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ORIGINAL RESEARCH

Changes in Serum Bilirubin and Total Bile Acids During Biologic Therapy in Patients with Ulcerative Colitis: A Retrospective Study

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Purpose: Ulcerative colitis (UC) requires new non-invasive serum biomarkers for assistance in monitoring due to the low rate of endoscopic follow-up. Previous research indicated reduced levels of serum indirect bilirubin (sIBIL), serum total bilirubin (sTBIL), and serum total bile acids (sTBAs) in UC patients. This study aims to assess their monitoring potential in UC during biologic therapy.

Methods: We conducted a retrospective single-center study including 138 UC patients and 150 controls with normal colonoscopy results. The receiver operating characteristic (ROC) curve was used to assess diagnostic value of sIBIL, sTBIL, and sTBAs. Spearman correlation analysis was performed to assess the association between these biomarkers and the severity of both endoscopic findings and clinical symptoms in UC patients. Additionally, changes in serum biomarkers were analyzed in 72 UC patients during biologic therapy, with stratified analyses based on endoscopic remission status.

Results: Patients with UC exhibited lower concentrations of sIBIL, sTBIL, and sTBAs compared to the controls ($P < 0.05$), and all these biomarkers demonstrated moderate diagnostic value in identifying UC from normal controls ($P < 0.05$). sIBIL concentration negatively correlated with disease severity and showed a progressive increase during biologic therapy, particularly in patients achieving endoscopic remission at week 52 ($P < 0.05$). The sIBIL concentration in the remission group was significantly higher than that in the non-remission group after week 26 ($P < 0.05$). For sTBAs, concentration initially increased and then decreased, with a turning point at week 14 in the remission group ($P < 0.05$) and at week 26 in the non-remission group ($P > 0.05$). No significant differences in sTBAs concentrations were found between remission and non-remission groups at any time ($P > 0.05$).

Conclusion: sIBIL may be used as a valuable serum biomarker for the clinical diagnosis and the monitoring of response to biologics. Additionally, the change trend of sTBAs may provide reference value for monitoring UC biologic therapy. However, further studies are needed to analyze the changes in its internal composition.

Keywords: ulcerative colitis, serum total bilirubin, serum indirect bilirubin, serum total bile acids, endoscopic remission

Introduction

Ulcerative colitis (UC) is a chronic, systemic immune-mediated disorder of uncertain etiology, that affects not only the gastrointestinal tract, but also overall health. It is characterized by progressive intestinal damage and functional impairment, which significantly reduces the quality of life for those affected. The incidence of UC has been rising in recent years, accompanied by significant treatment costs and the need for long-term management, resulting in a considerable disease burden.[1] At present, the diagnosis and monitoring of UC primarily depend on colonoscopy, which is constrained by its invasive nature and high costs. Many patients with inflammatory bowel disease (IBD) report moderate to severe discomfort during colonoscopy and express a preference for noninvasive methods, which results in a lower rate of endoscopic review.[2] Fecal calprotectin is a reliable non-invasive biomarker for evaluating mucosal

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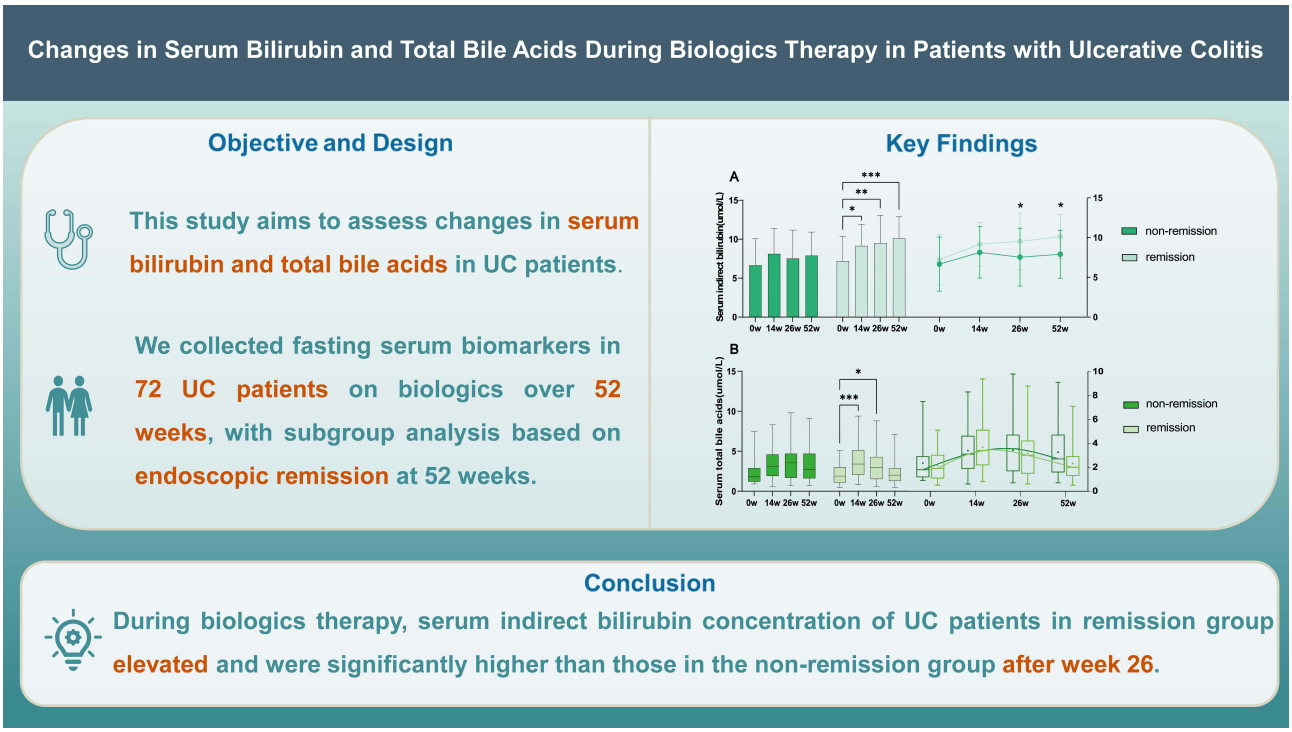
Graphical Abstract



inflammation in UC. However, its practical use is hampered by the collection and processing of fecal samples. Additionally, traditional serum inflammatory markers, including high-sensitivity C-reactive protein (hs-CRP), show limited diagnostic effectiveness.[3] Therefore, the search for additional non-invasive and practical serum biomarkers to assist in disease diagnosis and monitoring of disease activity has become the focus of current research.

