SCINDEKS
Serbian Citation Index
J Med Biochem 2026; 45 (7) DOI: 10.5937/jomb0-63357

UDK 577.1 : 61 **ISSN 1452-8258**

J Med Biochem 45: 1484–1491, 2026

Original paper
Originalni naučni rad

IDENTIFICATION OF NEW MARKERS FOR POOR RECOVERY IN INFLAMMATORY BOWEL DISEASE BASED ON INTESTINAL BARRIER FUNCTION AND INFLAMMATORY STRESS RESPONSE

IDENTIFIKACIJA NOVIH MARKERA U VEZI SA LOŠIM OPORAVKOM KOD INFLAMATORNE BOLESTI CREVA NA OSNOVU FUNKCIJE INTESTINALNE BARIJERE I ODGOVORA NA INFLAMATORNI STRES

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Summary

Background: Inflammatory bowel disease (IBD) is one of the common diseases in the department of gastroenterology. We conducted a cross-sectional study of 74 IBD patients admitted to our hospital from January 2024 to April 2025, focusing on changes in serum cytokines. Subsequently, based on these cytokines, we developed a novel risk model for poor recovery in IBD.
Methods: Based on sample size estimation and screening for inclusion and exclusion criteria, we included 74 IBD patients as research subjects (January 2024 to April 2025) for a cross-sectional survey. Key indicators were measured, including intestinal barrier markers (I-FABP, DAO, FC), inflammatory cytokines (IL-6, TNF-α, IL-10), Th17/Treg ratio, and stress hormones (Cor, ACTH). Subsequently, logistic regression was used to develop a risk model to predict poor patient recovery.
Results: Patients with IBD exhibit significant impairment of intestinal barrier function: intestinal barrier integrity markers I-FABP, DAO, and FC declined by 31.42%, 45.11%, and 37.31%, respectively ($P<0.05$). In addition, the patient had a significant inflammatory response that decreased after treatment: inflammatory modulation improved, characterised by a rise in IL-10 and declines in IL-6, TNF-α, and the Th17/Treg ratio ($P<0.05$). Finally, the patient's stress response was significant and relieved after treatment: Cor and ACTH decreased after treatment ($P<0.05$). After follow-up, 25 patients had poor recovery.

Kratak sadržaj

Uvod: Inflamatorna bolest creva (IBD) jedna je od čestih bolesti u gastroenterologiji. Sproveli smo studiju preseka na 74 pacijenta sa IBD hospitalizovanih u našoj ustanovi u periodu od januara 2024. do aprila 2025. godine, sa fokusom na promene u serumskoj koncentraciji citokina. Na osnovu dobijenih podataka razvijen je novi model rizika za loš oporavak kod IBD.
Metode: Na osnovu procene veličine uzorka i kriterijuma za uključenje i isključenje, u studiju je uključeno 74 pacijenta sa IBD (januar 2024 – april 2025) u okviru istraživanja preseka. Mereni su ključni pokazatelji, uključujući markere intestinalne barijere (I-FABP, DAO, FC), inflamatorne citokine (IL-6, TNF-α, IL-10), odnos Th17/Treg ćelija i hormone stresa (kortizol – Cor, ACTH). Zatim je primenjena logistička regresija radi razvoja modela rizika za predikciju lošeg oporavka pacijenata.
Rezultati: Pacijenti sa IBD su pokazali značajno oštećenje funkcije intestinalne barijere: markeri integriteta intestinalne barijere I-FABP, DAO i FC smanjeni su za 31,42%, 45,11% i 37,31%, redom ($P<0,05$). Takođe, prisutan je bio izražen inflamatorni odgovor koji se nakon terapije smanjio: inflamatorna regulacija se poboljšala, što se ogledalo u porastu IL-10 i smanjenju IL-6, TNF-α i odnosa Th17/Treg ($P<0,05$). Na kraju, stresni odgovor je bio izražen, ali je nakon lečenja ublažen: vrednosti Cor i ACTH su se smanjile ($P<0,05$). Tokom praćenja, kod 25 pacijenata je zabeležen loš oporavak. Logistička regresiona ana-

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Logistic regression analysis showed that ACTH was an independent factor affecting the poor recovery of IBD patients. The ROC curve showed that the sensitivity and specificity of ACTH in predicting poor recovery from IBD were 79.59% and 68.00%, respectively (AUC=0.710).
Conclusions: Patients with IBD have obvious intestinal barrier function impairment, and their inflammatory and stress responses are intensified. After the treatment, all these conditions improved. The detection of ACTH can effectively assess IBD recovery.

