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ORIGINAL RESEARCH

Circulating Neutrophil Extracellular Traps as Diagnostic and Prognostic Markers for Inflammatory Bowel Disease: A Case-Control Study

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Objective: Current challenges in inflammatory bowel disease (IBD) treatment include the invasive nature of endoscopic evaluation, the gold standard for diagnosis, and the limited prognostic value of traditional inflammatory markers such as CRP and IL-6. This study aimed to explore the potential role of neutrophil extracellular traps (NETs) as biomarkers for the diagnosis, disease monitoring, and prognosis of IBD.

Methods: A total of 100 patients with Crohn's disease or ulcerative colitis and 100 healthy controls were recruited between June 2020 and September 2022. Clinical and laboratory data were collected, and patients with inactive IBD were followed for two years to assess factors influencing disease relapse.

Results: Significant differences were observed in the levels of NETs markers and inflammatory cytokines among the three groups. Cell-free DNA (cfDNA), myeloperoxidase (MPO)-DNA complexes, and citrullinated histone 3 (CitH3) levels were significantly elevated in the active IBD group compared to the inactive IBD and healthy control groups ($P < 0.001$). Additionally, inflammatory cytokines such as C-reactive protein (CRP), vascular endothelial growth factor (VEGF), IL-1β, and IL-6 were also higher in the active IBD group ($P < 0.001$). A positive correlation was observed between circulating NETs markers and inflammatory cytokines. Multivariate analysis identified cfDNA (OR = 1.045), MPO-DNA (OR = 1.084), and CitH3 (OR = 2.871) as independent risk factors for IBD. Furthermore, patients with higher NETs scores experienced more frequent relapses. At the 1-year follow-up, the high-NETs group had 13 relapses compared to 5 in the low-NETs group ($P = 0.026$), and at the 2-year follow-up, 22 versus 14 relapses ($P = 0.044$).

Conclusion: These findings suggest that NETs biomarkers may serve as effective diagnostic and prognostic tools for IBD, enabling early intervention and improved long-term management.

Keywords: circulating NETs markers, inflammatory bowel disease, prognosis

Introduction

Inflammatory bowel disease (IBD) encompasses a group of chronic, progressive, disabling, and non-specific intestinal inflammatory diseases with unclear etiology and pathogenesis. IBD primarily includes ulcerative colitis (UC) and Crohn's disease (CD).[1] The disease is characterized by unpredictable episodes of onset and remission, leading to malnutrition, psychological issues, and intestinal or extraintestinal tumors, necessitating lifelong medical follow-up and treatment.[2] Globally, the incidence of IBD continues to rise, posing significant challenges to public health systems, including in China.[3] It is estimated that approximately 7 million individuals worldwide suffer from IBD, with the highest prevalence in North America and Europe. The incidence of IBD in China has increased dramatically over the past 30 years, with annual prevalence rates of approximately 1.2–3.0 per 100,000 in UC and 0.5–1.5 per 100,000 for CD. The overall prevalence increased from 1.93/100,000 in 1990 to 34.3/100,000 in 2016, a trend potentially associated with

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dietary and environmental changes as well as improved diagnostic capabilities.[4] Currently, endoscopy with tissue biopsy remains the gold standard for diagnosing IBD, distinguishing CD from UC, and assessing diseases severity. However, it's invasive, causing discomfort and potential risks.[5,6] Given the recurrent nature of IBD, patients often require repeated endoscopies and biopsies, which can be difficult to tolerate.[7]

Novel IBD biomarkers provide new tools for diagnosis, follow-up, prognosis, and potential therapeutic interventions, providing fresh perspectives on IBD management.[8–10] In the inflamed intestinal mucosal tissue, a significant infiltration of activated leukocytes has been observed, including polymorphonuclear neutrophils (PMNs), T cells, B cells, macrophages (Mφ), and dendritic cells.[11–14] PMNs play a crucial role in IBD immune responses. During intestinal inflammation, they migrate to the affected site and contribute to the local immune defense.[15] In IBD, inflammatory cytokines disrupt tight junction proteins, increasing intestinal permeability and allowing bacteria and endotoxins to penetrate the mucosa and enter circulation, thereby triggering severe inflammation.[16,17]

