Predictive Value of CD8+CD28+/CD8+CD28- T Cell Balance for Gastrointestinal Bleeding in Patients with Inflammatory Bowel Disease: Post-print

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Abstract

Objective: To evaluate the role and value of CD8 + CD28 + /CD8 + CD28 - T-cell balance in predicting gastrointestinal hemorrhage (GH) in patients with inflammatory bowel disease (IBD).

Methods: A total of 49 IBD patients were enrolled, including 30 with ulcerative colitis (UC) and 19 with Crohn' s disease (CD). Flow cytometry was used to detect the percentage of CD8 + CD28 + and CD8 + CD28 - T cells in peripheral blood. Patients were followed up for 1 year. Receiver operating characteristic (ROC) curve analysis was used to evaluate the efficacy of CD8 + CD28 + /CD8 + CD28 - T-cell balance (ratio) in predicting GH in IBD patients. Kaplan-Meier survival analysis was employed to compare differences in lasting time of remission (LTR) under different factors, and correlation analysis was performed on relevant indicators.

Results: (1) The utilization rates of immunosuppressants, corticosteroids, and biologic agents (BA) in the CD group were significantly higher than those in the UC group (P=0.003, 0.043, and 0.002, respectively); (2) CD8 + CD28 + T cells in UC patients were significantly higher than those in CD patients (t=3.022, P=0.004); (3) ROC results showed that CD8 + CD28 + T cells, CD8 +CD28 - T cells, and the CD8 + CD28 + /CD8 +CD28 - ratio all demonstrated good efficacy in predicting GH (all P<0.01), with the CD8 + CD28 + /CD8 +CD28 - ratio being optimal [area under the curve (AUC) = 0.977, P=0.000]. Cutoff analysis revealed that when the CD8 + CD28 + /CD8 + CD28 - ratio was 1.14 (13.95%/12.24%), the corresponding sensitivity reached 93.3% and specificity

was 91.2%; (4) The mean and median LTR were significantly longer in IBD patients who did not use BA than in those who used BA (^{2} = 9.730, P=0.002), and significantly longer in patients who did not undergo surgery than in those who had undergone surgery (^{2} = 15.981, P=0.000); (5) Spearman analysis showed that CD8 + CD28 + /CD8 + CD28 - was significantly correlated with both BA and surgery (P=0.009 and 0.038, respectively).

Conclusion: Decreased peripheral blood CD8 + CD28 + T cells or increased CD8 + CD28 - T cells are closely associated with GH in IBD patients. The CD8 + CD28 + /CD8 + CD28 - balance demonstrates high sensitivity and specificity for predicting GH, particularly when the ratio is 1.14. This balance is significantly correlated with biologic agents and surgery.

Full Text

Preamble

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Abstract

Objective: To evaluate the sensitivity and specificity of the CD8 CD28 /CD8 CD28 T lymphocyte balance in predicting gastrointestinal hemorrhage (GH) in patients with inflammatory bowel disease (IBD).

Methods: Forty-nine IBD patients, including 30 with ulcerative colitis (UC) and 19 with Crohn' s disease (CD), were enrolled to test peripheral blood CD8 CD28 and CD8 CD28 T cells using flow cytometry. All patients were followed up for one year. Receiver-operating characteristic (ROC) curves were used to evaluate the efficiency of the CD8 CD28 /CD8 CD28 T lymphocyte balance for predicting GH. Differences in lasting time of remission (LTR) under various factors were compared using Kaplan-Meier survival analysis, and correlations between CD8 T lymphocytes and clinical factors were analyzed.

Results: The utilization rates of immunosuppressants, steroids, and biological agents (BA) were significantly higher in CD patients than in UC patients (P=0.003, 0.043, and 0.002, respectively). The frequencies of CD8 CD28 T cells were significantly higher in UC patients than in CD patients (t=3.022, P=0.004). CD8 CD28 T cells, CD8 CD28 T cells, and especially the CD8 CD28 /CD8 CD28 ratio (area under curve of 0.977, P=0.000; cut-off value of 1.14 [13.95%/12.24%] with a sensitivity of 93.3% and specificity of 91.2%) showed good efficiency in predicting GH (P<0.01). The mean and median LTR of IBD patients who did not receive BA or surgical treatment were significantly longer (^{2}=9.730, P=0.002; ^{2}=15.981, P=0.000). The CD8 CD28 /CD8 CD28 ratio was significantly related to both BA (P=0.009) and surgery (P=0.038).