Bilirubin, once thought to indicate cholestatic liver damage when elevated, has recently been found to possess protective effects on the body.[4] In the intestine, bilirubin serves as an antioxidant, diminishes inflammation, and adjusts the immune response.[5,6] Studies have shown that there is a correlation between serum bilirubin levels and the onset of UC.[4,7] Serum bilirubin levels were significantly lower in patients with UC than in healthy controls, with further reductions observed during active disease compared to remission phase.[7] Importantly, lower bilirubin levels were associated with elevated inflammatory markers and higher disease activity scores in UC patients.[3] However, changes in serum bilirubin concentration during biologic therapy of UC have not been reported.

Bile acids (BAs) are generally considered emulsifiers that promote the emulsification and absorption of dietary fat in the intestinal lumen. Notably, BAs also function as signaling molecules that play important roles in gut signaling pathways and promote enterohepatic circulation. This process is essential for the maintenance of hepatic and intestinal physiology.[8,9] Bile acids are an integral part of metabolism, immune regulation, and gut microbiota homeostasis.[10–12] Studies have shown that disorder of BA metabolism is associated with the onset of IBD.[11–13] In UC patients, reduced serum BAs levels alongside elevated luminal concentrations may disrupt intestinal mucosal integrity, increasing permeability and structural damage that contribute to diarrhea, which is a dysregulation implicated in UC pathogenesis.[8,14,15] However, the clinical significance of BAs in UC patients has not been fully researched.

The aim of this study is to evaluate the diagnostic value of serum total bilirubin (sTBIL), serum indirect bilirubin (sIBIL), and serum total bile acids (sTBAs) in patients with UC, while observing their changes during the biologic therapy.

Methods

Participants

We conducted an observational retrospective study initially enrolled 171 UC patients from the Department of Gastroenterology, the First Affiliated Hospital of Xi'an Jiaotong University from December 2022 to October 2024. New and detailed cases were selected as much as possible to control for recall bias. The diagnosis of UC was confirmed in all patients according to the European Crohn's and Colitis Organisation (ECCO) diagnostic guidelines, based on comprehensive assessment of clinical manifestations, endoscopic findings, and histopathological criteria. To evaluate the diagnostic value of sTBIL, sIBIL, and sTBAs in UC, we also retrospectively enrolled 174 controls with normal colonoscopy results during the study period. These participants were matched to UC patients by age and sex. The exclusion criteria for the control and UC groups were the same and included the following: lack of baseline data, excessive alcohol consumption, a history of hepatobiliary surgery, hematological disorders, hepatobiliary diseases, systemic autoimmune diseases, other gastrointestinal disorders, malignancies, and the use of medications that could influence bilirubin and BA metabolism (sulfonamides, among others).

Patients with UC who did not respond to oral and suppository treatments were administered biologic therapy after signing informed consent. Both vedolizumab (VDZ) and infliximab (IFX) were included in this study. The standard dosing regimen of VDZ consisted of induction therapy (300 mg IV at weeks 0, 2, and 6) followed by maintenance therapy (300 mg IV every 8 weeks after induction). The dose of IFX was adjusted according to the patient's body weight at 5mg /kg. Serum biomarker concentrations were measured in UC patients at weeks 0, 14, 26, and 52 during biologic therapy using wet chemistry analysis (Hitachi LST008 biochemical analyzer).

Disease Activity Assessment

We utilized a modified Mayo score system to assess the severity of UC, which incorporated several parameters, including stool frequency, rectal bleeding, overall physician assessment, and the endoscopic appearance of the mucosa. Clinical remission was defined as a combined part Mayo score ≤1 for stool frequency and rectal bleeding. The Mayo endoscopic score (MES) was employed to assess the severity of endoscopic lesions. Endoscopic remission was defined as a MES ≤1.[16] The location of disease was classified according to the Montreal classification.

Clinical and laboratory data were retrieved from the medical record system, and data on all participants including age, sex, BMI, smoking, fasting serum direct bilirubin (sDBIL), sTBIL, sIBIL, and sTBA concentrations were collected. For UC patients, the Mayo score for diarrhea (0–3) and hematochezia (0–3), MES (0–3), Montreal classification of disease extent (E1/E2/E3), hs-CRP, and erythrocyte sedimentation rate (ESR), were additionally collected. Current treatment regimens for UC patients were also recorded, encompassing infliximab (IFX), vedolizumab (VDZ), and non-biological agents. We evaluated the concentrations of sTBIL, sIBIL, and sTBAs in UC patients stratified by endoscopic findings, disease location, rectal bleeding, stool frequency, and inflammatory status (hs-CRP of >10 mg/L or ESR of >20 mm/h were classified as having an elevated inflammatory response). We also stratified UC patients into endoscopic remission/non-remission groups according to week-52 endoscopic outcomes post-biologic therapy and retrospectively evaluated changes in sTBIL, sIBIL, and sTBAs concentrations from baseline to week 52.

