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Peripheral differentials by Cytodiff flow cytometric system predict disease activity in Chinese patients with inflammatory bowel disease☆

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ABSTRACT

Background: This study aimed at evaluating whether leukocyte differentials could serve as effective biomarkers for disease activity in IBD.
Methods: A total of 100 subjects were prospectively enrolled, including 36 patients with CD, 34 patients with UC, and 30 healthy controls (HC). Leukocyte differentials were determined by CytoDiff Flow Cytometry analysis.
Results: Total neutrophil counts, monocyte/lymphocyte ratio (M/L), and $CD16^-$ monocyte/lymphocyte ratio ($CD16^-$ M/L) were significantly higher in active UC patients compared with quiescent UC patients and HC. A cut-off value of 0.25 in M/L exhibited the best overall accuracy of 82.4% with an AUC of 0.846 in differentiating active UC from quiescent UC. Total leukocyte counts were significantly decreased in active CD patients, while total monocyte counts and total $CD16^-$ monocyte counts were significantly increased in active CD patients compared with quiescent CD patients and HC. A cut-off value of 0.25 in $CD16^-$ M/L displayed the best AUC of 0.886 (overall accuracy of 86.1%) in differentiating active CD from quiescent CD.
Conclusions: Our data suggest that $CD16^-$ M/L could serve as promising biomarkers for distinguishing active disease from quiescent disease in both UC and CD. In addition, they could be used as supplements to other disease activity indicators, such as hsCRP and ESR.

1. Introduction

Inflammatory bowel disease (IBD) is a group of chronic immune-mediated relapsing-remitting intestinal inflammatory diseases [1–3]. Crohn's disease (CD) and ulcerative colitis (UC) are two major phenotypes. Of note, CD and UC exhibit considerable differences in term of lesion locations and treatment options [1–3]. Currently, the etiology of CD and UC remain partially understood. Aberrant activation of immune response against the microorganisms in the intestine, together with genetic and environmental risk factors, has been regarded to initiate or contribute to disease pathogenesis [3].

For patients with IBD, assessment of disease activity is crucial for monitoring and tailoring the therapies [4–5]. However, the most frequently used index for the assessing disease activity, such as Crohn's Disease Activity Index (CDAI) for CD and Truelove and Witts Severity Index for UC are often subjective, and may not accurately reflect the true disease status [6]. In addition, the gold standards, such as histopathological or endoscopical examinations, are invasive and time consuming, and are unsuitable for routine practice [6–7]. Moreover, although C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are routinely checked in patients with IBD as indicators for disease activity, they are only partially effective [6–7]. Thus, continuous efforts have been made to identify easily accessible, inexpensive and efficacious biomarkers that can be commonly used in clinical practice to reflect disease severity and predict disease progression.

Dysregulation of both innate and adaptive immune responses

Abbreviations: AUC, area under the curve; CRP, C-reactive protein; CD, Crohn's disease; ESR, erythrocyte sedimentation rate; HC, healthy controls; IBD, inflammatory bowel disease; M/L, monocyte/lymphocyte ratio; ROCs, receiver operator curves; UC, ulcerative colitis

☆ The authors have no conflicts of interest to disclose.

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contributes significantly to IBD pathogenesis [3], thus alterations of leukocyte differentials may have potentials to serve as indicators for disease activity. Indeed, neutrophil counts have been identified to correlate with disease activity in patients with rheumatoid arthritis and ankylosing spondylitis [8–9], and increased monocytes counts and increased neutrophil-lymphocyte ratios have been observed in patients with UC [10–12]. In the inflamed bowel, monocytes/macrophages produce pro-inflammatory cytokines/chemokines, which are crucial in modulating T-cell mediated inflammation, while neutrophils directly cause tissue damage [3]. Interestingly, delayed neutrophil apoptosis has been reported in patients with IBD, which may result in increased periphery neutrophil counts [13].

CytoDiff Flow Cytometric system has been displayed good performance in characterizing leukocytes into 16 subpopulations [14–15]. Using this system, we aimed to investigate the alterations of leukocyte differentials between quiescent and active UC and CD, and assess whether the dysregulated leukocyte differentials could serve as effective biomarkers for disease activity in Chinese patients with UC and CD.

2. Materials and methods

2.1. Subjects and specimen collections

From May 2014 to July 2015, a total of 100 subjects were prospectively enrolled in this study, including 36 patients with CD, 34 patients with UC, and 30 healthy controls (HC). HCs were defined as subjects without any signs of infection or inflammation or other significant illnesses. All patients were diagnosed and managed at the Department of Gastroenterology, Peking Union Medical College Hospital (PUMCH). IBD was defined by the Lennard-Jones criteria [16]. Specifically, subjects were diagnosed with CD or UC based on a combination of standard criteria that included clinical symptoms, physical examination, colonoscopy, imaging (bariums studies and CT enterography), and histopathology. Infections (including enteric infections), intestinal tuberculosis, ischemia, non-steroidal anti-inflammatory drug induced ulceration, and radiation colitis were excluded. Clinical phenotypes of the IBD patients were determined based on the Montreal Classification [17]. CD is described by A, L and B classifications. A represents age at diagnosis (A1, below 17 years; A2, between 17 and 40 years; A3, above 40 years), L represents the location of disease (L1, ileal; L2, colonic; L3, ileocolonic; L4, upper disease), and B represents disease behavior (B1, non-stricturing, non-penetrating; B2, stricturing; B3, penetrating; P, perianal disease modifier). UC is described by E classifications (E1, proctitis, lesions limited to the rectum; E2, left-sided colitis, lesions below the splenic flexure; E3, pancolitis, lesions exceeded the splenic flexure). The activity of UC was defined by the Simple Clinical Colitis Activity Index (SCCAI) as mild (3–5 scores), moderate (6–11 scores) and severe (above 12 scores). The activity of CD was defined by Crohn's Disease Activity Index (CDAI), as previously described with CDAI scores < 150 as disease remission and CDAI scores ≥ 150 as active disease [18]. Specifically, the activity of CD was defined as mild (CDAI scores of 150–220), moderate (CDAI scores of 221–450) and severe (CDAI scores of > 450). The demographics and clinical characteristics of the UC and CD patients are shown in Table 1. Study protocols were reviewed and approved by the Ethical Committee of PUMCH and informed consents were obtained from all participants (Institutional Board Review (IRB), S-615).

