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Peripheral Blood Lymphocyte Subsets as Biomarkers for the Severity of Inflammatory Bowel Disease Subtypes: A Cross-Sectional Study

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Abstract

Objective: This study aimed to assess IBD severity and its prognostic activity using peripheral blood lymphocyte subsets.

Methods: The study was conducted on 46 patients with Crohn's disease (CD), 55 with ulcerative colitis (UC), and 20 healthy controls (HCs). Based on the Harvey-Bradshaw Index (HBI) and Mayo score, patients were classified into remission or active status. Blood samples were collected from patients and analyzed for CD3+, CD4+, CD8+, CD4+/CD8+ ratio, and CD19+ proportions. Differential leukocytes, platelets, albumin, globulin, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and faecal calprotectin were also assessed.

Results: CD4+, CD8+, and CD19+ proportions were increased in CD and UC patients, while CD3+ was declined. CD4+ and CD19+ were elevated in remission and active CD, but CD8+ was changed only in active CD. Among UC patients, lymphocyte subsets were elevated in remission and active status. CD19+ positively correlated with CD severity. Consequently, CD19+ combined with inflammatory markers was highly significant in CD.

Conclusion: These results suggest that peripheral blood lymphocyte subsets are good biomarkers for the IBD severity and prognostic activity with great clinical application values.

Keywords: Crohn's disease, ulcerative colitis, lymphocyte subsets, disease severity, inflammatory biomarkers

Introduction

Inflammatory bowel disease (IBD) is divided into two subtypes: Crohn's disease (CD) and ulcerative colitis (UC).[1] Accurate identification and diagnosis of IBD subtypes and their severity require a comprehensive approach, incorporating clinical assessments, laboratory tests, radiological imaging, endoscopic evaluations, and histological analyses.[2]

The complexity of IBD often leads to a progressive course, where patients can shift from remission to severe symptoms. Thus, effective monitoring of disease activity is pivotal for optimizing treatment strategies and improving patient outcomes.[3-5]

Current methods for assessing disease severity are predominantly invasive, presenting challenges such as discomfort, risk, and high costs.[6,7] These limitations underscore the necessity for noninvasive biomarkers that reliably indicate disease severity and anticipate flare-ups. Traditional inflammatory markers, including C-reactive protein (CRP), anti-Saccharomyces cerevisiae antibodies (ASCA), faecal calprotectin, and erythrocyte sedimentation rate (ESR), are frequently utilized in clinical settings. However, they exhibit varying sensitivities and specificities, complicating their clinical application.[1,8,9] Moreover, collecting and processing faecal samples can be inconvenient and costly.

Recent studies have increasingly focused on the role of immune dysregulation in IBD, highlighting the involvement of various immune cells, particularly lymphocytes, in disease pathogenesis.[10,11] The dysregulation of adaptive immune responses, especially regarding lymphocyte subsets, may play a pivotal role in intestinal inflammation among IBD patients. Investigating the relationships between specific lymphocyte subsets and IBD severity presents a promising avenue for enhancing diagnostic accuracy.[12,13]

The present study aimed to establish the proportional changes in peripheral lymphocyte subsets among CD and UC patients compared to healthy individuals. Additionally, it explored the relationship between lymphocyte subsets and disease severity and their potential utility as noninvasive biomarkers in conjunction with traditional inflammatory markers.

Materials and Methods

Study Design and Setting

This prospective cross-sectional study was conducted from October 2022 to July 2023 at the Teaching Hospital for Gastroenterology and Hepatology, Sulaimaniyah, Iraq. The study included a total of 121 participants, comprising of 20 healthy controls (HCs), 46 patients with confirmed CD, and 55 patients with UC that a professional gastroenterologist diagnosed (Figure 1).

Diagnostic Criteria

Diagnosis of CD and UC was confirmed through standard laboratory tests, clinical evaluations, radiological imaging, endoscopic examinations, and histological methods.[3,14,15] CD severity was determined using the Harvey-Bradshaw Index (HBI),[16] with patients categorized as remission (HBI ≤4) or having active disease (HBI >4).[3] UC severity was assessed using the Mayo score,[15] where a score of up to 5 indicated remission, and scores >5 indicated active disease.[3] Healthy participants showed no signs of immune-mediated diseases or IBD. The Montreal classification guidelines assessed disease location and behaviour in CD and the extent of disease in UC.[17] CD behaviours were classified as B1 (non-structuring, non-penetrating), B2 (structuring), and B3 (penetrating),

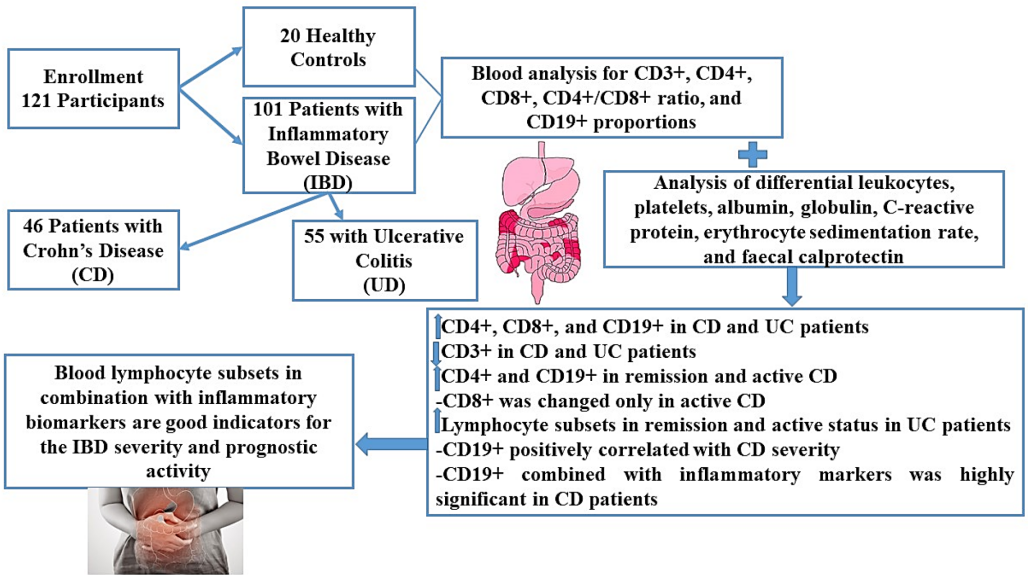

Fig. 1 **Flow diagram of studied participants.**

while UC extent was categorized into E1 (proctitis), E2 (left-sided colitis), and E3 (pancolitis).

Inclusion Criteria

This study included confirmed diagnosed IBD patients.

Exclusion Criteria

Patients with undetermined IBD, acute or chronic infections, immune-mediated diseases, autoimmune diseases, or histories of intestinal surgeries that may influence the leukocyte ratios were excluded.