Statistical Analysis

This study employed statistical analyses utilizing GraphPad Prism 9 (GraphPad Software, USA) and SPSS 26.0 (IBM SPSS Statistics, USA). Continuous variables exhibiting a normal distribution were reported as mean ± SD, while those with a non-normal distribution were presented as median (IQR). Categorical variables were expressed in terms of counts (percentages). The *t* test was used to compare the continuous variables with a normal distribution between two groups, and the Mann–Whitney test was applied to compare those not conforming to a normal distribution. In cases involving three or more groups with normally distributed continuous variables, one-way ANOVA was performed followed by Tukey's post hoc test. Continuous variables that were not normally distributed or had uneven variances were compared using the Kruskal–Wallis rank-sum test followed by Dunn's post hoc test. Spearman rank correlation coefficient (ρ) was employed to analysis the correlation between two variables when at least one of the variables did not conform to

a normal distribution or was non-continuous. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of serum biomarkers, and the area under the curve (AUC) was calculated to quantify the diagnostic accuracy of the model. AUC values ranged from 0.5 (indicating no diagnostic value) to 1.0 (indicating perfect diagnosis value), and more than 0.7 was generally considered to have good diagnostic value. Before all analyses, normality of variables was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using the Bartlett's test. In addition, we used the Chi-square test to compare the two groups of categorical variables. A P-value <0.05 was deemed statistically significant.

In the analysis of changes in biomarkers during biologic therapy, data from four time points are deemed missing if there is a lack of data from any one of the time points. If missing data were considered to be missing at random, missing data were deleted and the results were analyzed for consistency before and after deletion.

Results

Comparison of Demographics and Serum Biomarkers in Participants

After applying the inclusion criteria, 13 patients with UC and 24 controls were excluded from the study. In addition, 20 UC patients with a lack of endoscopic reports were excluded. Finally, 138 UC patients and 150 controls were enrolled in this study (Figure 1). We analyzed baseline characteristics and serum biomarker concentrations between the two groups. There were no statistically significant differences in age, gender, BMI, smoking history, or concentration of serum direct bilirubin (sDBIL) between the UC and the control group ($P > 0.05$). However, the UC group exhibited significantly lower concentrations of sTBIL (9.70 μmol/L, IQR: 7.50–12.43 vs 13.20 μmol/L, IQR: 11.10–15.80), sIBIL (6.95 μmol/L, IQR: 4.75–8.83 vs 10.20 μmol/L, IQR: 8.70–12.20), and sTBAs (1.55 μmol/L, IQR: 1.10–1.90 vs 2.85 μmol/L, IQR: 2.20–4.00) compared to the control group ($P < 0.05$) (Table 1).

Diagnostic Value of sIBIL, sTBIL, and sTBAs for UC

ROC curve analysis and AUC were used to evaluate the diagnostic value of sIBIL, sTBIL, and sTBAs for UC. Our results showed that sIBIL, sTBIL, and sTBAs all demonstrated moderate diagnostic value, and sIBIL (AUC = 0.7857) had a higher AUC than sTBIL (AUC = 0.7387) and sTBAs (AUC = 0.7514) (Figure 2).

Correlation of sIBIL with Endoscopic Activity and Clinical Characteristics of Patients with UC

We evaluated the concentrations of sTBIL, sIBIL, and sTBAs in UC patients stratified by endoscopic findings, disease location, rectal bleeding, stool frequency, and inflammatory status. The results showed UC patients with higher endoscopic disease activity scores, more severe diarrhea, more frequent hematochezia, as well as ESR >20 mm/h and hs-CRP >10 mg/L had significantly lower sIBIL concentration (Table 2). Further Spearman rank correlation coefficient analysis revealed that sIBIL concentration was negatively correlated with the MES ($\rho = -0.41$, 95% CI: −0.54 to −0.25, $P < 0.001$), the Mayo Stool Frequency Score ($\rho = -0.32$, 95% CI: −0.44 to −0.18, $P < 0.001$), the Mayo Rectal Bleeding Score ($\rho = -0.33$, 95% CI: −0.49 to −0.17, $P < 0.001$), hs-CRP ($\rho = -0.31$, 95% CI: −0.45 to −0.16, $P < 0.001$), and ESR ($\rho = -0.31$, 95% CI: −0.45 to −0.12, $P < 0.001$) (Table 3).

Changes in sTBIL, sIBIL, and sTBAs During the Biologic Therapy in Patients with UC

Among 138 UC patients, 41 patients were effective in oral and suppository therapy, 14 patients refused biologic therapy because of economic reasons, and 83 UC patients were finally treated with biological agents. 11 patients discontinued treatment or had data missing for various reasons, as shown in Figure 1. We conducted sensitivity analyses, which showed that the results before and after the removal of missing data were consistent (Supplementary Figure 1–3), so we considered the missing data to be random and removed the missing data.

We compared the rate of endoscopic and clinical remission at week 52 between UC patients treated with IFX and those treated with VDZ, with no significant difference observed ($P > 0.05$) (Supplementary Table 1). The sIBIL concentration of patients increased during biologic therapy. Significant differences in sIBIL concentrations were observed