In the early stages of IBD-related inflammation, neutrophils gather in the inflamed mucosa and play an irreplaceable role in its pathogenesis.[18] Recent studies have found that neutrophils can form extracellular reticular structures called neutrophil extracellular traps (NETs) in response to specific stimulatory factors. These structures consist of a DNA backbone embedded with proteins such as citrullinated histone 3 (CitH3), myeloperoxidase (MPO), neutrophil elastase (NE), and proteinase 3, which possess antimicrobial properties and enhance tissue permeability.[19] NETs may amplify IBD-related inflammation by attaching to proteolytic enzymes and granular proteins on cfDNA, enhancing macrophage cytokine production. They also aggravate tissue damage, harm endothelial cells, promote inflammation and ulcers, and lead to extraintestinal thrombosis. Elevated NETs expression has been observed in multiple disease models and serves as a biomarker for diagnosis and prognosis.[20–22] In summary, this study aims to explore circulating NETs as biomarkers for IBD diagnosis, monitoring, and prognosis. It could help assess relapse risk, optimize treatment, and improve disease management, enhancing outcomes and patient quality of life.

Materials and Methods

Participants

This study included 100 patients diagnosed with CD or UC from the Department of Gastroenterology and Endoscopy Center at Changshu No.2 People's Hospital from June 2020 to September 2022. Additionally, 100 healthy volunteers were randomly matched as controls. Participants were recruited by physicians from the project team, and informed consent was obtained on the day of endoscopic examination. This study was approved by the Ethics Committee of Changshu No.2 People's Hospital (2023-KY-SK01).

Inclusion Criteria

IBD group: (1) age ≥18 years; (2) diagnosis of UC or CD confirmed by colonoscopy combined with histopathology, in accordance with the World Gastroenterology Organization (WGO) IBD diagnostic criteria in 2019. Controls: (1) age and gender matched to the IBD patient group; (2) no history of chronic gastrointestinal disease, autoimmune disease, or recent infection (within 3 months).

Exclusion Criteria

(1) Presence of other autoimmune diseases (eg, rheumatoid arthritis, systemic lupus erythematosus) or primary immunodeficiencies; (2) use of glucocorticoid shock therapy or broad-spectrum antibiotics within 2 weeks prior to enrollment; (3) pregnant or breastfeeding women; (4) presence of malignant tumors, pr severe cardiac, hepatic, or renal insufficiency; (5) Patients using broad-spectrum antibiotics (including β-lactams such as ceftriaxone, carbapenems such as imipenem, and fluoroquinolones such as levofloxacin) or antifungals (eg, fluconazole) within 2 weeks before enrollment.

In this study, the area under the operating characteristic curve (AUC) of the diagnostic test was used as the primary endpoint, and the sample size was calculated based on the following formula:

$$N = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \cdot AUC(1 - AUC)}{(0.5 \cdot \nabla)^2}$$

Considering potential loss to follow-up and missing data (expected at 10%), the final sample size was set at 125 cases per group. However, due to resource constraints, the sample size was adjusted to 100 cases in the IBD group (60 cases in the active phase and 40 cases in the remission phase) and 100 cases in the healthy control group, resulting in a total sample size of 200 cases.

Disease Diagnosis and Classification

Diagnosis of UC

The diagnosis adhered to the *Chinese Consensus on Diagnosis and Treatment in Inflammatory Bowel Disease*.[23] The extent of lesions was classified using the Montreal Classification of UC,[1] and the disease activity and severity were graded using the modified Mayo Scores.

Diagnosis of CD

The diagnosis adhered to the *Chinese Consensus on Diagnosis and Treatment in Inflammatory Bowel Disease*.[23] The extent of lesions was classified using the Montreal Classification of CD, and the disease activity and severity were assessed using the simplified Crohn's Disease Activity Index (CDAI).

Collection of General Data

General data collected for participants included gender, age, body mass index (BMI), marital status, medical history (diabetes, hypertension, cardiovascular and cerebrovascular diseases), smoking and drinking history, use of nonsteroidal anti-inflammatory drugs (NSAIDs), blood in stool, lesion extent, disease behavior, severity, extraintestinal manifestations, complications, intestinal infections, and antibiotic use.