Keywords: inflammatory bowel disease, intestinal barrier, stress response, inflammatory factors, diagnosis

liza pokazala je da je ACTH nezavisan faktor koji utiče na loš oporavak pacijenata sa IBD. ROC kriva je pokazala da su senzitivnost i specifičnost ACTH u predikciji lošeg oporavka kod IBD iznosile 79,59% i 68,00%, respektivno (AUC=0,710).
Zaključak: Pacijenti sa IBD imaju izraženo oštećenje funkcije intestinalne barijere, uz pojačan inflamatorni i stresni odgovor. Nakon terapije dolazi do poboljšanja svih ovih parametara. Određivanje ACTH može efikasno doprineti proceni oporavka kod IBD.

Ključne reči: inflamatorna bolest creva, intestinalna barijera, stresni odgovor, inflamatorni faktori, dijagnostika

Introduction

Inflammatory bowel disease (IBD) is a collection of immune-driven conditions characterised by persistent, recurring inflammation of the intestinal tract. This category primarily includes two major disorders: ulcerative colitis (UC) and Crohn's disease (CD) (1). Recent years have witnessed a considerable global increase in IBD cases, which can be attributed to shifts in dietary patterns, improved sanitation conditions, and disruptions to the homeostasis of the gut microbiota (GMB) (2). For instance, epidemiological statistics from China reveal that the standardised incidence rate (SIR) of IBD rose from 1.5 cases per 100,000 population in 2010 to 3.2 cases per 100,000 by 2020. This upward trend has imposed a growing strain on public health systems (3). The mechanisms driving IBD development are complex, involving interconnected processes such as GMB imbalances, impairment of the intestinal mucosal barrier, and dysregulated immune responses (4).

At present, the treatment strategy for IBD aims to induce and maintain clinical remission and mucosal healing. Biological agents (such as anti-TNF-α monoclonal antibodies), immunosuppressants, and Microecological agents (MA) have all been proven to improve patient prognosis (5, 6). However, in clinical practice, problems persist, such as poor therapeutic response, short remission periods, and postoperative recurrence in some patients (7, 8). Most existing studies focus on macroscopic indicators such as post-treatment symptom scores and endoscopic mucosal healing rates but lack a systematic assessment of dynamic changes in intestinal barrier function (e.g., permeability and tight junction integrity) and inflammatory stress responses (e.g., cytokine networks and oxidative stress states) (9). Although some longitudinal cohort studies have observed improvements in certain serum indicators after treatment, they typically focus on a single dimension (e.g., measuring only serum LPS or TNF-α), lacking a comprehensive analysis of the interaction between barrier function and inflammatory stress (10, 11).

Therefore, in this study, we used a cross-sectional design to compare multidimensional changes in intestinal barrier function and the inflammatory stress response in IBD patients before and after standardised treatment. If it is confirmed that the treatment can improve prognosis by simultaneously repairing the intestinal barrier and inhibiting inflammatory stress, it suggests that dual-target intervention should be emphasised in clinical practice. If it is found that some patients only have inflammation relieved but the barrier has not been repaired, the risk of recurrence should be warned, and guidance should be provided for intensified mucosal protection treatment. In addition, we will develop a new diagnostic index for poor IBD recovery based on these indicators, to provide a direct, objective reference for clinical practice.