Samples and Data Collection

Peripheral blood samples were collected from patients with IBD and HCs in EDTA anticoagulant tubes. Using a BD FACS-Canto™ II flow cytometry (BD Biosciences, USA), the proportions of lymphocyte subsets (CD3+, CD4+, CD8+, and CD19+) were measured according to product protocol,[18] and the CD4+/CD8+ ratio was calculated. Clinical data included demographic information such as age, gender, body mass index (BMI), smoking history, and treatment regimens for CD and UC. Laboratory parameters were determined, including CRP, serum albumin, and serum globulin, using a Cobas c311 biochemical analyzer (Roche-Hitachi, Germany), with the serum albumin to globulin ratio (AGR) calculated. Haematological parameters, such as platelets (PLT), neutrophils, lymphocytes, and monocytes, were assessed using Medonic M51 Hematology Analyzer, Sweden. ESR and faecal calprotectin levels were also recorded. All assessments were conducted on the same day to preserve blood consistency.

Statistical Analysis

The Shapiro-Wilk test was used to assess the normality distribution of the data. T-tests and ANOVA tests were used to analyze the normally distributed data, while baseline nonparametric data from participants were compared to HCs using Mann-Whitney t-tests. One-way ANOVA was employed to compare disease severity in CD and UC with HCs, utilizing post-hoc analysis for continuous variables. Qualitative data were analyzed using the Fisher exact Chi-square test. The correlation between lymphocyte subsets and the HBI in CD and the Pearson correlation coefficient was used to evaluate Mayo scores in UC. The efficacy of lymphocytes and inflammatory biomarkers was determined via receiver operating characteristic (ROC) curves, identifying optimal cutoff points for sensitivity and specificity. Statistical significance was set at $P < 0.05$, and analyses were conducted using IBM SPSS Statistics 26.0 software.

Results

Characteristics of the Participants

Features of IBD patients were compared to those of HCs. Disease activity revealed that 18 CD patients were in remission and 28 were active. Among the 55 UC patients, 20 were in remission, and 35 were active. The mean age of patients with CD in remission (31.44 ± 15.81 years) and those with active disease (31.75 ± 13.51 years) did not differ significantly from HCs (32.15 ± 11.13 years) ($P = 0.87$ and $P = 0.91$, respectively). Gender distribution among CD patients showed 12 males (66.66%) in remission and 12 (42.85%) in active status, with no significant difference compared to HCs ($P = 0.28$). BMI was significantly lower in both remission (23.2 ± 4.41 kg/m^2) and active CD patients (20.7 ± 4.93 kg/m^2) compared to HCs (26.9 ± 2.28 kg/m^2) ($P = 0.003$ and $P < 0.0001$, respectively) (Table 1).

For UC patients, BMI values were 23.99 ± 3.5 kg/m^2 for remission and 22.35 ± 5.36 kg/m^2 for active status, significantly lower than HCs ($P = 0.0037$ and $P < 0.0001$, respectively). Other characteristics such as age, gender, smoking history, disease extension classification, and treatment were not significant between remission and active states for either CD or UC ($P > 0.05$) (Table 2).

Laboratory Parameters

Serum levels of CRP and ESR were significantly elevated in both the remission ($P = 0.005$ and $P = 0.002$, respectively) and the active CD group ($P < 0.001$ and $P < 0.001$, respectively)

Table 1. **Baseline characteristics of Crohn's disease (CD) patients and healthy controls (HC)**

Variable	Control ($n = 20$)	CD-Remission ($n = 18$)	CD-Active ($n = 28$)	Post-hoc		
				*P*1	*P*2	*P*3
Age (Years) (Mean ± SD)	32.15 ± 11.13	31.44 ± 15.81	31.75 ± 13.51	0.87	0.91	0.94
Gender						0.28
Male	10 (50%)	12 (66.66%)	12 (42.85%)			
Female	10 (50%)	6 (33.3%)	16 (57.14%)			
BMI (kg/m²)	26.9 ± 2.28	23.2 ± 4.41	20.7 ± 4.93	0.003	<0.0001	0.08
History of smoking *n* (%)						0.83
Non-smoker	15 (75%)	14 (77.7%)	24 (85.7%)			
Smoker	3 (15%)	3 (16.6%)	2 (7.1%)			
Shisha	2 (10%)	1 (5.5%)	2 (7.1%)			
Disease location						0.66
- L1		8	9			
- L2		4	8			
- L3		6	11			
- L4		6	5			
Disease phenotypes						0.97
- B1		5	8			
- B2		5	7			
- B3		8	13			
Treatment						0.28
- 5-ASA		3	4			
- AZA		2	10			
- Infliximab		7	8			
- AZA+Infliximab		3	3			
CRP (mg/L)	1.61 ± 0.99	14.82 ± 19.75	31.26 ± 25.7	0.005	<0.0001	0.018
ESR (mm/hour)	8.4 ± 2.87	31.11 ± 20.65	51.39 ± 25.42	0.001	<0.0001	0.005
Alb. (g/dL)	5.3 ± 0.66	4.31 ± 0.62	3.78 ± 0.77	0.0002	<0.0001	0.04
Glo. (g/dL)	1.3 ± 0.53	2.78 ± 0.74	3.39 ± 0.84	<0.0001	<0.0001	0.02
AGR	4.91 ± 2.49	1.68 ± 0.59	1.23 ± 0.6	<0.0001	<0.0001	0.56
Fecal Calprotectin (µg/g)	10.59 ± 3.67	288.7 ± 287.4	548.5 ± 374.5	0.011	<0.0001	0.01
PLT (10^3/ml)	221.2 ± 23.9	346.3 ± 186.1	342.3 ± 126.5	0.01	0.005	0.99
Neutrophil (10^3/ml)	4.34 ± 0.64	4.93 ± 2.17	4.87 ± 1.88	0.54	0.544	0.99
Lymphocyte (10^3/ml)	3.22 ± 0.47	2.24 ± 1.25	1.59 ± 0.68	0.0018	<0.0001	0.029
Monocytes (10^3/ml)	0.46 ± 0.14	0.39 ± 0.17	0.59 ± 0.26	0.53	0.1	0.006
NLR	1.37 ± 0.28	2.85 ± 2.37	3.64 ± 2.00	0.037	0.0002	0.32
LMR	7.45 ± 2.17	6.54 ± 4.17	3.01 ± 1.36	0.54	<0.0001	0.0001

BMI, Body mass index; L1; Ileal, L2; Colonic, L3; Ileocolonic, L4; Including upper GI, B1; Inflammatory, B2; Structuring, B3; Penetrating, 5-ASA; 5-Aminosalicylic Acid, AZA, Azathioprine; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; Alb., Albumin; Glo., Globulin; AGR, Albumin to globulin ratio; PLT, platelets; NLR, neutrophil to lymphocyte ratio; and LMR, Lymphocyte to monocyte ratio.