Definition of Disease Remission

Definition of Remission

Remission of UC: Complete remission was defined as the absence of symptoms (normal bowel movements without blood in stool or tenesmus) combined with endoscopic evidence of mucosal healing (normal intestinal mucosa or no active inflammation) and a modified Mayo score ≤ 2. Remission of CD: Remission was defined as the near-complete disappearance of clinical symptoms following treatment, with a simplified CDAI ≤ 4. Sustained remission: After achieving remission naturally or through treatment, sustained remission was defined as the absence of related clinical symptoms during follow-up.

Definition of Relapse

Relapse of UC: Relapse was defined as the reappearance of symptoms such as blood in stool and diarrhea after achieving remission naturally or through treatment, confirmed by colonoscopy and a modified Mayo score ≥ 3. Relapse of CD: Relapse was defined as the recurrence of CD-related clinical symptoms after achieving remission through treatment, with supporting evidence from laboratory inflammatory markers, endoscopic findings, or imaging. Relapse criteria included a CDAI >150 with an increase of at least 100 points from the previous score.

Laboratory Parameter Analysis

Detection of Circulating NETs Markers

Circulating NETs markers were quantified through the following methods: cfDNA quantification: Plasma cfDNA was quantified using the PicoGreen dsDNA Quantification Kit (Invitrogen, USA) according to the manufacturer's instructions. Measurement of MPO-DNA complex: The MPO-DNA complexes were measured using solid-phase modified specific antibodies targeting MPO and dsDNA, as described in the study by Middleton et al.[16] Measurement of citH3: Plasma citH3 levels were determined using anti-histone antibodies from the Cell Death Detection ELISA Kit (Roche Diagnostics, Switzerland).

Detection of Inflammatory Markers

CRP concentration was detected by an immunofluorescence analyzer (FS-112 Flying Test II). Serum levels of inflammatory markers such as VEGF, IL-1β, and IL-6 were measured by using commercially available ELISA kits (R&D Systems, Minneapolis, USA). Laboratory testing was conducted by trained technicians following the manufacturers' protocols.

Statistical Analyses

SPSS 22.0 statistical software was used to analyze data. For measurement data, the comparisons were conducted using the *t*-test between two groups and the one-way ANOVA for multiple groups, results were expressed as mean ± standard deviation. For count data, the comparisons were made using the chi-square test. The correlation between NETs and inflammatory factors was analyzed using Pearson correlation analysis. Logistic regression analysis was employed to explore the influencing factors of IBD diagnosis. The receiver operating characteristic (ROC) curve was used to evaluate the sensitivity and specificity of plasma NETs as biomarkers for IBD. After inclusion of all candidate variables (eg, NETs markers, inflammatory factors, demographic characteristics), LASSO regression was used to screen for the most predictive variables in high-dimensional data to avoid overfitting. A P-value <0.05 was considered statistically significant.

Results

Comparison of Clinical Characteristics

In this study, IBD patients were divided into two groups based on their pathological features: active (N=36) and inactive (N=64). The specific grouping details are shown in Figure 1. Baseline general clinical characteristics, as well as NETs markers and inflammatory factor levels, were compared among the two IBD groups and healthy controls. The results are shown in Table 1. The results showed that there were no statistical differences in the general clinical characteristics of gender, age, or BMI among the three groups. However, there were significant differences in the levels of NETs markers and inflammatory factors among the three groups (Figure 2).

For the levels of NETs markers, the cfDNA levels in the active IBD group (197.69±18.67 ng/mL) were significantly higher than that in the inactive IBD group (165.94±18.67 ng/mL) and the healthy controls (132.88±32.81 ng/mL) (P <0.001). The MPO-DNA levels in the active IBD group (67.27±12.01 mAU/mL) were significantly higher than that in the inactive IBD group (55.16±12.61 mAU/mL) and the healthy controls (35.98±16.28 mAU/mL) (P <0.001). The citH3 levels in patients with active IBD (4.12±1.36 ng/mL) were significantly higher than that in patients with inactive IBD (3.15 ±1.16 ng/mL) and healthy controls (1.88±1.03 ng/mL) (P <0.001).

Regarding inflammatory factors, CRP levels in the active IBD group (0.45 ± 0.25 mg/L) were higher than that of the inactive IBD group (0.34 ± 0.28 mg/L) and healthy controls (0.15 ± 0.51 mg/L) (P <0.001). VEGF levels in active IBD group (446.08 ±46.60 pg/L) were also higher than that of the inactive IBD group (302.60 ± 43.54 pg/L) and healthy controls (166.05 ±26.51 pg/L) (P <0.001). Similarly, IL-1β levels and IL-6 levels in the active IBD group were higher than those in the inactive IBD group and the healthy controls (P <0.001).