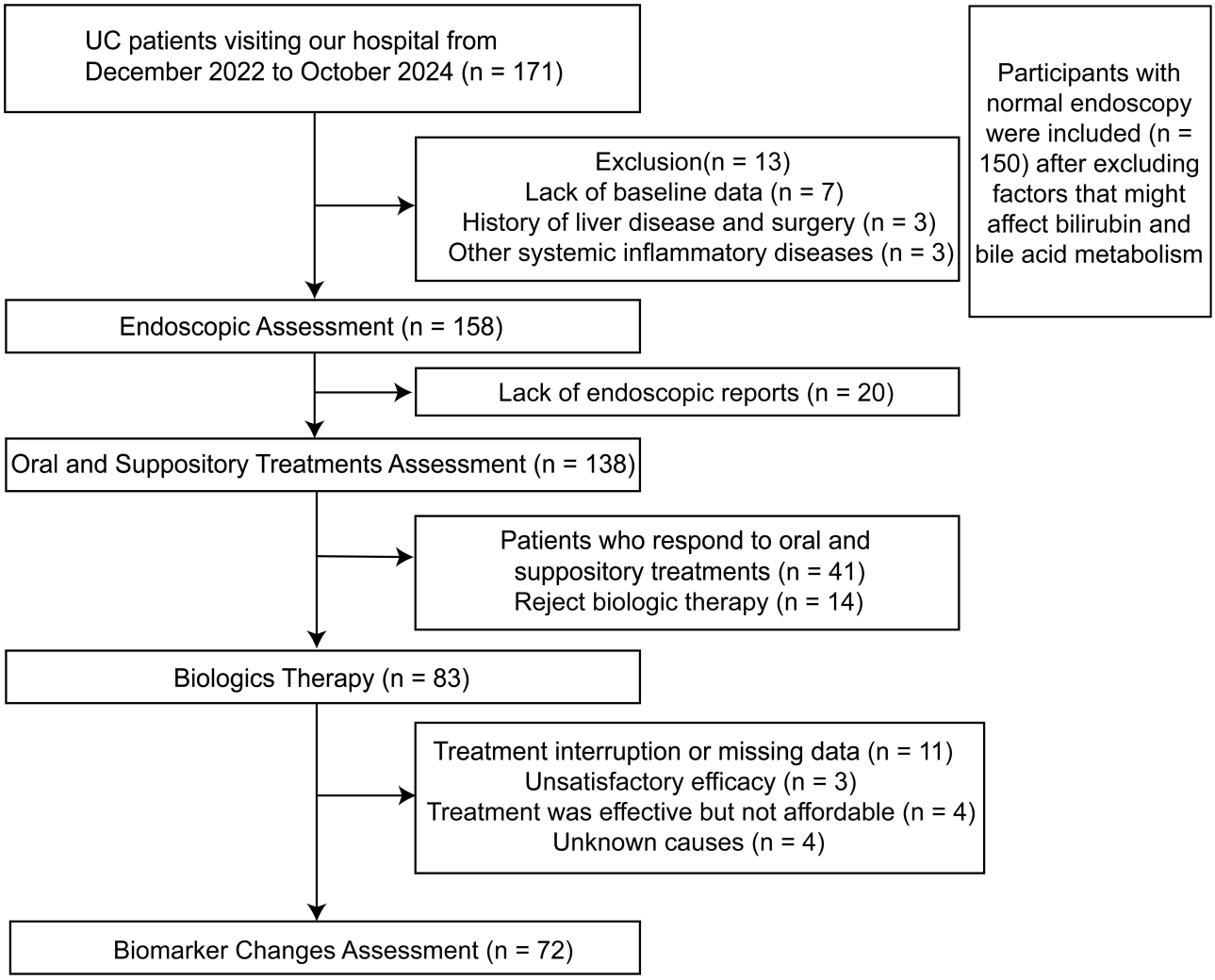


Figure 1 Participant inclusion and exclusion flowchart. We included 171 patients with ulcerative colitis (UC) who visited our hospital from December 2022 to October 2024. After first excluding patients with liver disease or surgery, other systemic immune disorders, and missing data at baseline, we evaluated endoscopic data from 158 patients, of whom 20 patients with lacking of endoscopic reports were excluded. Next, we excluded 55 patients who did not receive biologics as well as 11 patients who interrupted treatment, we finally investigated changes in biomarkers in 72 patients who received complete 52 week of biologic therapy. In addition, we collected 150 endoscopically normal participants who met inclusion exclusion criteria as a control group.

at weeks 14, 26, and 52 compared to baseline ($P < 0.05$) (Figure 3A). Although the concentration of sTBIL also showed an upward trend with extended therapy duration, this change did not reach statistical significance ($P > 0.05$) (Figure 3B). Due to the non-normal distribution of the data, we analyzed the changes in median sTBAs concentration. The median

Table 1 Comparison of Demographics and Serum Biomarkers in Participants

	UC	Controls	*P*
N	138 (100%)	150 (100%)	
Male	91 (65.9%)	96 (64.0%)	0.480
Age, y	45 (35–49)	43 (38–54)	0.328
BMI, kg/m^2	21.7 ± 3.2	21.2 ± 2.6	0.171
Smoking history	24 (17.9%)	21 (14.0%)	0.480
Disease course, m	57.5 (24,96)		

(*Continued*)

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Table 1 (Continued).

	UC	Controls	*P*
Montreal classification			
E1	40 (29.0%)		
E2	35 (25.3%)		
E3	63 (45.7%)		
Mayo Endoscopic Score			
1	18 (13.0%)		
2	90 (65.2%)		
3	30 (21.7%)		
Modified Mayo Score	9 (8,10)		
hs-CRP, mg/L	3.27 (0.85–9.24)		
ESR, mm/h	14 (7–24)		
sDBIL, μmol/L	2.90 (2.00–4.10)	3.00 (2.20–3.70)	0.986
sIBIL, μmol/L	6.95 (4.75–8.83)	10.20 (8.70–12.20)	0.000
sTBIL, μmol/L	9.70 (7.50–12.43)	13.20 (11.10–15.80)	0.000
sTBAs, μmol/L	1.55 (1.10–1.90)	2.85 (2.20–4.00)	0.000
Current treatment			
IFX	55 (39.9%)		
VDZ	28 (20.2%)		
Non-biological agents	55 (39.9%)		

Notes: Data were presented as mean ± SD, counts (percentages) or median (IQR) when applicable. $P < 0.05$ considered statistically significant.
Abbreviations: hs-CRP, high-sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate; UC, ulcerative colitis; sDBIL, serum direct bilirubin; sIBIL, serum indirect bilirubin; sTBIL, serum total bilirubin; sTBAs, serum total bile acids; IFX, infliximab; VDZ, vedolizumab.

sTBAs concentration peaked at week 14 ($P < 0.05$) and remained elevated at week 26 ($P < 0.05$) despite a subsequent decline (Figure 3C).