compared to HCs. Additionally, active CD patients exhibited higher levels of CRP and ESR than those in remission ($P = 0.018$ and $P = 0.005$, respectively). Other biomarkers, such as serum globulin, faecal calprotectin, PLT, and neutrophil-to-lymphocyte ratio (NLR), were also significantly altered in both remission ($P < 0.0001$, $P = 0.11$, $P = 0.01$, and $P = 0.037$, respectively) and active CD groups ($P < 0.0001$, $P < 0.0001$, $P = 0.005$, and $P = 0.0002$, respectively) compared to HCs. Globulin, faecal calprotectin, and monocytes were significantly higher in the active CD group than in the remission group ($P = 0.02$, $P = 0.01$, and $P = 0.006$, respectively). Serum albumin, AGR, and lymphocyte counts were lower in both remission ($P = 0.0002$, $P < 0.0001$, and $P = 0.0018$, respectively) and active CD groups ($P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively).

In UC patients, serum CRP, ESR, globulin, and faecal calprotectin levels were also significantly altered compared to HCs in both remission ($P = 0.07$, $P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively) and active UC groups ($P = 0.0041$,

Table 2. **Baseline characteristics of ulcerative colitis (UC) patients and healthy controls (HC)**

Variable	Control (*n* = 20)	UC-Remission (*n* = 20)	UC-Active (*n* = 35)	Post-hoc		
				*P*1	*P*2	*P*3
Age (Years) (Mean ± SD)	32.15 ± 11.13	31.45 ± 12.93	30.49 ± 12.56	0.98	0.87	0.95
Gender						0.21
Male *n* (%)	10 (50%)	11 (55%)	17 (48.57%)			
Female *n* (%)	10 (50%)	9 (45%)	18 (51.42%)			
BMI (kg/m²)	26.9 ± 2.28	23.99 ± 3.5	22.35 ± 5.36	0.0037	<0.0001	0.17
History of smoking *n* (%)						0.4
- Non-smoker	15 (75%)	15 (75%)	25 (71.42%)			
- Ex-Smoker	0 (0%)	1 (5%)	6 (17.14%)			
- Smoker	3 (15%)	2 (10%)	2 (5.71%)			
- Shisha	2 (10%)	2 (10%)	2 (5.71%)			
Classification						0.72
- E1		5	8			
- E2		8	11			
- E3		7	16			
Treatment						0.71
- 5-ASA		7	10			
- AZA		6	7			
- Infliximab		4	9			
CRP (mg/L)	1.61 ± 0.99	20.14 ± 19.83	25.93 ± 35.14	0.07	0.0041	0.71
ESR (mm/hour)	8.4 ± 2.87	51.55 ± 25.09	53.44 ± 26.96	<0.0001	<0.0001	0.95
Alb. (g/dL)	5.3 ± 0.66	4.12 ± 0.6	3.67 ± 0.89	<0.0001	<0.0001	0.102
Glo. (g/dL)	1.3 ± 0.53	2.94 ± 0.75	3.27 ± 0.81	<0.0001	<0.0001	0.23
AGR	4.91 ± 2.49	1.53 ± 0.57	1.24 ± 0.56	<0.0001	<0.0001	0.73
F. Calprotectin (µg/g)	10.59 ± 3.67	520.7 ± 3521	573.6 ± 444.7	<0.0001	<0.0001	0.85
PLT (10^3/ml)	221.2 ± 23.9	256 ± 62.89	339 ± 120	0.43	<0.0001	0.004
Neutrophil (10^3/ml)	4.34 ± 0.64	4.94 ± 1.21	5.22 ± 2.36	0.53	0.044	0.83
Lymphocyte (10^3/ml)	3.22 ± 0.47	2.008 ± 0.63	1.75 ± 1.08	<0.0001	<0.0001	0.53
Monocytes (10^3/ml)	0.46 ± 0.14	0.39 ± 0.26	0.37 ± 0.24	0.55	0.35	0.97
NLR	1.37 ± 0.28	2.62 ± 0.78	4.81 ± 5.17	0.51	0.002	0.081
LMR	7.45 ± 2.17	6.91 ± 3.68	4.23 ± 4.03	0.88	0.005	0.022

BMI, Body mass index; E1; Proctitis, E2; Left side, E3; Pancolitis (Extensive), 5-ASA, 5 Aminosalicylic Acid; AZA, Azathioprine; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; Alb., Albumin; Glo., Globulin; AGR, Albumin to globulin ratio; PLT, platelets; NLR, neutrophil to lymphocyte ratio; and LMR, Lymphocyte to monocyte ratio.

$P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively). Furthermore, the active UC group's PLT count, neutrophils, and NLR significantly differed from HCs ($P < 0.0001$, $P = 0.044$, and $P = 0.002$, respectively). The serum albumin, AGR, and lymphocyte count in the remission and active UC groups were lower than in HCs ($P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively). The lymphocyte-to-monocyte ratio (LMR) was significantly lower in the active UC group than in the remission UC group and HCs ($P = 0.022$ and $P = 0.005$, respectively).

Distribution of Lymphocyte Subsets in CD and UC Patients Compared to HC

The mean lymphocyte subset proportions in HCs were as follows: CD3+ (79.37 ± 3.007), CD4+ (36.59 ± 3.641), CD8+ (36.78 ± 3.987), CD4+/CD8+ ratio (1.007 ± 0.15), and CD19+ (7.61 ± 1.045). Compared to HCs, the proportion of CD3+ cells in the CD group was significantly lower at 75.75 ± 6.71 ($P = 0.037$) (Figure 2A). In contrast, CD4+ (47.16 ± 8.75), CD8+ cell proportions (43 ± 8.91), and the proportion of CD19+ cells in the CD group (12.68 ± 3.97) was significantly higher in the CD group ($P < 0.0001$, $P = 0.0009$, and $P < 0.0001$, respectively) (Figure 2B, C, and E). The CD4+/CD8+ ratio in the CD group (1.17 ± 0.46) did not show a significant difference compared to HCs ($P = 0.13$) (Figure 2D).