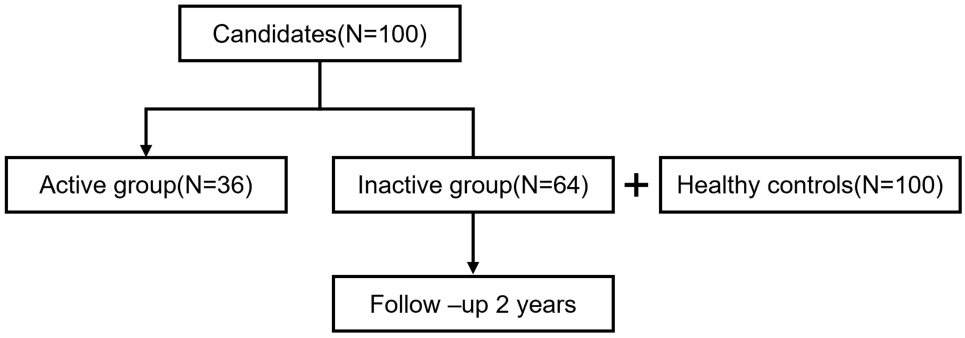

Figure 1 Enrollment of the study participants in the primary cohort.

Table 1 Comparison of Clinical Characteristics Among the Three Groups

Characteristic	Active IBD Group (N=36)	Inactive IBD Group (N=64)	Healthy Controls (N=100)	*P* Value
Age (years)	49.35 ± 11.05	48.24 ± 14.35	49.25 ± 14.10	0.882
Male, n (%)	18 (50.00%)	34 (53.12%)	58 (58%)	0.664
BMI (kg/m^2)	22.96 ± 3.39	23.27 ± 2.76	23.44 ± 3.02	0.712
Living alone, n (%)	4 (11.11%)	8 (12.5%)	10 (10%)	0.883
Medical history (diabetes, hypertension, cardiovascular and cerebrovascular diseases), n (%)	6 (16.67%)	12 (18.75%)	–	0.795
Smoking, n (%)	12 (33.33%)	28 (43.75%)	37 (37%)	0.631
Drinking, n (%)	14 (38.89%)	27 (42.18%)	41 (41%)	0.950
NSAIDs Use, n (%)	2 (5.55%)	5 (7.81%)	–	0.671
Blood in stool, n (%)			–	0.830
None	8 (22.22%)	16 (25%)		
Bloody purulent stool	17 (47.22%)	32 (50%)		
Fresh blood in stool	11 (30.56%)	16 (25%)		
Lesion extent, n (%)			–	0.976
Rectum	4 (11.11%)	8 (12.5%)		
Left colon	10 (27.78%)	18 (28.12%)		
Extensive colon	22 (61.11%)	38 (59.38%)		
Severity, n (%)			–	0.003
Mild	8 (22.22%)	34 (53.12%)		
Moderate	13 (36.11%)	20 (31.25%)		
Severe	15 (41.67%)	10 (15.63%)		
Endoscopic score, n (%)			–	0.922
≤ 1	1 (2.78%)	2 (3.12%)		
> 1	35 (97.22%)	63 (96.88%)		
Extraintestinal manifestations, n (%)	28 (77.78%)	26 (40.62%)	–	<0.001
Complications, n (%)	16 (44.44%)	12 (18.75%)	–	0.006
Gastrointestinal infection, n (%)	17 (47.22%)	12 (18.75%)	–	0.003
Antibiotic use, n (%)	9 (25%)	10 (15.62%)	–	0.251

Notes: One-way ANOVA: $F=MS_{between} / MS_{withen.}$

When comparing the clinical characteristics of the active IBD group and the inactive IBD groups, the active IBD group showed a higher prevalence of extraintestinal manifestations (P <0.001), gastrointestinal infections (P =0.003), complications (P =0.006), and more severe disease (P =0.003) than the inactive IBD group, and the differences were statistically significant.