Materials and Methods

Study subjects

A single-centre cross-sectional study was conducted involving 74 IBD patients admitted to our hospital from January 2024 to April 2025. The primary endpoint was the change in intestinal fatty acid-binding protein (I-FABP) expression, a biomarker of intestinal barrier function. For sample size calculations, we assumed a 20% difference in I-FABP expression between pre- and post-treatment, with a 15% standard deviation. A two-sided α of 0.05 and a of 0.2 were adopted for the calculation, indicating that 67 patients per group were necessary. To account for a projected 10% attrition rate, attributed to factors like lost follow-up and early study withdrawal, each group was ultimately enrolled with 74 patients.

Criteria for participant selection

Inclusion criteria: Patients aged 18–65 years; confirmed IBD diagnosis via clinical examination (12); in the active phase of the disease; no history of antibiotic, probiotics, or immunosuppressant use in the previous 4 weeks; signed informed consent; and agreed to complete the 12-week treatment and monitoring period. Exclusion criteria: Coexisting infectious enteritis, ischemic bowel disease, or intestinal tuberculosis; presence of severe underlying conditions (e.g., heart failure, decompensated liver cirrhosis,

malignancies); pregnancy or breastfeeding; psychiatric conditions hindering assessment cooperation; hypersensitivity to Azathioprine (AZA) or MA.

Treatment

Patients received an intestinal examination upon admission and were initiated on Live Combined Bifidobacterium and Lactobacillus Tablets (Shanghai Sine Pharmaceutical Laboratories Co. Ltd., approval number S10950032). 2 tablets per dose, 3 times daily. Subsequently, the patient received AZA (Shanghai Sine Pharmaceutical Laboratories Co. Ltd., Approval Number H31021422) at a starting daily dose of 1 mg/kg, administered post-breakfast. Poor recovery was defined as: (1) persistence of symptoms (abdominal pain, diarrhoea, or hematochezia) after 12-week treatment, assessed by IBD Symptom Severity Score 4; or (2) relapse within 3 months after initial relief, confirmed by endoscopic evaluation (Mayo Score 2 for UC or SES-CD 4 for CD).

Sample collection and detection

Before treatment and 12 weeks after treatment initiation, 4–5 mL of fasting venous blood was collected from each patient in the early morning and divided into three aliquots:

First aliquot: Serum was separated via centrifugation for the determination of I-FABP, diamine oxidase (DAO), interleukin (IL)-6, TNF-α, and IL-10 using enzyme-linked immunosorbent assay (ELISA) kits (Wuhan Huamei Biotechnology Co., Ltd.) according to the manufacturer's instructions: Centrifugation was performed at 3000 rpm for 10 minutes at 4 °C. The serum layer was carefully pipetted into EP tubes. Detection steps included: ① Coating: 50 μL of standard solution or sample was added to each well of the microplate. ② Blocking: 300 μL of blocking solution (1% BSA-PBS) was added to each well. ③ Antibody addition: 50 μL of biotin-labelled detection antibody was introduced. ④ Colour development: 100 μL of streptavidin-HRP was added. ⑤ Reaction termination: 50 μL of stop solution was added. ⑥ Reading: optical density (OD) was read at 450 nm. A standard curve was used to calculate concentrations.

Second aliquot: For Th17/Treg ratio analysis via flow cytometry (Beckman Coulter CytoFLEX), peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque PLUS density gradient centrifugation (2000 rpm, 20 min) and adjusted to 1×10 cells/mL. Detection steps were as follows: ① Surface labelling: PBMCs were incubated with 1 μL each of CD4-FITC and CD25-APC at 4 °C for 20 minutes, protected from light. ② Fixation and membrane permeabilisation: 1 mL of fixation/permeabilisation solution was added, and the mixture was incubated at 4 °C for 30 minutes; after centrifugation to discard the supernatant, the cells were resuspended in 2 mL of PBS. ③ Intracellular labelling: 0.5 μL each of IL-17-PE and Foxp3-PE-Cy7 were added, followed by incubation in darkness at 4 °C for 30 minutes. ④ Washing: Cells were washed twice with 2 mL of PBS, centrifuged to remove the supernatant, and resuspended in 500 μL of PBS. ⑤ Detection: The CD4 T-cell population was gated after excluding debris based on the FSC/SSC threshold. Th17 (CD4 IL-17) and Treg (CD4 CD25 Foxp3) subsets were identified, and their ratio was computed.