Conclusion: Both decreased CD8 CD28 T cells and elevated CD8 CD28 T cells are closely correlated with GH, and their ratio can predict the occurrence of GH with high sensitivity and specificity and is correlated with BA and surgery at the cut-off value of 1.14.

Keywords: inflammatory bowel disease; active stage; gastrointestinal hemorrhage; CD8 CD28 /CD8 CD28 T lymphocytes

Introduction

Inflammatory bowel disease (IBD), comprising ulcerative colitis (UC) and Crohn' s disease (CD), has shown a 逐年 increasing incidence worldwide with a trend toward younger onset [1]. Epidemiological data indicate that the incidence of IBD among children aged 0-14 years in Shanghai increased 12-fold over the past decade, from 0.5 to 6.1 per million [2]. In Hong Kong, the incidence of IBD was reported as 3 per 100,000 in 2013, a threefold increase

since 2001 [3]. IBD can be classified into remission and active stages based on disease activity, with the active stage representing disease progression that may manifest symptoms such as gastrointestinal hemorrhage (GH). GH can exacerbate anemia and infection in IBD patients, posing significant clinical risk [4]. Unfortunately, there is currently a lack of highly sensitive and specific markers for predicting GH in IBD patients. Furthermore, limited research has identified factors that contribute to disease progression from remission to active stage.

Our previous study found that UC patients exhibited decreased peripheral blood CD8 CD28 T cells and increased CD8 CD28 T cells compared with healthy controls, with the resulting CD8 CD28 /CD8 CD28 ratio significantly reduced [5]. We therefore hypothesized that this immunological balance might serve as a predictor for active disease, particularly GH, in IBD patients. To test this hypothesis, we examined changes in these two T cell subsets and their balance in 49 IBD patients. Our findings demonstrate that all three parameters correlate significantly with GH, with the CD8 CD28 /CD8 CD28 ratio showing the highest sensitivity and specificity for GH prediction, underscoring its significant clinical value.

Methods

1.1 Patient Data

According to the "Consensus on Diagnosis and Treatment of Inflammatory Bowel Disease (2012)" established by the IBD Group of the Chinese Society of Gastroenterology, we selected IBD patients from the Emergency Department and Department of Gastroenterology at Nanfang Hospital, Southern Medical University, between October 2012 and October 2013. Inclusion criteria were: (1) diagnosis of UC or CD confirmed by colonoscopy and pathological biopsy; (2) for UC patients, disease extent classified as E1-E3 according to the Montreal classification and severity classified as mild to severe according to the Truelove and Witts criteria; (3) for CD patients, diagnosis meeting WHO-recommended criteria (including six items such as non-continuous or segmental changes and perianal lesions) and disease severity scoring 4-9 on the simplified Harvey-Bradshaw CDAI; and (4) good patient compliance with follow-up visits. Exclusion criteria included other chronic colitides (e.g., radiation enteritis), malignancy, tuberculosis, chronic infection, autoimmune disease, or pregnancy [6].

A total of 49 patients were enrolled, comprising 30 UC and 19 CD cases. The cohort included 31 males and 18 females, aged 13-69 years (mean 39.31±14.75 years). Fourteen patients were in remission and 35 in active disease. No statistically significant differences were observed between UC and CD groups in terms of gender distribution, disease stage composition, or age (P=0.551, 0.711, and 0.481, respectively) .

1.2 Flow Cytometry

CD8-FITC and CD28-PE antibodies were purchased from Santa Cruz Biotechnology (USA). Approximately 5 mL of fasting elbow venous blood was collected in heparin-anticoagulated tubes. Samples were diluted with an equal volume of Hank' s solution, and peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient centrifugation. After washing, PBMC concentration was adjusted to 2×10 cells/L, followed by incubation with CD8-FITC and CD28-PE antibodies [7]. Samples were analyzed using a Beckman multicolor flow cytometer with gating based on CD8 and CD28 expression. A total of 1,000 cells were acquired per tube, and Beckman Coulter Epics XL software was used to calculate the percentages of CD8 CD28 and CD8 CD28 T cells among all lymphocytes [5].