Correlation Analysis Between Circulating NETs Markers and Inflammatory Factors (IL-1β, IL-6, CRP, and VEGF)

The study analyzed the correlation between circulating NETs markers and inflammatory factors (IL-1β, IL-6, CRP, and VEGF). The results showed that cfDNA was positively correlated with IL-1β, IL-6, and VEGF (IL-1β: r =0.542, P <0.001; IL-6: r =0.634, P <0.001; VEGF: r=0.655, P <0.001), MPO-DNA was positively correlated with IL-1β, IL-6, CRP and VEGF (IL-1β: r =0.487, P <0.001; IL-6: r =0.625, P <0.001; CRP: r=0.188, P =0.008; VEGF: r=0.622, P <0.001), citH3 showed positive correlation with IL-1β, IL-6 and VEGF (IL-1β: r =0.429, P <0.001; IL-6: r =0.577, P <0.001; VEGF: r=0.576, P <0.001). The results are shown in Table 2.

Logistic Regression Analysis of Independent Predictors for IBD and Evaluation of Predictive Ability Using ROC Curve

The study employed Logistic regression analysis to assess the relationship between the circulating NETs markers (cfDNA, MPO-DNA, citH3) and the occurrence of IBD. The results indicated that cfDNA, MPO-DNA, and citH3 are