UC patients were classified into endoscopic remission and non-remission group based on endoscopic findings at week 52 (Supplementary Figure 4). No significant differences in demographics, sTBIL, sIBIL, or sTBAs concentrations were observed between the two groups ($P > 0.05$) (Table 4). Changes in sIBIL and sTBAs concentrations (baseline, weeks 14,

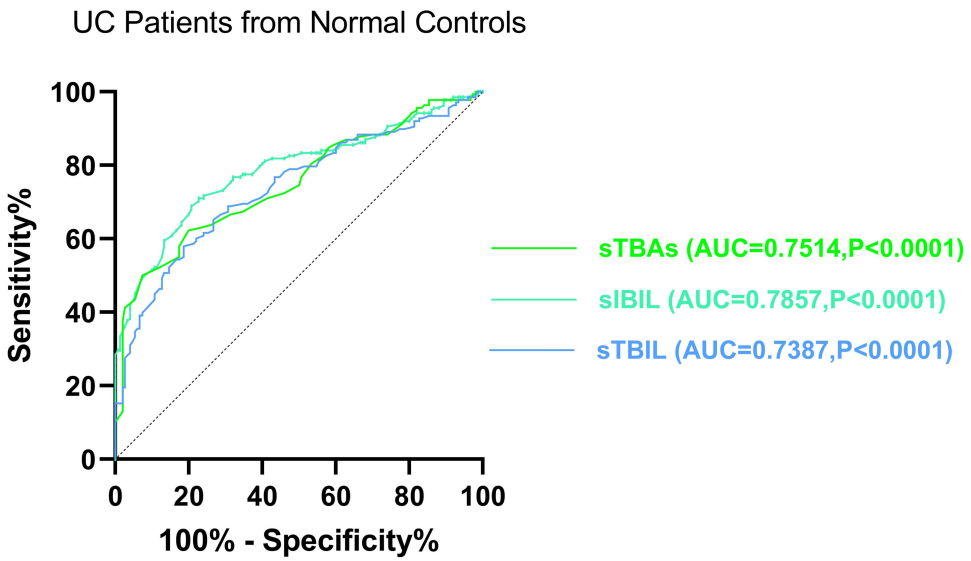

Figure 2 The receiver operating characteristic (ROC) curve and the area under the curve (AUC) were used to evaluate the diagnostic value of serum indirect bilirubin (sIBIL), serum total bilirubin (sTBIL), and serum total bile acids (sTBAs) for UC. Optimal diagnostic thresholds: sTBAs 2.05 μmol/L (AUC: 0.7514, 95% CI: 0.6946–0.8083, Sensitivity: 62.32%, Specificity: 80.00%), sIBIL 8.25 μmol/L (AUC: 0.7857, 95% CI: 0.7317–0.8398, Sensitivity: 68.84%, Specificity: 79.33%), sTBIL 10.55 μmol/L (AUC: 0.7387, 95% CI: 0.6807–0.7967, Sensitivity: 49.63%, Specificity: 81.33%). All markers demonstrated moderate predictive value for UC ($P < 0.001$).

Table 2 Concentrations of sTBIL, sIBIL, and sTBA in UC Patients with Different Disease Extent, Endoscopic Findings, Hematochezia, Diarrhea and Inflammatory Status

	N	sIBIL, μmol/L	*P*	sTBIL, μmol/L	*P*	sTBAs, μmol/L	*P*
Montreal classification							
E1	40(29.0%)	8.00(6.21–9.95)		10.85(9.13–13.4)		1.95(1.23–2.90)	
E2	35(25.4%)	7.00(5.00–8.80)	0.063	9.60(7.60–12.50)	0.079	1.70(1.00–3.40)	0.250
E3	63(45.6%)	6.20(4.30–8.00)		9.00(6.60–12.20)		1.40(1.10–2.60)	
Mayo Endoscopic Score							
1	18(13.0%)	10.50(9.30–12.15)		13.90(12.60–16.45)		2.50(1.30–3.00)	
2	90(65.2%)	6.85(4.80–8.23) **a**	0.000	9.50(7.65–11.73) **a**	0.000	1.70(1.10–3.00)	0.204
3	30(21.7%)	5.40(3.58–7.08) **a**		7.60(5.88–10.33) **a**		1.20(1.00–2.60)	
Mayo Stool Frequency Score							
1	28(20.3%)	9.10(7.35–11.03)		13.05(12.08–13.88)		2.20(1.23–3.00)	
2	78(56.5%)	6.75(4.78–9.13) **a**	0.000	12.40(11.70–14.55)	0.795	1.50(1.00–3.03)	0.325
3	32(23.2%)	5.55(3.65–7.85) **a**		12.55(12.03–13.85)		1.50(1.03–2.23)	
Mayo Rectal Bleeding Score							
0	13(9.4%)	11.10(8.70–12.95)		13.20(12.05–14.00)		1.50(0.70–2.85)	
1	26(18.8%)	7.90(5.38–9.85)	0.000	13.30(12.30–14.30)	0.378	1.95(1.00–3.05)	0.784
2	68(49.3%)	6.50(4.93–8.83) **a**		12.30(11.83–14.10)		1.50(1.03–2.75)	
3	31(22.5%)	5.50(3.20–7.90) **a**		12.60(11.80–13.90)		1.60(1.10–2.90)	
hs-CRP							
>10mg/L	32(23.2%)	5.10(3.23–6.70)	0.000	12.75(11.65–13.70)	0.487	1.50(1.03–2.75)	0.496
≤10mg/L	106(76.8%)	7.70(5.40–11.13)		12.75(11.90–14.13)		1.65(1.10–2.93)	
ESR							
>20mm/h	48(34.8%)	5.65(3.83–7.88)	0.002	12.30(11.73–13.70)	0.487	1.45(1.00.3.05)	0.621
≤20mm/h	90(65.2%)	7.55(5.40–11.13)		13.00(11.90–14.15)		1.70(1.10–2.83)	

Notes: $P < 0.05$ considered statistically significant. **a**Compared to the first group $P < 0.05$.
Abbreviations: hs-CRP, high-sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate; sIBIL, serum indirect bilirubin; sTBIL, serum total bilirubin; sTBAs, serum total bile acids.