In the remission and active CD group, CD4+ ($P = 0.0002$ and $P < 0.0001$, respectively) and CD19+ proportions ($P = 0.012$ and $P < 0.0001$, respectively) were significantly altered. The CD8+ proportion showed no significant change ($P = 0.128$). However, in the active CD group, CD8+ proportions were altered considerably ($P = 0.0087$). Additionally, the proportion

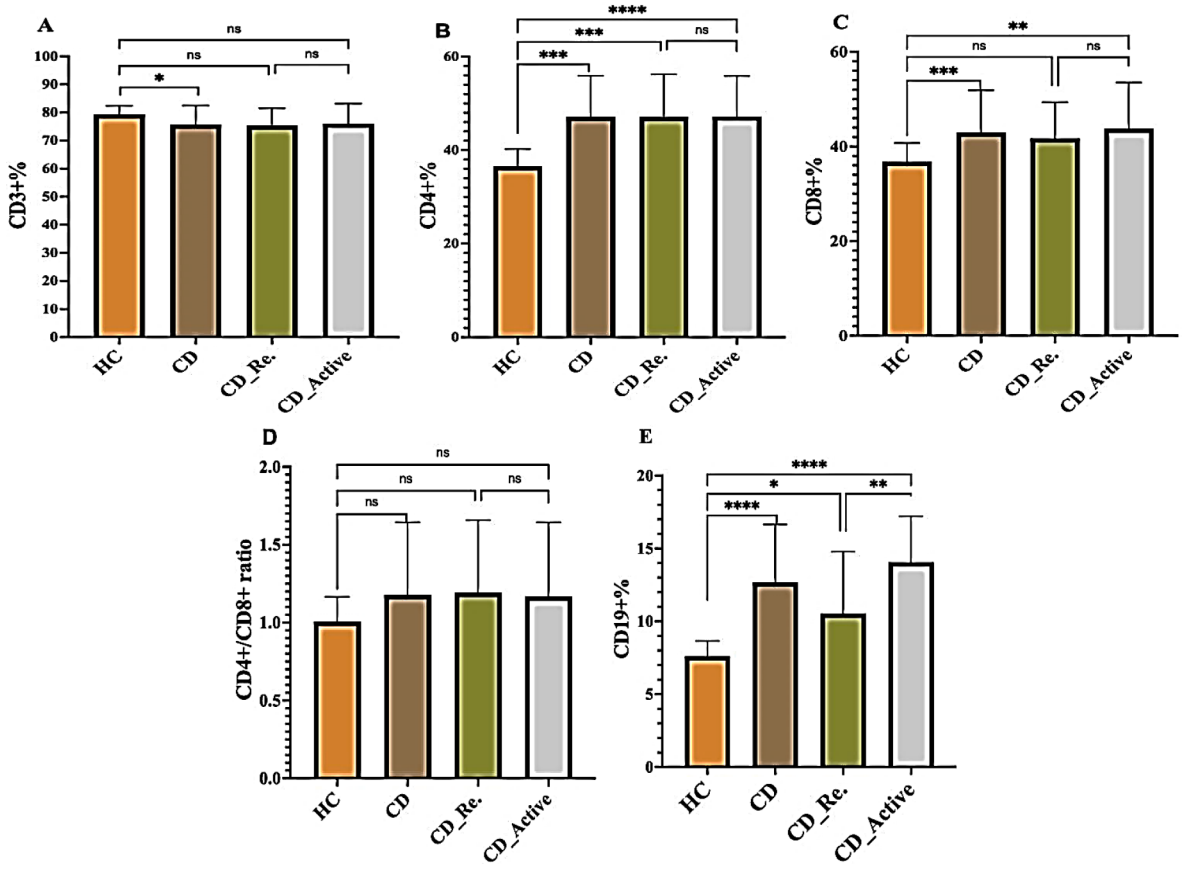

Fig. 2 **The proportion of lymphocyte subsets (CD3+, CD4+, CD8+, CD4+/CD8+ ratio, and CD19+) in patients with Crohn's disease and healthy controls.**

of CD19+ was significantly higher in the active CD group compared to the remission group ($P = 0.001$). Considered the UC group, the CD3+ (75.36 ± 9.43) was considerably lower than the HC ($P = 0.0067$) (Figure 3A). The proportion of CD4+ (47.34 ± 10.74), CD8+ (47.55 ± 10.15), and CD19+ (11.92 ± 5.93) were higher than HC ($P < 0.0001$, $P < 0.0001$, and $P = 0.0037$, respectively) (Figure 3B, C, and E). Moreover, the proportion of CD4+, CD8+, and CD19+ was significantly higher in the remission UC group ($P = 0.001$, $P = 0.003$, and 0.005, respectively) and active UC group ($P = 0.0005$, $P < 0.0001$, and 0.027, respectively) compared to HC (Figures 3B, C, and E).

Correlations Association of Lymphocytes Subset with the Disease Severity of CD and UC

The CD group's proportion of CD19+ ($r = 0.523$; $P = 0.0002$) (Figure 4E) positively correlates with HBI. Moreover, there was no correlation among other lymphocyte subsets (CD3+ $r = 0.12$; $P = 0.427$; CD4+ – $r = 0.066$; $P = 0.656$; CD8+ – $r = 0.217$; $P = 0.148$; CD4+/CD8+ r = -0.108; $P = 0.474$) (Figure 4A-D).

Further, the correlation between the UC group's lymphocyte subsets and Mayo scores was insignificant (CD3+ – $r = 0.12$; $P = 0.36$; CD4+ – $r = -0.03$; $P = 0.82$; CD8+ – $r = 0.018$; $P = 0.89$; CD4+/CD8+ – r = -0.022; $P = 0.87$; CD19+ $r = -0.136$; $P = 0.37$) (Figure 5).

The Lymphocyte Subset Value in Assessing Disease Severity Among CD and UC Groups

The analysis revealed that the AUC for the proportion of CD19+ cells was 0.770 ($P = 0.002$), with a cutoff value of 11.1% (Figure 6A). For the inflammatory markers, the AUC for CRP, ESR, and faecal calprotectin were 0.764 ($P = 0.003$), 0.731 ($P = 0.009$), and 0.737 ($P = 0.007$), respectively (Figure 6B). The cutoff values for CD3+, CD4+, CD8+, and the CD4+/CD8+ ratio were 81.8, 47.75, 48.75, and 1.18, respectively. The corresponding AUCs were 0.526 ($P = 0.770$), 0.528 ($P = 0.753$), 0.575 ($P = 0.392$), and 0.490 ($P = 0.910$) (Table 3).

Additionally, we evaluated the combined efficacy of the CD19+ proportion with CRP, ESR, and faecal calprotectin to predict CD severity using binary logistic regression. The combination of CD19+ and CRP demonstrated the most robust predictive efficacy, with a cutoff value of 0.611 ($P = 0.000082$) and an AUC of 0.847. Similarly, the combination of CD19+ with ESR showed an AUC of 0.819 at a cutoff of 0.612 ($P = 0.00029$), while the combination with faecal calprotectin had an AUC of 0.827 at a cutoff of 0.53 ($P = 0.00024$) (Figure 6C).