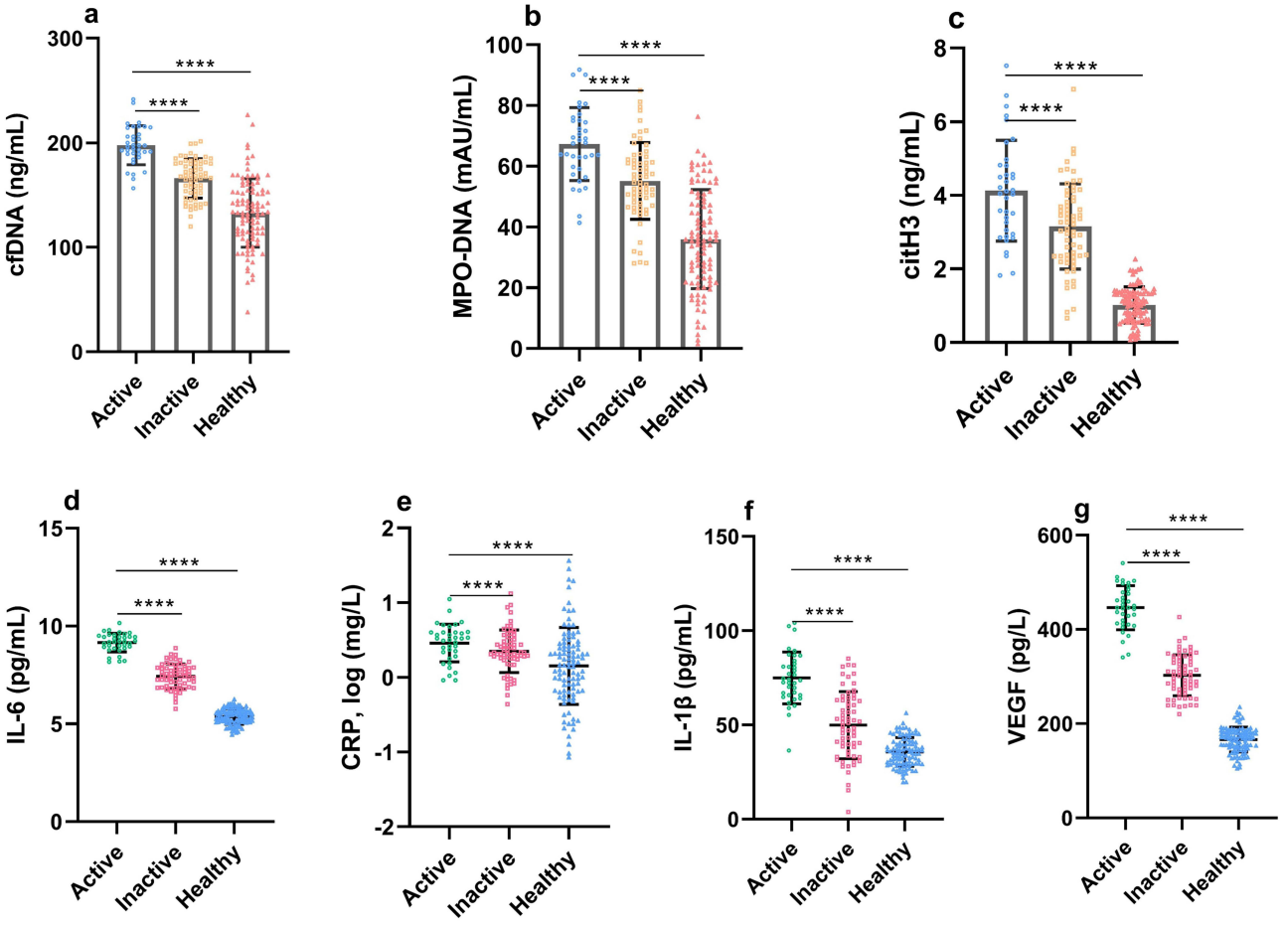

Figure 2 Comparison of NETs markers (**a–c**) and inflammatory factors (**d–g**) among the three groups. P<0.001: ****.

independent risk factors for the development of IBD (cfDNA: OR=1.045, 95% CIs: 1.025–1.067; MPO-DNA: OR=1.084, 95% CIs: 1.048–1.122; citH3: OR=2.871, 95% CIs: 1.786–4.616) (Table 3). The ROC curve evaluated the clinical value of these indicators in IBD, with the combination of all four markers showing the highest diagnostic efficacy (AUC=0.984) (Figure 3).

Table 2 Correlation Between Circulating NETs Markers and Inflammatory Factors

Characteristic	cfDNA		MPO-DNA		citH3	
	Correlation Coefficient	*P*- Value	Correlation Coefficient	*P*- Value	Correlation Coefficient	*P*- Value
IL-1β	0.542	<0.001	0.487	< 0.001	0.429	< 0.001
IL-6	0.634	<0.001	0.625	< 0.001	0.577	< 0.001
CRP	0.060	0.398	0.188	0.008	0.098	0.166
VEGF	0.655	<0.001	0.622	< 0.001	0.576	< 0.001

Table 3 Logistic Regression Analysis Reveals Independent Predictors for IBD

Predictor	OR	95% CI	*P*-Value
cfDNA	1.045	1.025–1.067	< 0.001
MPO-DNA	1.084	1.048–1.122	< 0.001
citH3	2.871	1.786–4.616	< 0.001

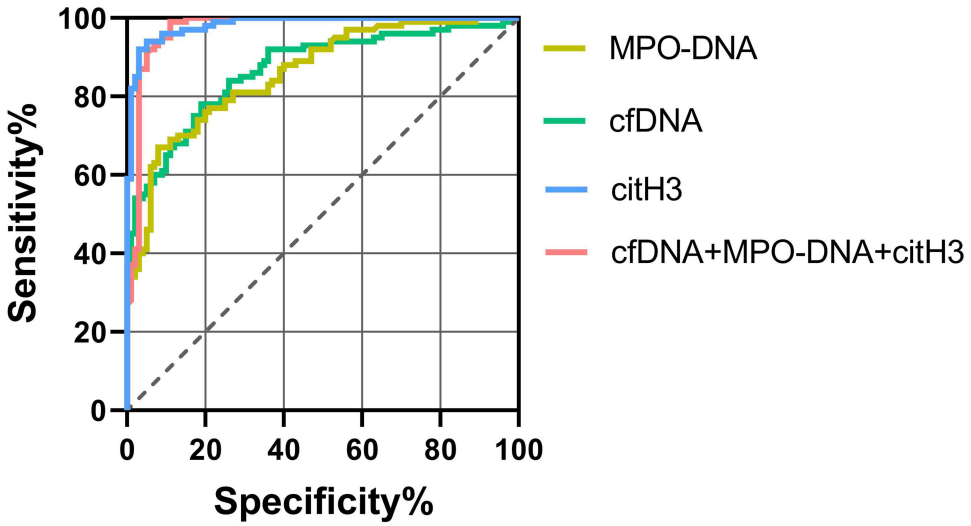

Figure 3 ROC curve analysis of cfDNA, MPO-DNA and citH3 as predictors of IBD.

The Impact of NETs on the Prognosis of IBD Patients

First, the study established a NETs scoring system based on prior research. In simple terms, each NETs marker was categorized as 0 or 1, and corresponding weights were assigned based on the AUC-ROC results. If the AUC was greater than 0.9, the weight was doubled, and the results were later converted to percentages.[24] The AUC results for each NETs marker are shown in Table 4. Subsequently, the study stratified the inactive group of IBD patients based on the median NETs score to analyze the impact of NETs scores on the prognosis of IBD patients. The results showed that compared with patients with lower NETs scores, patients with higher NETs scores may have a worse prognosis, and the probability of recurrence is significantly higher than that of patients with lower NETs scores (1-year follow-up: P = 0.026; 2-year follow-up: P = 0.044). These results are presented in Table 5.