Table 3 Spearman Correlation Analysis of sIBIL Concentration with Endoscopic Activity and Clinical Characteristics of Patients with UC

	ρ (95% CI)	*P*	N
sIBIL and Mayo Endoscopic Score	−0.41 (−0.54, −0.25)	0.000	138
sIBIL and Mayo Stool Frequency Score	−0.32 (−0.44, −0.18)	0.000	138
sIBIL and Mayo Rectal Bleeding Score	−0.33 (−0.49, −0.17)	0.000	138
sIBIL and hs-CRP	−0.31 (−0.45, −0.16)	0.000	138
sIBIL and ESR	−0.31 (−0.45, −0.12)	0.000	138

Notes: Mayo Endoscopic Score (range: 1–3); Mayo Stool Frequency Score (range: 1–3); Mayo Rectal Bleeding Score (range: 0–3); sIBIL, hs-CRP and ESR (specific value). $P < 0.05$ considered statistically significant.
Abbreviations: sIBIL, serum indirect bilirubin; hs-CRP, high-sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate; ρ, Spearman rank correlation coefficient, 95% CI, 95% Confidence Interval.

26, 52) in UC patients were analyzed separately by endoscopic remission status. In the remission group, sIBIL concentrations significantly increased from baseline at weeks 14, 26, and 52 ($P < 0.05$), whereas no significant changes occurred in the non-remission group. Furthermore, sIBIL concentrations in the remission group were significantly higher than those in the non-remission group at weeks 26 and 52 ($P < 0.05$) (Figure 3D). For sTBAs, concentration increased transiently and then decreased, with a turning point at week 14 in the remission group ($P < 0.05$) and at week 26 in the non-remission group ($P > 0.05$). No significant differences in sTBAs concentrations were found between remission and non-remission groups at any time ($P > 0.05$). (Figure 3E).