2.2. Leukocyte differentials by CytoDiff flow cytometry analysis

CytoDiff Flow Cytometry analysis was performed as previously described [14,19]. Briefly, whole peripheral blood was incubated with a pre-mixed cocktail, which contained a combination of six antibodies (CD36-FITC, CD2-PE, CD294 (CRTH2)-PE, CD19-ECD, CD16-PC5 and CD45-PC7). After red blood cell lysis, the cell suspensions were analyzed by a flow cytometer (FC500, Beckman Coulter). The gating and results analysis were performed as previously described [19]. A representative CytoDiff Flow Cytometry analysis is shown in Supplemental Fig. 1.

Table 1
Demographics of Patients with Inflammatory Bowel Disease and controls.

	CD (n = 36)	UC (n = 34)	HC (n = 30)
Female, n (%)	8 (22.2)	14 (41.2)	13 (43.3)
Median age at study (max, min)	30.5 (60, 16)	37.5 (67, 22)	38 (65, 22)
Median duration (max, min)	6 (20, 0.3)	3.5 (13, 0.1)	NA
Median age at diagnosis (max, min)	24.3 (55, 13)	35.9 (66, 16)	NA
Remission, n (%)	14 (38.9)	15 (44.1)	NA
Active disease, n (%)	22 (61.1)	19 (55.9)	NA
Disease severity, n (%)			
Symptomatic remission	14 (38.9)	15 (44.1)	NA
Mild	7 (19.4)	1 (2.9)	NA
Moderate	7 (19.4)	2 (5.9)	NA
Severe	8 (22.2)	16 (47.1)	NA
Disease location, n (%)			
Proctitis (E1)	NA	0 (0)	NA
Left-sided colitis (E2)	NA	6 (17.6)	NA
Pancolitis (E3)	NA	28 (82.4)	NA
Ileal (L1)	4 (11.1)	NA	NA
Colonic (L2)	22 (61.1)	NA	NA
Ileocolonic(L3)	25 (69.4)	NA	NA
Upper disease, modifier (L4)	3 (8.3)	NA	NA
Disease behavior, n (%)			
Non-stricturing, non-penetrating (B1)	8 (22.2)	NA	NA
Stricturing(B2)	13 (36.1)	NA	NA
Penetrating (B3)	16 (41.7)	NA	NA
Perianal disease (p)	13 (36.1)	NA	NA
Extraintestinal manifestations			
Musculoskeletal	9 (25.0)	9 (26.5)	NA
Dermatologic	8 (22.2)	10 (29.4)	NA
Ocular	1 (2.8)	2 (5.9)	NA
Treatment, n (%)			
Previous surgery	20 (55.6)	7 (20.6)	NA
Anti-TNF therapy	17 (47.2)	5 (14.7)	NA
Immunosuppressive	22 (61.1)	12 (35.3)	NA
Steroids	31 (24.2)	31 (91.2)	NA
5-ASA	26 (72.2)	25 (73.5)	NA
ESR (mm/h), median (max, min)	20 (79, 2)	20 (77, 3)	NA
hsCRP (mg/L), median (max, min)	12 (140.2, 0.5)	8.3 (201.6, 0.2)	NA

CD, Crohn's disease; UC, ulcerative colitis; HC, health controls; NA, not applicable; ESR, erythrocyte sedimentation rate; hsCRP, high-sensitivity C-reactive protein.

2.3. Statistical analysis

SPSS 20.0 (SPSS Inc., IL, USA) and Prism 5.02 (GraphPad, CA, USA) were utilized for all statistical tests. Multiple tests were determined by Dunnett's tests of one-way ANOVA. Receiver operator curves (ROCs), which were constructed by logistic regression models, were used to determine the optimal cut-off values. p values of < 0.05 were considered significant.

3. Results

3.1. Leukocyte differentials in patients with active UC patients, quiescent UC patients and HC

As shown in Fig. 1A–C, total neutrophil counts were significantly increased in active UC patients, compared with UC patients in remission ($p < 0.05$) and HC ($p < 0.0001$). In addition, total monocyte counts were significantly elevated in active UC patients compared with HC ($p < 0.05$), and a trend of higher levels of total monocyte counts was observed in active UC patients compared with quiescent UC patients. No significant difference was observed in total lymphocyte counts among active UC patients, UC patients in remission and HC.