Among UC patients, the ROC curve for CD3+ cell proportions yielded an AUC of 0.68 ($P = 0.027$), with a cutoff value of 78.75%, resulting in a sensitivity of 48.6% (Figure 7A). For inflammatory markers, the AUC of CRP was 0.709 ($P = 0.011$), and ESR was 0.763 ($P = 0.001$). The cutoff values were 13.42 mg/dL and 55.5 mm/hr for ESR (Figure 7B). Furthermore, for lymphocyte subsets, the AUCs were as follows: CD4+ at 0.495, CD8+ at 0.533, CD4+/CD8+ ratio at 0.470, and CD19+ at 0.434. Moreover, the efficacy of the CD3+ proportion combined with CRP, ESR, and faecal calprotectin was assessed (Figure 7C).

The combination of CD3+ with CRP had an AUC of 0.821 ($P < 0.001$), with a cutoff value of 0.618. The combination with ESR showed a higher AUC of 0.854 ($P < 0.001$) at a cutoff of

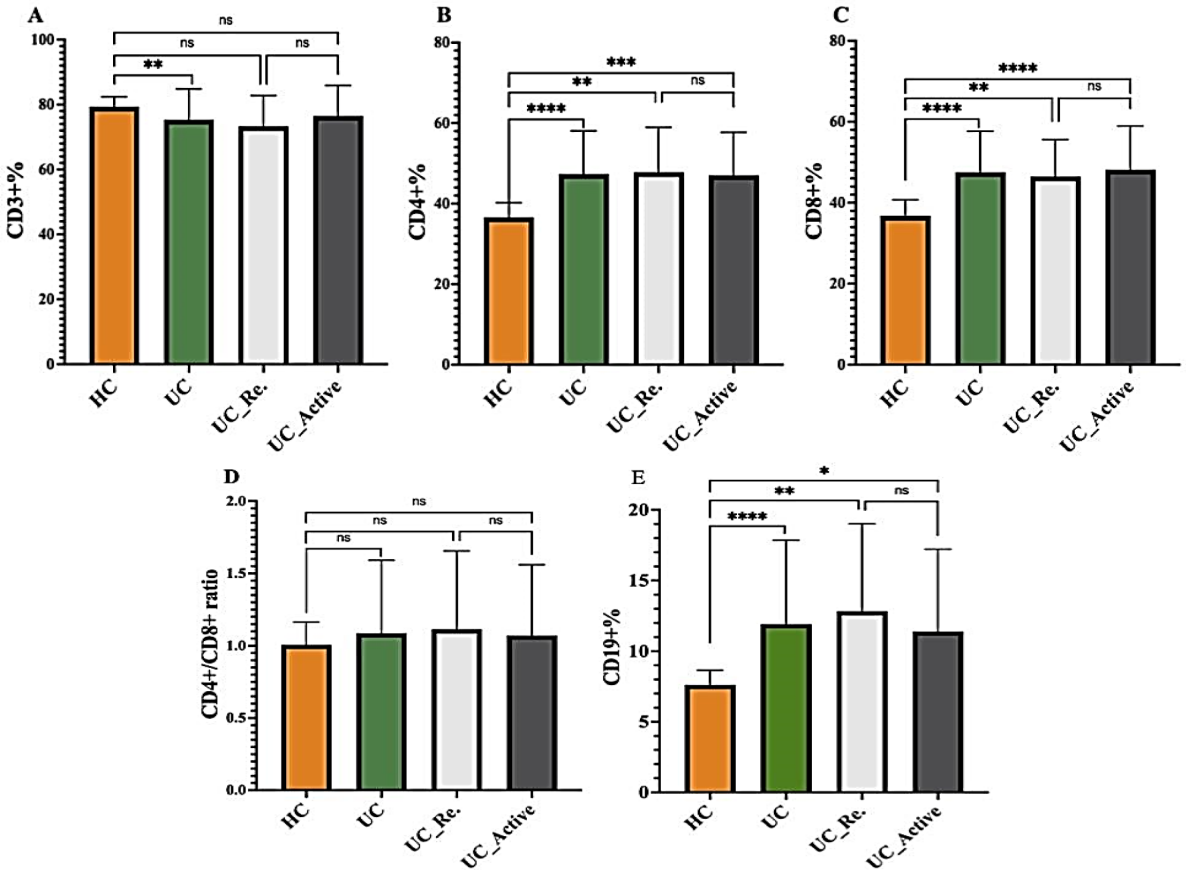

Fig. 3 **The proportion of lymphocyte subsets (CD3+, CD4+, CD8+, CD4+/CD8+ ratio, and CD19+) in patients with ulcerative colitis and healthy controls.**