Third aliquot: We used an automatic electrochemiluminescence analyser (cobas e 801, Roche) to measure cortisol (Cor) and adrenocorticotropic hormone (ACTH) levels. Blood samples were centrifuged at 3000 rpm for 10 minutes at 4 °C. The resulting serum was aliquoted into EP tubes at 4 °C, with detection completed within 24 hours. The assay procedure consisted of: ① Instrument calibration: A two-point calibration was carried out using manufacturer-provided calibrators (two concentrations). ② Quality control testing: Two levels of quality control materials (normal and pathological) were tested to ensure results fell within the acceptable range. ③ Sample testing: Serum specimens were dispensed into reaction cups; the analyser automatically aspirated each sample, added reagents, conducted the immunoassay, performed luminescence detection, and recorded Cor (nmol/L) and ACTH (pg/mL) concentrations.

Faecal calprotectin (FC) concentrations were also quantified by ELISA: 1 g of fresh stool was homogenised in appropriate normal saline, diluted, and centrifuged to obtain a clarified supernatant. The identical methodology employed for the serum ELISA was used for this measurement.

Quality control

Stringent quality control protocols were applied across all assays. In ELISA, each run included a five-point standard curve, blank controls, and low-, medium-, and high-concentration quality controls, and accepted only batches with a coefficient of variation (CV)$<10\%$. For flow cytometry, laser intensity and fluorescence compensation were calibrated daily before testing; the same batch of fluorescent antibodies was used, and isotype controls were set for each batch. Electrochemiluminescence testing required daily instrument self-checks and utilised proprietary Roche quality controls (Levels 1 & 2) with CV ≤ 5%.

Statistical analysis

Analyses were carried out using SPSS 30.0. Categorical data are reported as frequencies (percentages) and evaluated using chi-square tests. Normality of continuous data was determined by the

Shapiro-Wilk test. Normally distributed data are reported as ($\bar{x}\pm s$) and analysed with t-tests (independent or paired). Non-normal data are presented as median (IQR) and compared with Mann-Whitney U or Kruskal-Wallis tests. Diagnostic efficacy was analysed using an ROC curve, and the cut-off value and AUC were determined at the maximum Youden index. A P-value < 0.05 was considered significant.

Results

The clinical characteristics of the research subjects

Summarising the demographic characteristics of the subjects in this study, it can be seen that the IBD patients included in this study present the clinical features of »predominantly male, mainly urban residents, high proportion of ulcerative colitis, heavy BMI, more than half of the smoking proportion, and no history of alcohol consumption is more common« (*Table I*).

Change of intestinal barrier function, inflammatory reaction, and stress response

First, observe the biomarkers indicative of the patients' intestinal barrier integrity. It can be seen that the baseline I-FABP, DAO, and FC levels of the patients are all high. After treatment, the I-FABP, DAO, and FC of the patients decreased ($P<0.01$). Similarly, baseline inflammatory markers (IL-6, TNF-α, IL-10) in IBD patients are also elevated. After treatment, the patient's IL-6 and TNF-α levels decreased, while IL-10 levels increased ($P<0.01$). The Th17/Treg ratio decreased from 3.17 ± 0.95 to 2.63 ± 0.98 ($P<0.001$), suggesting a shift toward a

Table I Clinical characteristics of the study participants.

Variables		n	Percentage
Age		53.97±10.33	
Duration of disease (months)		3.30±1.40	
Sex	male	50	67.57
	female	24	32.43
Body mass index (kg/m^2)		24.46±1.89	
Place of abode	city	62	83.78
	rural	12	16.22
Types of IBD	ulcerative colitis	60	81.08
	Crohn's disease	14	18.92
Smoking	yes	42	56.76
	no	32	43.24
Drinking alcohol	yes	28	37.84
	no	46	62.16

Table II Change of intestinal barrier function, inflammatory reaction, and stress response.