1.3 Index Comparison

We compared family history, medication use (5-aminosalicylic acid [5-ASA], immunosuppressants, steroids, intestinal microecological preparations [probiotics], and biological agents [BA]), and surgical rates during follow-up between UC and CD groups [8].

1.4 Follow-up and Observation

Patients were followed for one year, with GH recorded as a positive event. We compared differences in lasting time of remission (LTR) between UC and CD groups [9], evaluated the predictive efficacy of CD8 CD28 T cells, CD8 CD28 T cells, and the CD8 CD28 /CD8 CD28 ratio for GH, and compared LTR differences under various factors between UC and CD patients.

1.5 Statistical Processing

Continuous variables are expressed as mean±standard deviation, and categorical variables as n. Two independent samples t-tests were used for intergroup comparisons, while ^{2} or rank-sum tests were used for categorical data. Receiver-operating characteristic (ROC) curve analysis evaluated sensitivity and specificity for GH prediction. Kaplan-Meier analysis compared LTR differences across factors [10]. Correlation analysis employed Spearman' s method. SPSS 17.0 software was used for all analyses, with $P<0.05$ considered statistically significant.

Results

2.1 Comparison of General Factors

No statistically significant differences were observed between UC and CD patients in family history, 5-ASA or probiotic use, surgery, LTR, or GH ($P>0.05$).

However, CD patients showed significantly higher utilization rates of immunosuppressants, steroids, and BA compared with UC patients (P=0.003, 0.043, and 0.002, respectively) .

2.2 Differences in CD8 T Cells and Their Ratio Between UC and CD Patients

Both CD8 CD28 and CD8 CD28 T cells were expressed in both groups, with each subset comprising over 10% of lymphocytes and CD8 CD28 T cells slightly outnumbering CD8 CD28 T cells [Figure 1: see original paper]A,B. UC patients exhibited significantly higher CD8 CD28 T cell frequencies (14.32±6.17%) compared with CD patients (9.40±4.38%) (t=3.022, P=0.004) [Figure 2: see original paper]A. No statistically significant differences were observed in CD8 CD28 T cells or the CD8 CD28 /CD8 CD28 ratio (P=0.985 and 0.094, respectively) [Figure 2: see original paper]B,C.

2.3 ROC Analysis

ROC analysis demonstrated that CD8 CD28 T cells, CD8 CD28 T cells, and the CD8 CD28 /CD8 CD28 ratio all showed good predictive efficacy for GH (all P<0.01) . The CD8 CD28 /CD8 CD28 ratio exhibited the largest area under the curve (AUC) at 0.977, followed by CD8 CD28 cells at 0.791 [Figure 3: see original paper]. Cut-off analysis revealed that when the CD8 CD28 /CD8 CD28 ratio was 1.14 (13.95%/12.24%), sensitivity reached 93.3% and specificity 91.2%.

2.4 Survival Analysis

All 49 patients completed follow-up without loss to follow-up or censoring. Kaplan-Meier analysis showed that IBD patients who did not receive BA or surgical treatment had significantly longer mean and median LTR compared with those who received BA or underwent surgery (^{2}=9.730, P=0.002; ^{2}=15.981, P=0.000, respectively) , [Figure 4: see original paper].

2.5 Correlation Analysis

Spearman analysis revealed that the CD8 CD28 /CD8 CD28 ratio correlated significantly with both BA (P=0.009) and surgery (P=0.038). CD8 CD28 T cells correlated only with BA (P=0.001), while CD8 CD28 T cells showed no significant correlation with either BA or surgery (P=0.307 and 0.058, respectively) .

Discussion

Identifying risk factors for progression from remission to active disease holds significant clinical value, as the active stage directly causes progressive mucosal

destruction and deteriorating clinical status. Numerous factors influence IBD outcomes, broadly categorized into patient-related and external intervention factors, with treatment being the primary external factor [11]. Treatment factors can be further divided into pharmacological and non-pharmacological interventions, with the latter including surgery, endoscopic therapy, and the emerging fecal microbiota transplantation (FMT) [12].

