20.i39.14393

17. Liverani E, Scaioli E, Digby RJ, Bellanova M, Belluzzi A. How to predict clinical relapse in inflammatory bowel disease patients. *World J Gastroentero.* (2016) 22:1017–33. doi: 10.3748/wjg.v22.i3.1017

18. Langhorst J, Boone J, Lauche R, Rueffer A, Dobos G. Faecal lactoferrin, calprotectin, PMN-elastase, CRP, and white blood cell count as indicators for mucosal healing and clinical course of disease in patients with mild to moderate ulcerative colitis: post hoc analysis of a prospective clinical trial. *J Crohns Colitis.* (2016) 10:786–94. doi: 10.1093/ecco-jcc/jjw044

19. Däbritz J, Langhorst J, Lügering A, Heidemann J, Mohr M, Wittkowski H, et al. Improving relapse prediction in inflammatory bowel disease by neutrophil-derived S100A12. *Inflamm Bowel Dis.* (2013) 19:1130–8. doi: 10.1097/MIB.0b013e318280b1cd

20. De Vos M, Louis EJ, Jahnsen J, Vandervoort JG, Noman M, Dewit O, et al. Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. *Inflamm Bowel Dis.* (2013) 19:2111–7. doi: 10.1097/MIB.0b013e31829b2a37

21. Naismith GD, Smith LA, Barry SJ, Munro JI, Laird S, Rankin K, et al. A prospective evaluation of the predictive value of faecal calprotectin in quiescent Crohn's disease. *J Crohns Colitis.* (2014) 8:1022–9. doi: 10.1016/j.crohns.2014.01.029

22. García-Sánchez V, Iglesias-Flores E, González R, Gisbert JP, Gallardo-Valverde JM, González-Galilea A, et al. Does fecal calprotectin predict relapse in patients with Crohn's disease and ulcerative colitis? *J Crohns Colitis.* (2010) 4:144–52. doi: 10.1016/j.crohns.2009.09.008

23. Magro F, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis part 1: definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. *J Crohns Colitis.* (2017) 11:649–70. doi: 10.1093/ecco-jcc/jjx008

24. Gomollón F, Dignass A, Annese V, Tilg H, Van Assche G, Lindsay JO, et al. 3rd European evidence-based consensus on the diagnosis and management of crohn's disease 2016: part 1: diagnosis and medical management. *J Crohns Colitis.* (2017) 11:3–25. doi: 10.1093/ecco-jcc/jjw168

25. Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. *Gut.* (2006) 55:749–53. doi: 10.1136/gut.2005.082909

26. D'Haens G, Sandborn WJ, Feagan BG, Geboes K, Hanauer SB, Irvine EJ, et al. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology.* (2007) 132:763–86. doi: 10.1053/j.gastro.2006.12.038

27. Scaioli E, Sartini A, Bellanova M, Campieri M, Festi D, Bazzoli F, et al. Eicosapentaenoic acid reduces fecal levels of calprotectin and prevents relapse in patients with ulcerative colitis. *Clin Gastroenterol Heptol.* (2018) 16:1268–75.e2. doi: 10.1016/j.cgh.2018.01.036

28. Best WR, Becktel JM, Singleton JW, Kern F. Development of a Crohn's disease activity index. national cooperative crohn's disease study. *Gastroenterology.* (1976) 70:439–44. doi: 10.1016/S0016-5085(76)80163-1

29. Bourreille A, Cadiot G, Le Dreau G, Laharie D, Beaugerie L, Dupas JL, et al. Saccharomyces boulardii does not prevent relapse of Crohn's disease. *Clin Gastroenterol H.* (2013) 11:982–7. doi: 10.1016/j.cgh.2013.02.021

30. Ciccocioppo R, Klersy C, Russo ML, Valli M, Boccaccio V, Imbesi V, et al. Validation of the Italian translation of the inflammatory bowel disease questionnaire. *Dig Liver Dis.* (2011) 43:535–41. doi: 10.1016/j.dld.2010.12.014

31. Knowles SR, Graff LA, Wilding H, Hewitt C, Keefer L, Mikocka-Walus A. Quality of life in inflammatory bowel disease: a systematic review and meta-analyses-part I. *Inflamm Bowel Dis.* (2018) 24:742–51. doi: 10.1093/ibd/izx100