Indicators	Baseline (n=74)	After treatment (n=74)	t	P
I-FABP (ng/mL)	25.08±2.87	17.20±2.89	16.63	<0.001
DAO (U/mL)	4.50±0.96	2.47±0.73	14.39	<0.001
FC (mg/g)	67.14±10.46	42.09±8.36	16.09	<0.001
IL-6 (pg/mL)	18.72±5.64	9.68±2.51	12.60	<0.001
TNF-α (pg/mL)	14.02±3.65	9.83±2.36	8.30	<0.001
IL-10 (pg/mL)	6.39±1.66	9.60±2.58	9.00	<0.001
Th17/Treg	3.17±0.95	2.63±0.98	3.40	<0.001
Cor (nmol/L)	314.55±65.46	224.99±55.85	8.95	<0.001
ACTH (pg/mL)	63.12±12.85	47.26±7.18	9.27	<0.001

Table III Comparison of post-treatment biomarkers between patients with good and poor recovery.

Indicators	Baseline (n=74)	After treatment (n=74)	t	P
I-FABP (ng/mL)	18.54±2.96	16.52±2.63	3.00	0.004
DAO (U/mL)	2.78±0.73	2.32±0.69	2.65	0.010
FC (mg/g)	45.79±8.36	40.20±7.78	2.85	0.006
IL-6 (pg/mL)	10.77±2.59	9.12±2.29	2.80	0.007
TNF-α (pg/mL)	10.86±2.37	9.30±2.20	2.82	0.006
IL-10 (pg/mL)	8.56±2.54	10.13±2.47	2.55	0.013
Th17/Treg	3.03±0.99	2.43±0.92	2.59	0.012
Cor (nmol/L)	250.44±56.89	212.00±51.16	2.94	0.004
ACTH (pg/mL)	50.64±7.15	45.54±6.62	3.06	0.003

Table IV Multivariate analysis of factors influencing poor recovery in IBD.

Indicators	B	S.E.	Wals	Sig.	Exp (B)	95% C.I.	
						Lower limit	Upper limit
I-FABP (ng/mL)	-1.787	1.001	3.183	0.074	0.168	0.024	1.193
DAO (U/mL)	-12.861	7.71	2.782	0.095	0.000	0.000	9.494
FC (mg/g)	0.454	0.302	2.270	0.132	1.575	0.872	2.845
IL-6 (pg/mL)	2.141	1.957	1.198	0.274	8.51	0.184	393.913
TNF-α (pg/mL)	1.534	1.416	1.173	0.279	4.635	0.289	74.361
IL-10 (pg/mL)	1.346	1.156	1.355	0.244	3.841	0.398	37.043
Th17/Treg	-7.607	3.997	3.623	0.057	0.000	0.000	1.254
Cor (nmol/L)	0.022	0.09	0.062	0.804	1.023	0.857	1.22
ACTH (pg/mL)	1.692	0.679	6.206	0.013	5.430	1.434	20.555

more favourable immunoregulatory balance, which is favourable for IBD remission. Finally, the baseline Cor and ACTH of the patients were also relatively high and decreased after treatment (P<0.01) (*Table II*).

The relationship between intestinal barrier function, inflammatory reaction, stress response and rehabilitation

According to statistics, 25 (33.78%) patients had poor rehabilitation. Compared with patients with good recovery, patients with poor recovery had higher levels of I-FABP, DAO, FC, IL-6, TNF-α, Th17/Treg, Cor and ACTH, and lower levels of IL-10 after treatment (P<0.05) (*Table III*).