Following this framework, we selected gender, age, and family history as patient-related factors; 5-ASA, immunosuppressants, steroids, probiotics, and BA as pharmacological interventions; and intestinal resection surgery as a non-pharmacological intervention. Using IBD type (UC vs CD) as the grouping variable, we found no significant differences between UC and CD patients in family history, 5-ASA or probiotic use, surgery, LTR, or GH, suggesting that genetic factors do not differ substantially between UC and CD and that surgical rates and outcomes (LTR and GH) are similar between groups.

Regarding medication patterns, no significant differences were observed between UC and CD in 5-ASA or microecological agent use. However, CD patients showed significantly higher utilization rates of immunosuppressants, steroids, and BA. This likely reflects the fact that CD often presents with extensive, skip lesions requiring more intensive drug combinations [13]. In such cases, immunosuppressants and steroids are typically first-line therapies, with BA reserved for cases refractory to first-line agents, resulting in higher usage rates of all three drug classes in CD.

Nevertheless, many IBD patients who adhere to immunosuppressant, steroid, and BA therapy still progress to active disease, implicating additional factors, particularly immunological factors that represent the core pathogenic mechanism of IBD. Building on our previous research, we examined the value of CD8 T immune cells and their ratio for predicting GH, a direct clinical manifestation of active disease. CD8 T cells are cytotoxic T lymphocytes, while CD28 serves as a co-stimulatory molecule. CD8 T cells expressing CD28 become CD8 CD28 T cells with cytotoxic and phagocytic functions [14], whereas those lacking CD28 expression become CD8 CD28 T cells with immunosuppressive and bidirectional regulatory functions, representing a subset of regulatory T cells (Treg) [15].

Intergroup comparisons revealed that UC patients had significantly higher CD8 CD28 T cells than CD patients, though no differences were observed in CD8 CD28 T cells or the CD8 CD28 /CD8 CD28 ratio. However, this does not necessarily indicate superior predictive value of CD8 CD28 T cells for GH compared with CD8 CD28 T cells or the CD8 CD28 /CD8 CD28 balance, which required further ROC analysis for validation. ROC analysis confirmed that the CD8 CD28 /CD8 CD28 ratio achieved the largest AUC, followed by CD8 CD28 cells. Cut-off analysis demonstrated that when the CD8 CD28 /CD8 CD28 ratio was 1.14 (corresponding to 13.95% CD8 CD28 T cells and 12.24% CD8 CD28 T cells), sensitivity and specificity both exceeded 90%, establishing it as an ideal diagnostic indicator with important clinical

implications. These findings confirm the superior predictive performance of the CD8 CD28 /CD8 CD28 ratio.

Although no significant difference in LTR was observed between UC and CD groups (30.0±13.9 vs 24.5±10.3 weeks), simple t-tests cannot capture differences in LTR under various influencing factors. Therefore, we employed Kaplan-Meier survival analysis to compare LTR differences across factors, finding that IBD patients who did not receive BA or surgery had significantly longer mean and median LTR, consistent with reports by Papp et al. [16]. This phenomenon likely occurs because CD patients, with their extensive, aggressive lesions and often atypical symptoms leading to diagnostic delays [17], experience more severe disease progression than UC patients, necessitating BA use. Additionally, higher rates of fistulas and perforation in CD require surgical intervention [18].

Correlation analysis revealed that the CD8 CD28 /CD8 CD28 ratio correlated significantly with both BA and surgery, while CD8 CD28 T cells correlated only with BA and CD8 CD28 T cells showed no significant correlation with either factor. This indicates that the CD8 CD28 /CD8 CD28 ratio has the strongest association with IBD intervention modalities, which directly influence disease outcomes. Collectively, our results demonstrate that decreased peripheral blood CD8 CD28 T cells or increased CD8 CD28 T cells are closely associated with GH in IBD patients. The CD8 CD28 /CD8 CD28 balance shows ideal predictive efficacy for GH, and when this ratio falls below 1.14, clinicians should closely monitor for possible progression to active disease and advise patients to intensify follow-up, providing valuable clinical guidance. This study has limitations, including the small sample size and lack of long-term follow-up, which will be addressed in future research.

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Note: Figure translations are in progress. See original paper for figures.