32. Marinelli C, Savarino E, Inferrera M, Lorenzon G, Rigo A, Ghisa M, et al. Factors influencing disability and quality of life during treatment: a cross-sectional study on IBD patients. *Gastroent Res Pract.* (2019) 2019:5354320. doi: 10.1155/2019/5354320

33. Casellas F, López-Vivancos J, Casado A, Malagelada JR. Factors affecting health related quality of life of patients with inflammatory bowel disease. *Qual Life Res.* (2002) 11:775–81. doi: 10.1023/A:1020841601110

34. Casellas F, López Vivancos J, Malagela JR. Previous experience and quiality of life in patients with inflammatory bowel disease during relapse. *Rev Esp Enferm Dig.* (2003) 95:476–9.

35. Zhang CK, Hewett J, Hemming J, Grant T, Zhao H, Abraham C, et al. The influence of depression on quality of life in patients with inflammatory bowel disease. *Inflamm Bowel Dis.* (2013) 19:1732–9. doi: 10.1097/MIB.0b013e318281f395

36. Gower-Rousseau C, Sarter H, Savoye G, Tavernier N, Fumery M, Sandborn WJ, et al. Validation of the inflammatory bowel disease disability index in a population-based cohort. *Gut.* (2017) 66:588–96. doi: 10.1136/gutjnl-2015-310151

37. Soares JB, Pereira R, Costa JM, Arroja B, Gonçalves R. The inflammatory bowel disease-disability index: validation of the portuguese version according to the cosmin checklist. *Eur J Gastroen Hepat.* (2016) 28:1151–60. doi: 10.1097/MEG.0000000000000701

38. Zhao D, Ye C, Zhang S, Lv X, Yang B. Analysis of risk factors for early clinical recurrence of inflammatory bowel disease after fecal microbiota transplantation. *Am J Transl Res.* (2021) 13:12875–86.

39. Larsson K, Lööf L, Rönnblom A, Nordin K. Quality of life for patients with exacerbation in inflammatory bowel disease and how they cope with disease activity. *J Psychosom Res.* (2008) 64:139–48. doi: 10.1016/j.jpsychores.2007.10.007

40. van Gennep S, Evers SW, Rietdijk ST, Gielen ME, de Boer NKH, Gecse KB, et al. High disease burden drives indirect costs in employed inflammatory bowel disease patients: the WORK-IBD study. *Inflamm Bowel Dis.* (2021) 27:352–63. doi: 10.1093/ibd/izaa082

41. Greuter T, Rieder F, Kucharzik T, Peyrin-Biroulet L, Schoepfer AM, Rubin DT, et al. Emerging treatment options for extraintestinal manifestations in IBD. *Gut.* (2021) 70:796–802. doi: 10.1136/gutjnl-2020-322129

42. Rogler G, Singh A, Kavanaugh A, Rubin DT. Extraintestinal manifestations of inflammatory bowel disease: current concepts, treatment, and implications for disease management. *Gastroenterology.* (2021) 161:1118–32. doi: 10.1053/j.gastro.2021.07.042

43. Ott C, Schölmerich J. Extraintestinal manifestations and complications in IBD. *Nat Rev Gastro Hepat.* (2013) 10:585–95. doi: 10.1038/nrgastro.2013.117

44. Luchetti MM, Benfaremo D, Bendia E, Bolognini L, Fava G, Marini F, et al. Clinical and patient reported outcomes of the multidisciplinary management in patients with inflammatory bowel disease-associated spondyloarthritis. *Eur J Intern Med.* (2019) 64:76–84. doi: 10.1016/j.ejim.2019.04.015

45. Perera LP, Bhandari S, Liu R, Guilday C, Zadvornova Y, Saeian K, et al. Advanced age does not negatively impact health-related quality of life in inflammatory bowel disease. *Digest Dis Sci.* (2018) 63:1787–93. doi: 10.1007/s10620-018-5076-6

46. Liu R, Tang A, Wang X, Shen S. Assessment of quality of life in Chinese patients with inflammatory bowel disease and their caregivers. *Inflamm Bowel Dis.* (2018) 24:2039–47. doi: 10.1093/ibd/izy099

47. Yoon JY, Shin JE, Park SH, Park DI, Cha JM. Disability due to inflammatory bowel disease is correlated with drug compliance, disease activity, and quality of life. *Gut Liver.* (2017) 11:370–6. doi: 10.5009/gnl16422