Analysis of independent factors affecting poor rehabilitation

Variables with P<0.1 in univariate analysis were included in the multivariate logistic regression model to identify independent predictors of poor recovery. The model included all biomarkers that showed significant differences in univariate analysis (continuous variables; no values were assigned), with rehabilitation status as the independent variable. The results showed that I-FABP, DAO, FC, IL-6, TNF-α, Th17/Treg, Cor, and IL-10 were not independent factors associated with poor recovery in IBD patients (P>0.05), while ACTH was the only independent risk factor affecting the recovery of IBD (OR=5.430, P=0.013) (*Table IV*).

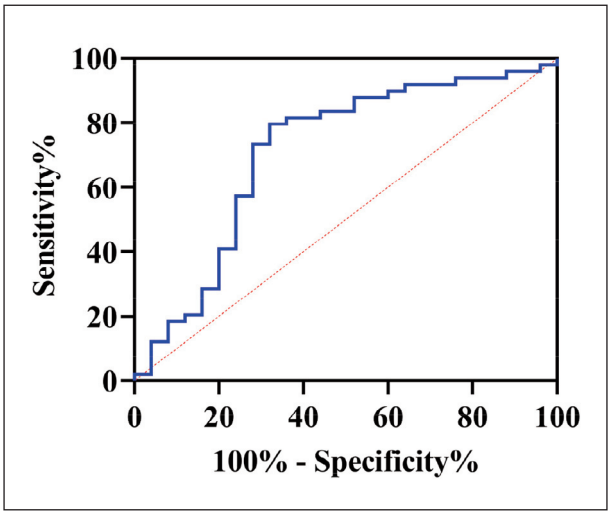

Figure 1 The predictive effect of ACTH on poor recovery by ROC curve analysis.

Predictive effect of ACTH on poor recovery

Therefore, we constructed ROC curves for poor recovery in IBD based on ACTH levels. The results showed that when ACTH was <49.09 pg/mL after treatment, the sensitivity and specificity for predicting poor recovery in IBD patients were 79.59% and 68.00%, respectively. The AUC of this curve was 0.710 (95% CI=0.575–0.846), which had a good reference value (*Figure 1*).

Discussion

The results of this study show that baseline I-FABP, DAO, FC, IL-6, TNF-α, IL-10, Cor, and ACTH levels are elevated in IBD patients, suggesting that intestinal barrier function is impaired and that inflammatory and stress responses are intensified. After treatment, the levels of the above indicators decreased, suggesting that reversing intestinal barrier function, the inflammatory response, and the stress response is key to improving IBD.

Among them, the baseline pathological status of IBD has been repeatedly reported in previous studies (13–15), and the results of this study are also consistent with these conclusions. Changes in patients after treatment were associated with the following factors: ① As an immunosuppressant, AZA primarily reduces lymphocyte proliferation by inhibiting DNA synthesis (16). In IBD, overactive lymphocytes damage intestinal mucosal epithelial cells, compromising the integrity of the intestinal barrier (17). By reducing lymphocyte numbers, AZA diminishes this immune attack, thereby partially alleviating intestinal barrier damage. However, AZA has limited direct effects on the intestinal barrier, as it primarily targets the immune system rather than acting directly on the barrier's structural components. We believe that restoration of intestinal barrier function relies more heavily on microbial agents (MS), a finding supported by multiple clinical studies (18, 19). Through the synergistic action of AZA and MS, in which AZA reduces immune-mediated damage, while MS directly repairs and strengthens the barrier structure, the combination group demonstrates superior improvement in intestinal barrier function. ② Similarly, AZA can also modulate immune responses by suppressing lymphocyte proliferation, thereby reducing pro-inflammatory cytokines (20). For example, IL-6 and TNF-α are key cytokines in inflammatory responses; they activate immune cells, promote inflammatory cascades, and lead to inflammation and damage in intestinal tissues (21). By reducing lymphocyte numbers, AZA controls the source of these pro-inflammatory cytokine production (22). Concurrently, AZA may also affect immune cell function, diminishing their ability to secrete pro-inflammatory cytokines (23). ③ As is well-documented, IBD patients often experience overactivation of the hypothalamic-pituitary-adrenal (HPA) axis (24). AZA indirectly influences the activation state of the HPA axis by modulating immune responses and alleviating intestinal inflammation. When intestinal inflammation is controlled, inflammatory signals reduce HPA axis stimulation, thereby partially decreasing stress hormone secretion.