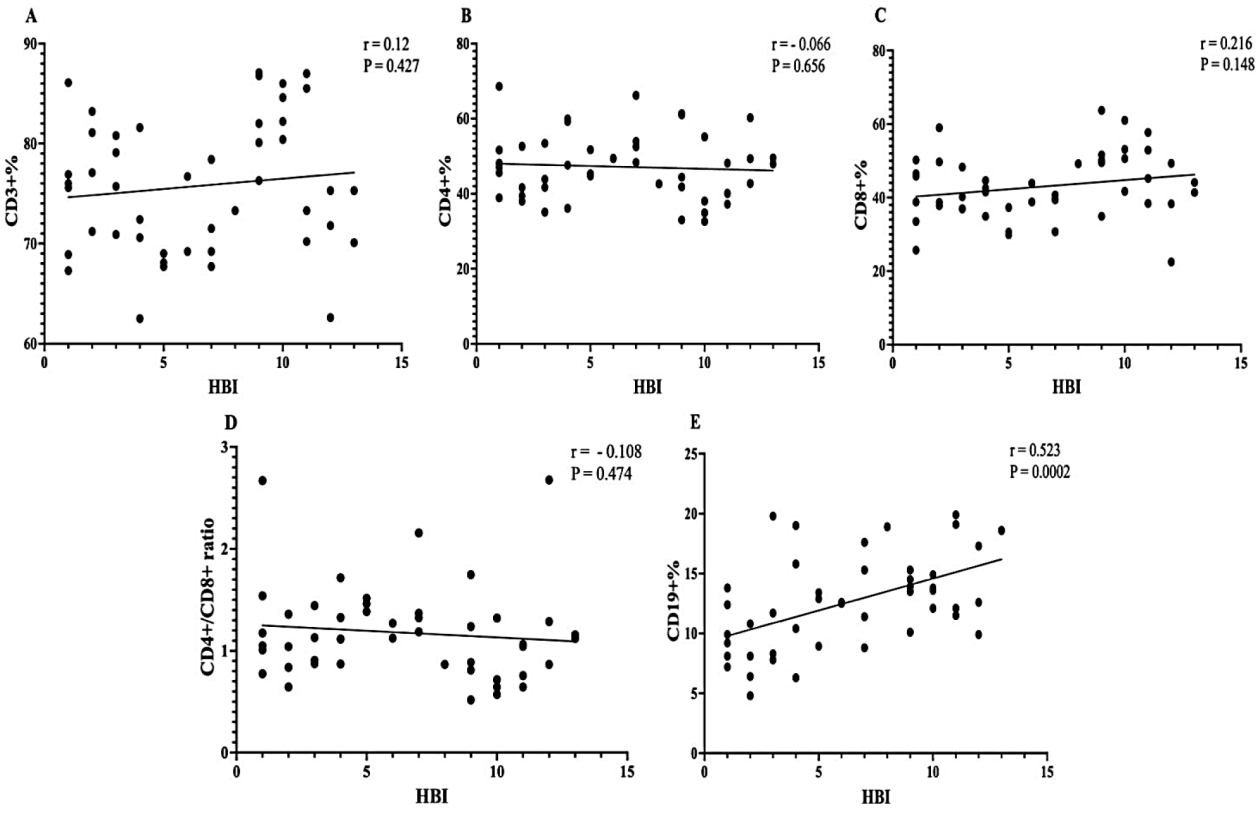

Fig. 4 **The correlations between CD patients' Harvey-Bradshaw Index (HBI) and proportional lymphocyte subset cells (CD3+, CD4+, CD8+, CD4+/CD8+ ratio, and CD19+).**

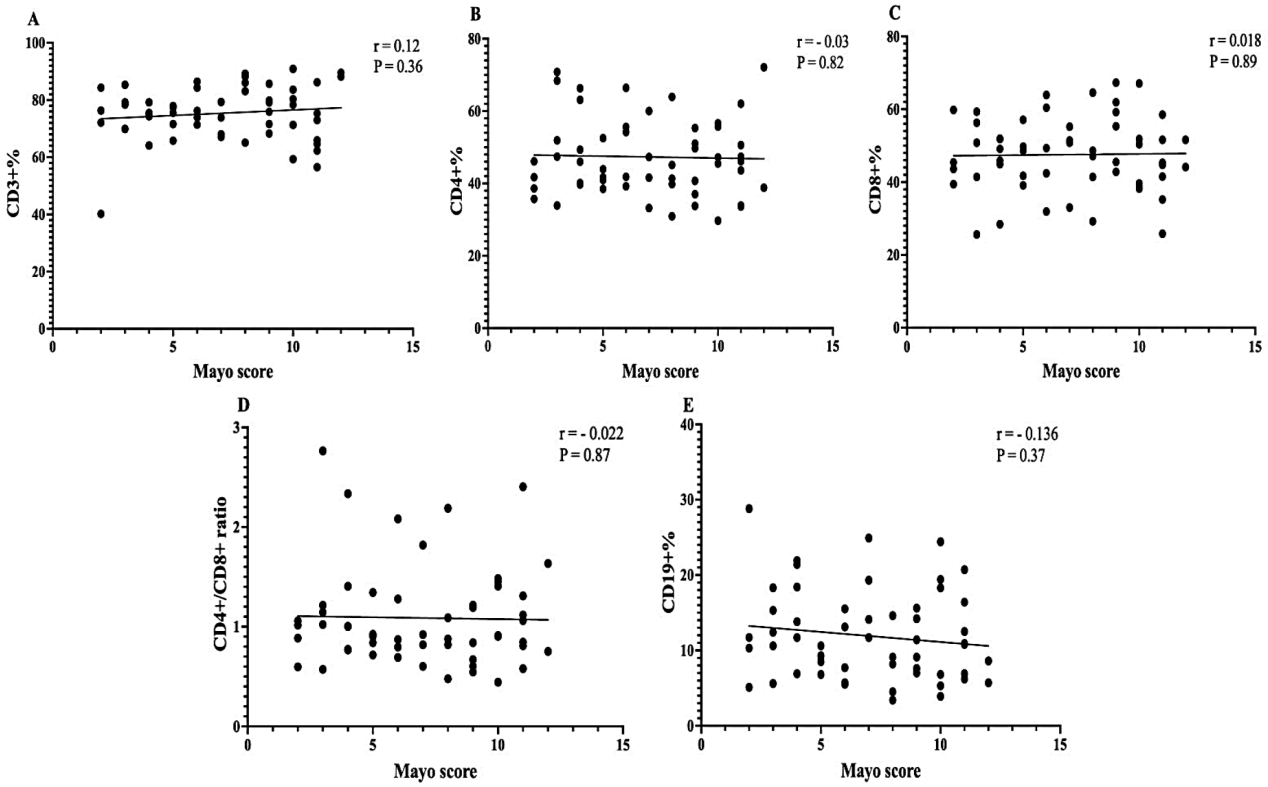

Fig. 5 **The correlations between the Mayo score of UC patients and proportional lymphocyte subset cells (CD3+, CD4+, CD8+, CD4+/CD8+ ratio, and CD19+).**