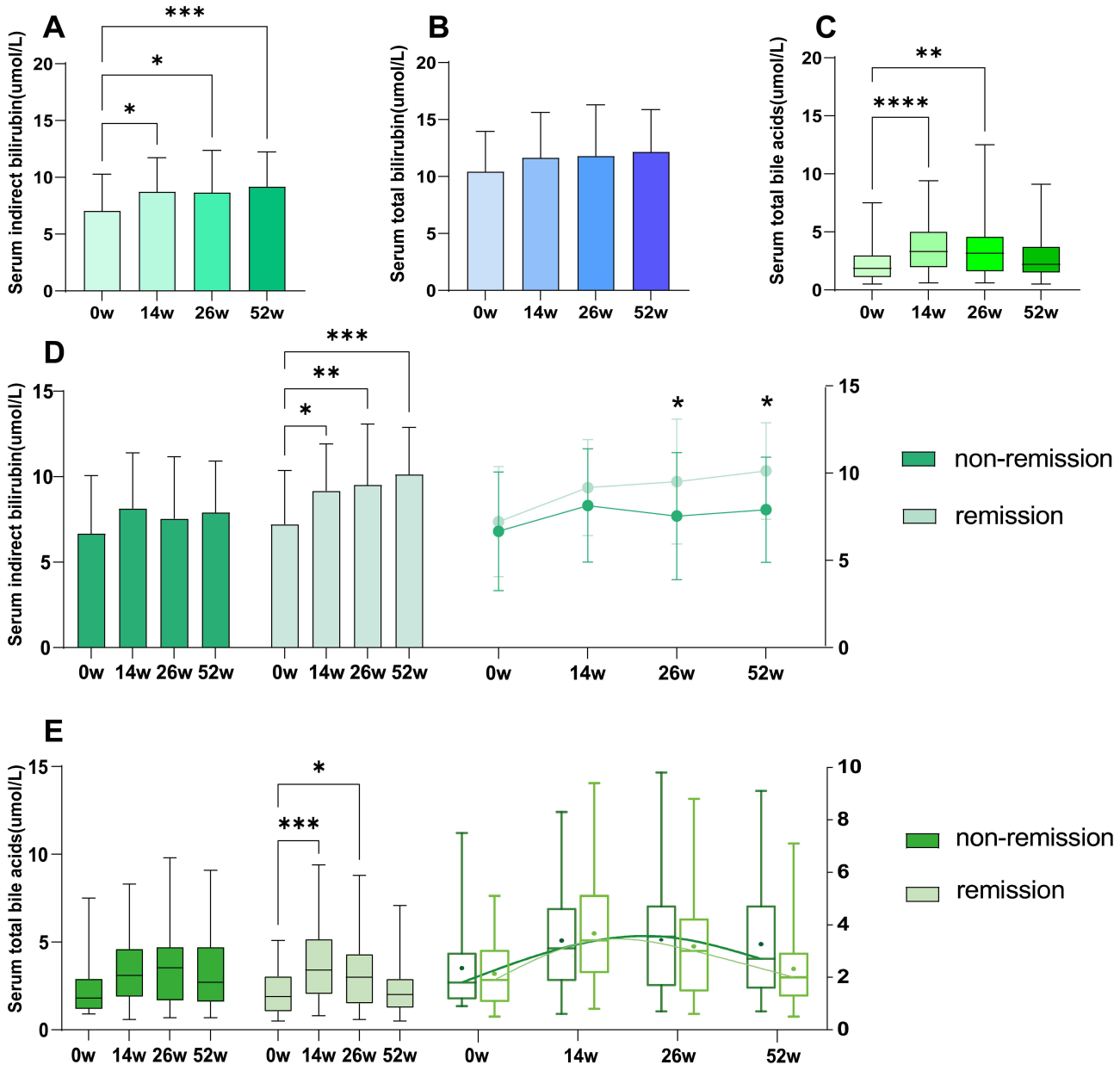

Figure 3 Serum biomarker changes during biologic therapy in ulcerative colitis (UC) patients. (**A**–**C**) Changes in concentrations of serum biomarkers in UC patients (n = 72) during biologic therapy: (**A**) serum indirect bilirubin (sIBIL), (**B**) serum total bilirubin (sTBIL), (**C**) serum total bile acids (sTBAs). (**D** and **E**) Comparative analysis during biologic therapy in concentrations of serum biomarkers between endoscopic remission (n = 41) and non-remission groups (n = 31): (**D**) sIBIL, (**E**) sTBAs (right panels: comparisons at matched timepoints); Box plots depict non-normally distributed data, bar plots depict normally distributed data. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

Discussion

Elevated bilirubin levels can induce pathological damage to multiple organ systems.[17] However, recent research has revealed that bilirubin at physiological levels can scavenge reactive oxygen species (ROS) and exhibit immunomodulatory effects. Thereby providing therapeutic benefits in animal models of inflammation, metabolic disorders and cancer.[18] In 1987, Stocker et al[19] demonstrated bilirubin has a superior antioxidant capacity in neutralizing hydrogen peroxide compared to vitamins C and E. Subsequently, bilirubin began to attract attention as a potent endogenous antioxidant.[6,20]

We observed that UC patients exhibited reduced concentrations of sIBIL and sTBIL, which demonstrated moderate diagnostic value in distinguishing UC patients from controls, consistent with the results of previous studies.[3,4,21–23] A negative linear correlation was found between sIBIL concentration and severity of both endoscopic activity and

Table 4 Comparison of Demographics and Serum Biomarkers Between Endoscopic Remission and Non-Remission Patients with UC

	Remission	Non-Remission	*P*
N	41(100%)	31(100%)	
Male	29(70.7%)	19(61.0%)	0.401
Age, y	43(34–57)	45(36–59)	0.495
BMI, kg/m^2	23.7±8.7	21.7±3.4	0.222
Disease course, m	60(36–96)	72(48–132)	0.493
Smoking history	8(19.5%)	3(9.6%)	0.331
Mayo Endoscopic Score			
2	35(85.4%)	24(77.4%)	0.538
3	6(14.6%)	7(22.6%)	
Modified Mayo score	9(8–10)	9(8–10)	0.654
sIBIL, μmol/L	7.22±3.15	6.67±3.40	0.481
sTBIL, μmol/L	10.97±3.24	9.74±3.85	0.156
sTBAs, μmol/L	1.90(1.05–3.05)	1.80(1.20–2.90)	0.724
Current treatment			
IFX	26(63.4%)	21(67.7%)	0.703
VDZ	15(36.6%)	10(32.3%)	

Notes: Data were presented as mean ± SD, counts (percentages) or median (IQR) when applicable. $P < 0.05$ considered statistically significant.
Abbreviations: sIBIL, serum indirect bilirubin; sTBIL, serum total bilirubin; sTBAs, serum total bile acids; IFX, infliximab; VDZ, vedolizumab.

clinical manifestations in patients with UC. In contrast, the concentration of sTBIL in patients with diverse degrees of bloody diarrhea and disparate levels of inflammation, showed no significant differences, which was different from previous study findings.[21,23] The discrepancy between our results and earlier studies may be attributed to the different proportion of sIBIL within sTBIL, considering that sIBIL is the main antioxidant component in sTBIL.[20] During biologic therapy, sIBIL concentration increased significantly, while changes in sTBIL were non-significant. Patients who achieved endoscopic remission at week 52 exhibited a significant increase in sIBIL concentration compared with their initial concentration. This finding aligns with prior research indicating that sIBIL levels are lower in active UC patients compared to those in remission.[22,23] Furthermore, the sIBIL concentration in the remission group increased more rapidly, was significantly higher than that in the non-remission group at week 26, and remained elevated at week 52. This suggests that changes in sIBIL concentration may be associated with the efficacy of UC biologic therapy.

In animal models, bilirubin and its derivatives have demonstrated potent therapeutic effects on colitis, surpassing those of 5-aminosalicylic acid. However, their clinical utility in treating UC remains unexplored.[18,24] The current study underscores that bilirubin exerts protective effects through its antioxidant, anti-inflammatory, and immunomodulatory properties. Bilirubin mitigates chronic inflammation by scavenging ROS,[25] while inhibiting the expression of adhesion molecules to reduce leukocyte aggregation and infiltration.[6] Notably, it decreases intercellular adhesion molecule-1, a critical factor in leukocyte recruitment and inflammation in the intestinal mucosa.[26] Meanwhile, bilirubin reduces Th17 cells and proinflammatory cytokines such as IL-22, TNF-α, IL-6, and IL-1, and promotes the regeneration of regulatory T cells to maintain intestinal homeostasis.[27]

Bilirubin has the potential to alleviate inflammation of UC through multiple mechanisms. Our study found that sIBIL concentration increased throughout biologic therapy. Consequently, elevated sIBIL within the physiological range may be beneficial for biologic therapy of UC and is expected to function as a novel serum biomarker for the dynamic monitoring of disease onset and progression. However, this hypothesis requires further validation through large-scale prospective studies. It is crucial to emphasize that changes in bilirubin levels should not be utilized in isolation as the basis for diagnosing or making treatment decisions regarding UC, given that reduced bilirubin levels are associated with various other medical conditions.[4]