Table 4 Performance of NETs Markers as Biomarkers in Patients with IBD

NETs Marker	AUC	95% CI	*P*-Value
cfDNA	0.868	0.819–0.918	< 0.001
MPO-DNA	0.861	0.811–0.910	< 0.001
citH3	0.839	0.784–0.894	< 0.001

Table 5 Effect of NETosis Score on the Prognosis of Patients with IBD

Characteristic	Low Level (N = 32)	High Level (N=32)	*P* Value
1-year follow-up			0.026
Relapse, n (%)	5(15.62%)	13(40.62%)	
Remission, n (%)	27(84.38%)	19(59.38%)	
2-year follow-up			0.044
Relapse, n (%)	14(43.75%)	22(68.75%)	
Remission, n (%)	18(56.25%)	10(31.25%)	

Notes: Low group (<6.7); high group (≥6.7).

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Discussion

IBD is a type of autoimmune disease characterized by chronic intestinal inflammation. Key clinical manifestations include recurrent abdominal pain, diarrhea, and bloody stools containing mucus and pus, severely impacting patients' quality of life. Its diagnosis, disease monitoring, and prognosis assessment have always been a hot topic in clinical research.[25] Traditional biomarkers such as CRP, VEGF, IL-1β, and IL-6 play an important role in the onset and progression of IBD and have been widely used in clinical practice. However, these markers have limitations regarding specificity, sensitivity, and their ability to predict disease progression.

The NETs markers cfDNA, MPO-DNA, and citH3 that this study focuses on are important components of NETs and show unique value in the pathogenesis of IBD.[26] NETs are web-like structures released by neutrophils under specific stimulation, possessing both antimicrobial and pro-inflammatory properties.[27] Our findings reveal that the levels of cfDNA, MPO-DNA, and citH3 are significantly elevated in active IBD patients compared to inactive IBD patients and healthy controls. This trend aligns with traditional inflammatory biomarkers but suggests that NETs biomarkers may be more sensitive and specific in reflecting IBD activity and severity. The levels of cfDNA, MPO-DNA, and CitH3 in the active IBD group were significantly higher than those in the remission group and the healthy control group. cfDNA is the structural backbone of NETs, and its release reflects neutrophil activation and the degree of NETs formation. MPO is a NET-specific enzyme, and its co-localization with DNA is a direct evidence of NETs formation. It is a product of histone citrullination and is necessary for NETs formation. IL-1β is secreted by macrophage/NETs-activated NLRP3 inflammatory vesicles and directly disrupts intestinal epithelial tight junctions. IL-6 promotes Th17 differentiation and inhibits Treg function, exacerbating mucosal immune imbalance. The significant correlation of both with NETs supports the hypothesis that NETs amplify inflammation through pro-inflammatory factors, which is consistent with the goal of the study to explore the NETs-inflammation axis. CRP is an acute-phase protein synthesized by hepatocytes that reflects systemic inflammation, but it lacks intestinal specificity. Its secretion promotes pathological vascular proliferation and intestinal wall fibrosis. Furthermore, NETs activate VEGF signaling by releasing molecules such as HMGB1, which aligns with the strong correlation observed between the two in the study.

Further analysis showed that there was a significant positive correlation between NETs markers and inflammatory factors (IL-1β, IL-6, CRP, and VEGF), indicating that the formation and release of NETs are closely related to the inflammatory response of IBD. Compared to traditional inflammatory factors, NETs markers may play a more direct role in the pathogenesis of IBD. NETs not only capture and eliminate pathogens through their reticular structure, but also amplify inflammatory responses and exacerbate tissue damage via proteolytic enzymes and granular proteins attached to the cfDNA backbone.[28] Additionally, NETs may contribute to aseptic inflammatory in tissues and organs. Intravascular NET formation can lead to small vessel inflammation, hepatitis, thrombosis, or lupus nephritis. In the lungs, NET formation is involved in the pathophysiological mechanism of cystic fibrosis.[29–33] Interleukin involvement in intestinal inflammatory responses may be associated with ROS system activation and regulation of the MAPK, NF-κB, and NLRP3 pathways.[34] The study confirms that NETs markers rise during IBD flares and correlate with inflammation, aligning with previous research. While NETs exhibit similar trends in chronic inflammatory diseases, their role in IBD is distinct. Unlike in RA (synovium) or SLE (kidney), IBD-related NETs interact with gut flora due to mucosal barrier damage, potentially amplifying inflammation via the TLR9/NF-κB pathway. While NETs in RA and SLE are linked to acute exacerbations, this study found baseline NETs scores predict 2-year IBD recurrence risk, emphasizing their unique role in chronic disease management.