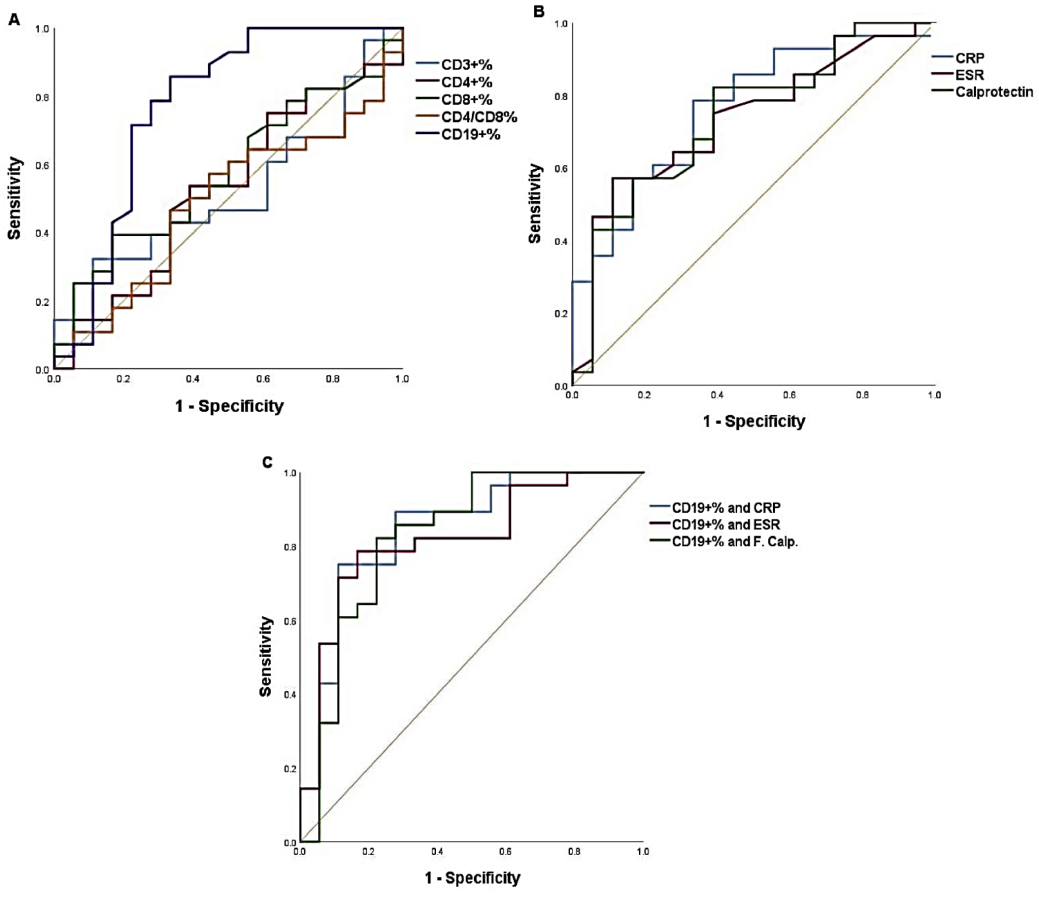

Fig. 6 **Presents ROC curves for lymphocyte subsets (CD3+, CD4+, CD8+, CD4+/CD8+ ratio, CD19+) and inflammatory biomarkers (ESR, CRP, faecal calprotectin), assessing their effectiveness in determining disease severity in ulcerative colitis patients.**

Table 3. **Area under ROC curves (AUC), cutoff value, sensitivity, specificity, and 95% confidence interval of lymphocyte subsets in Crohn's disease (CD) for determining the disease severity**

Variable	AUC	Cutoff	Sensitivity (%)	Specificity (%)	*P*-value	95% Confidence interval	
						Lower bound	Upper bound
CD3+%	0.526	81.8	51	89	0.770	0.356	0.695
CD4+%	0.528	47.75	59	61	0.753	0.354	0.701
CD8+%	0.575	48.75	61	83	0.392	0.409	0.742
CD4/CD8%	0.490	1.18	56	67	0.910	0.319	0.661
CD19+%	0.770	11.1	86	67	0.002	0.611	0.929
CRP (10^3/ml)	0.764	11.5	79	67	0.003	0.582	0.880
ESR (10^3/ml)	0.731	48.5	57	89	0.009	0.582	0.880
Calprotectin (µg/g)	0.737	201.35	82	61	0.007	0.588	0.886
CD19 % + CRP	0.847	0.611	75	88	<0.001	0.726	0.969
CD19 % + ESR	0.819	0.612	78	83	<0.001	0.692	0.947
CD19 % + Fecal Calprotectin	0.827	0.53	82	77	<0.001	0.691	0.963

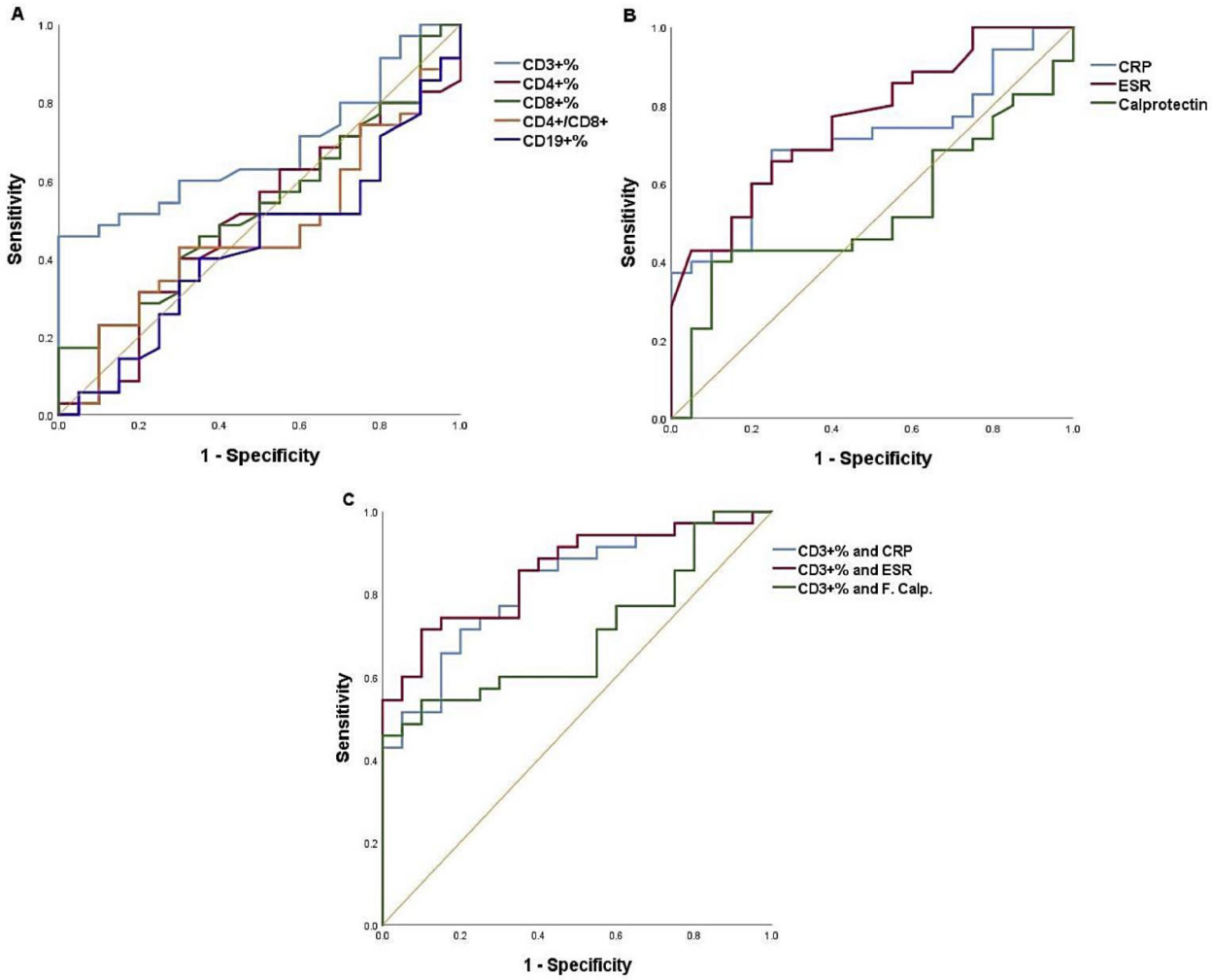

Fig. 7 **Presents ROC curves for lymphocyte subsets (CD3+, CD4+, CD8+, CD4+/CD8+ ratio, CD19+) and inflammatory biomarkers (ESR, CRP, faecal calprotectin), assessed separately in UC patients to determine disease severity.**