This study demonstrated that patients with poor recovery exhibited significantly elevated markers of intestinal barrier injury (I-FABP, DAO, FC), pro-inflammatory cytokines (IL-6, TNF-α), and Th17/Treg ratio following treatment, along with increased cortisol (Cor) and ACTH levels and reduced IL-10. These findings indicate a synergistic interplay among multiple pathological mechanisms: Intestinal barrier dysfunction: Persistent elevation of I-FABP and DAO suggests incomplete restoration of intestinal epithelial integrity, while elevated FC indicates abnormal intestinal permeability. This may contribute to a self-perpetuating cycle of bacterial translocation and immune activation. Imbalance in inflammatory response: As key mediators of inflammation, IL-6 and TNF-α promote Th17 cell differentiation and suppress Treg cell function, thereby exacerbating immune dysregulation. Although systemic inflammation decreased post-treatment, residual inflammatory activity in patients who poorly recovered may continue to impair mucosal healing. Hyperactivation of the stress response: Elevated Cor and ACTH levels reflect sustained activation of the hypothalamic-pituitary-adrenal (HPA) axis. Given that ACTH is the terminal hormone of this pathway, its prolonged elevation may interfere with intestinal mucosal repair through glucocorticoid receptor-mediated immunosuppression or direct cytotoxic effects.

Notably, although significant intergroup differences were observed for multiple biomarkers, only ACTH remained independently associated with prognosis in the multivariate model. This implies that the

prognostic relevance of other markers may be transiently influenced by therapeutic interventions, such as rapid IL-6 suppression via anti-inflammatory agents, whereas ACTH may represent a more stable pathophysiological state or a distinct biological pathway. For instance, chronic inflammation in IBD activates the HPA axis, leading to sustained ACTH release and glucocorticoid production. While initially anti-inflammatory, prolonged HPA activation may disrupt immune homeostasis by desensitising glucocorticoid receptors, impairing mucosal healing. This suggests that ACTH elevation may reflect a maladaptive stress response that perpetuates intestinal inflammation rather than merely being an epiphenomenon. The ROC curve indicated that post-treatment ACTH 49.09 pg/mL had moderate predictive accuracy (AUC=0.710) for poor recovery. Although this value is below the excellent threshold (AUC > 0.8), it remains clinically relevant for risk stratification in resource-limited settings, where ACTH testing is more readily accessible than invasive endoscopic monitoring. Based on these observations, we propose incorporating post-treatment ACTH levels into clinical efficacy assessments. For patients with ACTH 49.09 pg/mL, intensified mucosal protective strategies, such as probiotics combined with glutamine, or adjustments in immunosuppressive therapy should be considered.

Still, the constraints of the present study warrant attention. First, the single-centre design, in which all participants were sourced from a single geographic area, might limit the generalizability of the findings, necessitating further validation through collaborative multi-centre efforts. Second, the lack of metagenomic or metabolomic analysis limits our ability to elucidate microbial functional shifts or metabolite interactions underlying ACTH's role. Third, potential confounding factors such as psychological stress levels and medication adherence were not systematically assessed, which may influence recovery outcomes. Future studies should incorporate standardised assessments of these variables to isolate biomarker-outcome relationships better. In the future, we will conduct multi-centre, large-sample studies to summarise additional characteristics of IBD.

Conclusion

This study confirms that standardised treatment leads to significant improvements in intestinal barrier function, inflammatory status, and stress regulation in IBD patients. However, individuals with poor recovery exhibit a distinct elevation in ACTH levels. As an independent prognostic indicator, ACTH is significantly associated with disease outcomes through chronic stress-induced immune dysregulation. Future studies should investigate the direct role of ACTH in IBD pathophysiology and evaluate its potential as a target for precision medicine.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Funding

Not applicable.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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Received: November 12, 2025
Accepted: December 17, 2025