The study also found that cfDNA, MPO-DNA and citH3 are independent risk factors for IBD, and the combination of the four had the highest diagnostic efficacy (AUC=0.949). However, the molecular mechanisms and signaling pathways underlying the formation of NETs remain unclear. Research used HE staining of colon specimens from patients with UC has revealed the presence of numerous fragmented nuclei within crypts. Proteomic analysis further identified a significant increase in 11 protein components involved in NETs formation.[35] The mechanisms through which NETs cause intestinal mucosal damage are complex. Some studies suggest that NETs increase vascular endothelial cell permeability, leading to tissue edema and oxidative stress-induced injury.[36] Consequently, NETs markers may serve as novel biomarkers for IBD, offering more accurate and sensitive methods for disease diagnosis, monitoring, and prognosis evaluation.

Given the unique value of NETs markers in IBD, this study attempted to establish a novel diagnostic system based on NETs. The system first quantified cfDNA, MPO-DNA, and citH3 levels in patients' plasma, converting the values of these NETs markers into binary categories (0–1) and assigning corresponding weights based on receiver operating characteristic (ROC) curve analysis. Markers with an AUC exceeding 0.9 were assigned double weights to emphasize their significance in IBD diagnosis. The results were then transformed into percentages to derive a NETs score. Stratified analysis of NETs scores in inactive IBD patients revealed that patients with higher NETs scores had poorer prognoses, with significantly higher relapse rates compared to those with lower NETs scores. The study maximized the assurance that the results of NETs markers reflect the pathological activity of IBD itself by strictly excluding biologic and antibiotic interferences. Although previous studies have suggested that anti-TNF-α drugs can inhibit NETs release, NETs levels in patients with biologics discontinuation in the present cohort did not differ from those in the unexposed group, suggesting a negligible residual effect. These findings suggest that the novel diagnostic system based on NETs can not only aid in diagnosing and monitoring IBD but also provide crucial information for prognosis assessment. Early identification of patients with high NETs scores and enhanced therapeutic and long-term disease management may improve treatment outcomes and the quality of life for patients.

As a novel biomarker, NETs markers demonstrate unique advantages in the diagnosis, monitoring, and prognosis evaluation of IBD. The NETs-based diagnostic system offers new insights and methods for the clinical management of IBD. In the future, with deeper research into the role of NETs in the pathogenesis of IBD and advancements in detection technology, the NETs-based diagnostic system is expected to be further optimized, providing better treatment and management options for IBD patients. In addition, the disease duration of IBD patients in this study ranged from 0.5 to 21 years, with a median duration of 5.2 years. Patients with long disease duration may have elevated cfDNA levels due to repeated inflammation accumulation, while MPO-DNA and CitH3 more directly reflect current active inflammation.

However, this study has certain limitations. As a single-center study, it is restricted by the variables and the number of patients included. Future studies involving multi-center, large-sample cohorts are needed to validate these findings further.

Conclusion

The findings of this study suggest that circulating markers of NETs hold significant promise as versatile biomarkers for various aspects of IBD, including diagnosis, disease monitoring, and prognosis. These markers offer a unique opportunity for timely evaluation of patients' risk of relapse, enabling the implementation of more targeted and effective treatment strategies. Furthermore, their utilization can facilitate comprehensive long-term disease management, potentially leading to improved therapeutic responses and an enhanced quality of life for IBD patients. This underscores the transformative potential of NETs markers in the clinical management of IBD.

Data Sharing Statement

All relevant data are contained within the article. Additional data supporting the findings of this article are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of Changshu No.2 People's Hospital (2023-KY-SK01). Informed consent was obtained from all participants.

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Disclosure

The authors declare no conflict of this research.

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