Bile acids are widely utilized laboratory markers in biochemistry. Primary bile acids (PBAs), predominantly synthesized in the liver, are secreted into the intestines following meals and mostly reabsorbed in the ileum. Unabsorbed PBAs are metabolized by intestinal microbiota to secondary bile acids (SBAs) through dehydrogenation and dehydroxylation.[9] The PBAs in humans consist of cholic acid and chenodeoxycholic acid. SBAs mainly include deoxycholic acid (DCA), ursodeoxycholic acid (UDCA), and lithocholic acid (LCA).[9,10] Hydrophobic SBAs such as LCA and DCA may induce inflammatory cytokines and apoptosis in intestinal cells, while the more hydrophilic UDCA is considered to have anti-apoptotic effects.[9]

Typically, the concentration of PBAs in the intestinal lumen decreases from the small intestine to the large intestine due to reabsorption and microbial metabolism, accompanied by an increase in the proportion of SBAs.[10] Research has indicated that BA metabolism disorders are linked to the pathogenesis of IBD.[28,29] An imbalance in the ratio of SBAs to PBAs in the feces was observed in patients with IBD.[30] Compared with the controls, the serum SBAs concentration in UC patients was significantly reduced.[31] These observations suggest that a significant amount of unabsorbed BAs in the intestine of UC patients, particularly hydrophobic SBAs, may damage the intestinal barrier and disrupt intestinal physiology. Furthermore, increased lumen concentration of BAs can inhibit the growth of most bacterial species, thereby reducing intestinal microbiota diversity and contributing to dysbiosis.[9,11,28] Studies have established a correlation between high intestinal concentration of BAs and the onset of UC.[15] Notably, the role of BAs in intestinal diseases is not unidirectional. Insufficient BAs can impair lipid absorption, leading to diarrhea. Since lumen BAs inhibit the growth of pathogenic bacteria, reduced BAs concentration may enhance pathogen proliferation, thereby disrupting the microbiota balance.[11,14] Consequently, maintaining a balanced proportion and physiological concentration range of BAs within the intestinal lumen is essential for preserving intestinal homeostasis, which requires an accurate negative feedback mechanism.

Our research indicated that sTBAs concentration in UC patients was significantly reduced compared to controls and demonstrate moderate diagnostic value for the disease, which is consistent with previous studies.[31] Interestingly, in UC patients who have achieved endoscopic remission, sTBAs concentration initially increased but subsequently declined, failing to maintain elevated concentration. We hypothesize that elevated sTBAs in the early therapy phase may trigger a negative feedback mechanism, leading to downregulation of BAs synthesis or increased excretion,[10] thereby resulting in no significant difference in sTBAs concentrations between active and remission phases, both of which exhibit reduced concentrations. These results align with studies reporting increased fecal BAs levels in UC patients during both active and remission phases.[32,33] However, due to limitations in clinical examination, changes in sTBAs components remain unexplored, warranting further investigation.

We found that the endoscopic remission group exhibited a steeper peak and faster recovery to baseline in sTBAs concentration compared to the non-remission group. Despite the lack of statistical significance, we are intrigued by these trends and hypothesize that dynamic variations in sTBAs may reflect distinct states of enterohepatic circulation. The remission group likely experienced less severe disruption of enterohepatic circulation, leading to a more rapid response to fluctuations in serum BAs. It is well established that BAs synthesis and their enterohepatic circulation are tightly regulated by the farnesoid X receptor (FXR) and its downstream effector, fibroblast growth factor 19 (FGF19).[34] Patients with IBD exhibit a significant decrease in FXR transcriptional activity,[11,15] resulting in disruption of enterohepatic circulation.[35,36] FXR plays a crucial role in maintaining intestinal homeostasis.[9] On one hand, FXR accelerates enterohepatic circulation, preventing excessive accumulation of BAs in hepatocytes and the intestinal lumen, which could lead to direct cellular damage. On the other hand, FXR restricts mucosal inflammatory responses, modulates the intestinal immune system, regulates gut microbiota, and preserves the integrity of intestinal epithelial cells, thereby maintaining intestinal environment stability.[10,37]

Uridine diphosphate glucuronosyltransferase family 1 member A1 (UGT1A1) converts sIBIL to sDBIL by adding glucuronic acid. Peroxisome Proliferator-Activated Receptor-α (PPARα) induces the expression of UGT1A1, thereby decreasing the concentration of sIBIL.[38] In IBD animal model, the up-regulation of the PPARα-UGT axis is correlated with the down-regulation of FXR-FGF15 axis, both of which aggravate intestinal inflammation.[39] The interactions between sTBAs and sIBIL may be mediated by a variety of mechanisms, not limited to the above-mentioned mechanism, so we observed that the initial concentrations of sTBAs and sIBIL in UC patients were lower and showed the same

elevated trend early in biologic therapy. However, changes in concentrations of both moved in opposite directions over time, possibly due to negative feedback regulation that may have altered the internal composition of sTBAs.

This study has several limitations: 1. The sample size was small, which may affect the statistical power of the findings. 2. As a single-center retrospective study, recall bias and selection bias may affect the interpretation of the results. 3. The study was limited by the clinical examination, precluding a more detailed analysis of the sTBAs composition.

Conclusion

In summary, our study suggests that sIBIL may function as a valuable serum marker for the auxiliary clinical diagnosis and monitoring response to biologic therapy in UC, larger-scale studies are necessary to confirm these findings. Additionally, trends in sTBAs concentration may serve as a reference for monitoring UC biologic therapy response, further research is warranted to analyze the compositional changes within sTBAs.

Ethical Statement

This study was conducted in accordance with the Declaration of Helsinki and approved from the Institutional Review Board for Clinical Research of the First Affiliated Hospital of Xi'an Jiaotong University (No. 2022SF-135). All subjects were fully informed about the study and potential risks, and signed informed consent prior to biologic therapy, and all data were deidentified to protect patient privacy.

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Disclosure

The authors report no conflicts of interest in this work.

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