Table 4. **Area under ROC curves (AUC), cutoff value, sensitivity, specificity, and 95% confidence interval of lymphocyte subsets in Ulcerative colitis (UC) for determining the disease severity**

Test result variable(s)	AUC	Cutoff	Sensitivity (%)	Specificity (%)	*P*-value	95% Confidence interval	
						Lower bound	Upper bound
CD3+%	0.680	78.75	48.6	90	0.027	0.541	0.819
CD4+%	0.495	53.35	31.4	80	0.951	0.336	0.654
CD8+%	0.533	60.1	17.1	100	0.687	0.378	0.688
CD4/CD8%	0.470	1.059	42.9	70	0.713	0.314	0.626
CD19+%	0.434	12.45	40	65	0.416	0.278	0.589
CRP (10^3/ml)	0.709	13.42	68	75	0.011	0.573	0.844
ESR (10^3/ml)	0.763	55.5	68.6	80	0.001	0.638	0.888
Calprotectin (µg/g)	0.529	863	40	90	0.720	0.375	0.684
CD3% + CRP	0.821	0.618	71.4	80.7	0.00083	0.712	0.931
CD3% + ESR	0.854	0.72	71.4	90.1	0.00014	0.756	0.952
CD3% + Fecal Calprotectin	0.700	0.76	45.7	100	0.014	0.563	0.837

0.72. Lastly, the AUC for CD3+ combined with faecal calprotectin was 0.7 ($P = 0.014$), with a cutoff of 0.76 (Table 4).

Discussion

IBD is considered an autoimmune disease in which multifactors affect the immunoregulation of the disease, such as medication, environment, inherently, infection, or microbiota.[19] Any of the mentioned factors may have effects and results in the disorder of the lymphocytes subset. In the present study, we evaluated the proportion of CD3+, CD4+, CD8+, the ratio of proportional CD4+ to CD8+, and CD19+, as well as different laboratory inflammatory biomarkers. Many studies mentioned that the medication does not affect the proportion of lymphocytes among IBD patients, so the medicated patients were not considered an effector factor in this study.[20,21] The lymphocyte subsets in CD and UC patients were characterized by a decline in the proportion level of CD3+ and a higher proportion of CD4+, CD8+, and CD19+.

Moreover, the proportion of CD19+ cells was higher in active CD patients than in remission patients. The alteration of lymphocyte subsets indicates immune activation and dysregulation in both IBD subtypes and is significant in the progression of the disease. Additionally, a higher proportion of CD19+ can be assessed for CD severity as its value was higher than CRP, ESR, and faecal calprotectin.

The pathophysiological activation of the mentioned peripheral lymphocytes in IBD disease remains unclear. Some studies noted that activating CD4+ and CD8+ contributes to mucosal inflammation by attacking the intestinal cell layer and causing destruction and damaging the barrier of the mucosa. Moreover, other studies believe the activation of the peripheral lymphocyte subsets and recruitment to the intestinal barrier may be due to dysbiosis of intestinal microbiota, leading to mucosal inflammation in the intestine.[22] The other study mentioned the trafficking of mucosal dendritic cells that carry antigens from the inflamed intestine that caused by gut microbiota to the lymph node may promote the activation of the peripheral lymphocyte subsets, especially B cells.[22,23] So, the positive correlation between the proportion of peripheral CD19+ and disease severity in CD patients is linked to the intestine's inflammation rate. Previous studies have shown the proportion of CD4+ CD8+, and their ratio can be used to predict IBD disease severity and, more significantly, in UC.[24,25] However, the more recent study agreed with the result of this study, as the prognosis value and prediction were based on the proportion of CD4+ and CD8+, and the sensitivity and specificity were low.[26,27] More studies may be required to establish the potential use of such lymphocyte subsets to identify the severity of IBD disease.

For efficacy improvement in estimating the IBD disease severity, we used the highest AUC value of lymphocyte subsets among CD and UC patients with the combination of inflammatory biomarkers, furthermore, among CD disease, the proportion of CD19+, and among UC patients, the proportion of CD3+ were used for the combination. Surprisingly, the assessment of CD severity was enhanced and can be used as the prognostic value for CD severity as the AUC for the proportion of CD19+ in combination separately with CRP, faecal calprotectin, and ESR was 0.847, 0.827, and 0.819, respectively. Similarly, according to the results of this study, the combination of CD3+ with CRP or ESR can be used as a prognostic tool for the severity in UC patients as the sensitivity of the combined CD3+ with calprotectin was low. Our research shows no related study concerning the combination of lymphocyte subsets and inflammatory biomarkers in IBD patients. Furthermore, additional study may be required. However, the study used a combination of CD3+HLA-DR+ T cells and CRP in UC patients and agreed with the result of this study.[26] This is the first study that combines the peripheral lymphocyte subsets with inflammatory biomarkers for assessing IBD severity, as this is the strength of this study. Additionally, the used parameters are applicable and are not difficult to obtain. The study's limitation is the short sample collection period and the fact that collecting data from multiple regions is required to confirm the results.

Conclusions

The proportion of peripheral CD3+, CD4+, CD8+, and CD19+, and the CD4+/CD8+ ratio, was evaluated for CD and UC disease severity. Among CD patients, the proportion of CD19+ was more profitable for assessing disease severity, while among UC patients, the proportion of CD3+ can be used. All used inflammatory biomarkers for CD disease severity were given high value. However, the CRP and ESR may be usable for the severity of UC patients. We improved the efficiency of prognostic value for disease severity by combining the CD19+ proportion with inflammatory biomarkers in CD patients and the CD3+ proportion with inflammatory biomarkers in UC patients. The highest prognostic value with high sensitivity and specificity in CD patients was investigated in CD19+ with CRP, followed by ESR, and then with faecal calprotectin, whereas, in UC patients, it was shown in CD3+ combined with ESR, then with CRP. The proportion of CD3+ combined with faecal calprotectin had low sensitivity.

Ethical Approval and Consent to Participate

The study was approved by the Ethics Committee of the University of Sulaimani and the Sulaimani General Directorate of Health (No. 5217 on April 28, 2022). Written informed consent was obtained from all participants.

Authors' Contributions

KAHA: Methodology, data collection, data analysis, and writing the original manuscript. SAH: Study registration, supervision, validation, and data curation. MOMR: Study administration, investigation, and revising the final draft for submission.

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Availability of Data and Materials

The authors are ready to share the raw data upon reasonable request data policy.

Disclosure Statement

No potential conflict of interest was reported by the author(s).

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