48. Langbrandtner J, Steimann G, Reichel C, Bokemeyer B, Hüppe A. Inflammatory bowel disease - challenges in the workplace and support for coping with disease. *Rehabilitation.* (2022) 61:97–106. doi: 10.1055/a-1581-6497

49. Adamina M, Bonovas S, Raine T, Spinelli A, Warusavitarne J, Armuzzi A, et al. ECCO guidelines on therapeutics in crohn's disease: surgical treatment. *J Crohns Colitis.* (2020) 14:155–68. doi: 10.1093/ecco-jcc/jjz187

50. Burisch J, Weimers P, Pedersen N, Cukovic-Cavka S, Vucelic B, Kaimakliotis I, et al. Health-related quality of life improves during one year of medical and surgical treatment in a European population-based inception cohort of patients with inflammatory bowel disease–an ECCO-EpiCom study. *J Crohns Colitis.* (2014) 8:1030–42. doi: 10.1016/j.crohns.2014.01.028

51. Habibi F, Habibi ME, Gharavinia A, Mahdavi SB, Akbarpour MJ, Baghaei A, et al. Quality of life in inflammatory bowel disease patients: a cross-sectional study. *J Res Med Sci.* (2017) 22:104. doi: 10.4103/jrms.JRMS_975_16

52. Blondel-Kucharski F, Chircop C, Marquis P, Cortot A, Baron F, Gendre JP, et al. Health-related quality of life in Crohn's disease: a prospective longitudinal study in 231 patients. *AmJ Gastroenterol.* (2001) 96:2915–20. doi: 10.1111/j.1572-0241.2001.04681.x

53. McCombie AM, Mulder RT, Gearry RB. Coping strategies and psychological outcomes of patients with inflammatory bowel disease in the first 6 months after diagnosis. *Inflamm Bowel Dis.* (2015) 21:2272–80. doi: 10.1097/MIB.0000000000000476

54. Knowles SR, Keefer L, Wilding H, Hewitt C, Graff LA, Mikocka-Walus A. Quality of life in inflammatory bowel disease: a systematic review and meta-analyses-part II. *Inflamm Bowel Dis.* (2018) 24:966–76. doi: 10.1093/ibd/izy015

55. Stanton AL, Revenson TA, Tennen H. Health psychology: psychological adjustment to chronic disease. *Annu Rev Psychol.* (2007) 58:565–92. doi: 10.1146/annurev.psych.58.110405.085615

56. Luo XP, Mao R, Chen BL, Qiu Y, Zhang SH, He Y, et al. Over-reaching beyond disease activity: the influence of anxiety and medical economic burden on health-related quality of life in patients with inflammatory bowel disease. *Patient Prefer Adherence.* (2017) 11:23–31. doi: 10.2147/PPA.S118589

57. Moradkhani A, Beckman LJ, Tabibian JH. Health-related quality of life in inflammatory bowel disease: psychosocial, clinical, socioeconomic, and demographic predictors. *J Crohns Colitis.* (2013) 7:467–73. doi: 10.1016/j.crohns.2012.07.012

58. Neuendorf R, Harding A, Stello N, Hanes D, Wahbeh H. Depression and anxiety in patients with inflammatory bowel disease: a systematic review. *J Psychosom Res.* (2016) 87:70–80. doi: 10.1016/j.jpsychores.2016.06.001

59. Stone ML, Feng M, Forster EM. COVID-19 Pandemic increased anxiety among patients with inflammatory bowel disease: a patient survey in a tertiary referral center. *Digest Dis Sci.* (2022) 67:2876–81. doi: 10.1007/s10620-021-07095-y

60. Mitra D, Hodgkins P, Yen L, Davis KL, Cohen RD. Association between oral 5-ASA adherence and health care utilization and costs among patients with active ulcerative colitis. *BMC Gastroenterol.* (2012) 12:132. doi: 10.1186/1471-230X-12-132

61. Lachaine J, Yen L, Beauchemin C, Hodgkins P. Medication adherence and persistence in the treatment of Canadian ulcerative colitis patients: analyses with the RAMQ database. *BMC Gastroenterol.* (2013) 13:23. doi: 10.1186/1471-230X-13-23

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