Clinical Characteristics and Risk Factors of Intestinal Involvement in Behçet’s Syndrome Patients—A Cross-Sectional Study from a Single Center

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Clinical characteristics and risk factors of intestinal involvement in Behçet’s syndrome patients: a cross-sectional study from a single center

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Abstract

Background: Intestinal Behçet’s syndrome (BS) has high morbidity and mortality rates with serious complications. This study aimed to investigate the clinical characteristics and laboratory indicators of intestinal BS compared with mucocutaneous BS patients in China and analyzed the risk factors of intestinal complications in BS patients.

Methods: A retrospective analysis was used to collect the demographic data and laboratory results from 97 patients newly diagnosed with intestinal BS and 154 patients newly diagnosed with mucocutaneous BS. Univariate and multivariate logistic regression analyses were used to analyze the demographic data and laboratory indexes whether a risk factor of intestinal involvement in BS patients.

Results: The most common clinical manifestations of first onset in intestinal BS patients were oral ulceration (100.00%), followed by genital ulcers (62.89%) and erythema nodule (28.87%), gastrointestinal lesions (28.87%), pseudofolliculitis (25.77%), fever (17.53%), arthritis (16.49%), ocular involvement (5.15%), while the least common were vascular involvement (2.06%) and blood system involvement (2.06%). The most common intestinal segment involved in intestinal BS patients was terminal ileum (30.9%), followed by ileocecal (18.6%), colon (15.5%). Most intestinal BS patients (89.7%) used ≥ 3 immunosuppressants to control disease while most mucocutaneous BS patients (92.9%) used 1 or 2 immunosuppressant. By univariate logistic regression analysis, we found gender, age at hospitalization, age of disease onset, BDCAF, T-SPOT, fever, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), white blood cell (WBC), red blood cells (RBC), hemoglobin (HGB), neutrophil-to-lymphocyte ratio (NLR), serum amyloid A (SAA), complement 3 (C3), albumin, total cholesterol(TCH), high-density lipoprotein(HDL) and interleukin 6 (IL-6) were all risk factors of intestinal involvement in BS patients (P<0.05 or P=0.00). Additionally, gender (male), BDCAF (≥2), ESR (≥15mm/H), CRP (>10mg/L), HGB (<130g/L) and IL-6 (>7pg/ml) were the independent risk factors of intestinal involvement in BS patients (all P<0.05).

Conclusions: More attention should be paid to gender, BDCAF, ESR, CRP, HGB and
IL-6 of intestinal involvement in BS patients and therapeutic regiment should be adjusted timely to prevent the occurrence of serious clinical complication in BS patients.

Key words: Behçet’s syndrome, Intestinal ulcers, Risk factors

Introduction

Behçet’s syndrome (BS), also known as Behçet’s disease, is a chronic relapsing multisystemic disease, which is considered a unique vasculitis that can cause inflammation of vessels of all size with involvement of several organs and systems(1). It is characterized by recurrent oral and genital ulcers, ocular lesions, skin manifestations, and arthritis as well as vascular, neurological, and intestinal involvement(2; 3). BS is also known as the Silk Rout disease with high incidence in the Mediterranean, the Middle East, and the Far East, and the incidence rate in China is about 14/100,000(3).

Intestinal BS is diagnosed when there is a typically shaped ulcer in the gastrointestinal tract and clinical findings meet the diagnostic criteria for BS(4). The frequency of intestinal BS shows a wide variation across countries, ranging from 1% to 50%, being much more common in the Far East compared with the Middle East and Europe(1). There were few reports on the incidence of intestinal BS in China, and the frequency of intestinal involvement among BS patients was about 17% reported from a small-sized case series(5). Our previous study found that some BS patients have typical ulcers under colonoscopy although they have no clinical symptoms of the gastrointestinal tract(6). Therefore, the frequency of intestinal BS in China may be higher than 17%. Additionally, intestinal BS has high morbidity and mortality rates with serious complications, such as intestinal perforations, fistulas, infections and massive bleeding(7). Most intestinal BS patients underwent surgery or repeated surgery(8). However, there are few studies on biomarkers of intestinal involvement of BS. Some studies have found elevated C-reactive protein (CRP) levels can predict reactivation and postsurgical relapse of intestinal involvement in BS(1; 8). In some other studies, interleukin 6 (IL-6) were found significantly higher in BS patients(9-11), suggesting that it was related to clinical activity of BS. In addition, fecal calprotectin
is a useful marker of active gastrointestinal involvement in BS\cite{12}. BS patients who have gastrointestinal symptoms such as bleeding, abdominal pain, diarrhea, nausea or vomiting, will underwent colonoscopy to confirm whether they have intestinal involvement. However, in our daily clinical work, we found some BS patients have typical ulcers under colonoscopy although they have no clinical symptoms of the gastrointestinal tract. Therefore, to find the early biomarkers which can identify whether BS patients complicated with intestinal involvement will prevent serious gastrointestinal complications occurred in BS patients.

In this retrospective study, we focused on the clinical characteristics and laboratory indicators in intestinal BS and mucocutaneous BS patients in China, aiming to find the risk factors of intestinal involvement in BS patients.

**Patients and methods**

**Patients**

A cross-sectional study was performed based on well-organized electronic medical records. We conducted a retrospective analysis about the clinical characteristics of a total of 412 BS patients hospitalized in Huadong Hospital affiliated with Fudan University between August 1, 2018 and August 30, 2020. All subjects underwent a colonoscopy as part of their routine checkup. Intestinal BS is diagnosed when there is a typically shaped ulcer in the gastrointestinal tract and clinical findings meet the diagnostic criteria for BS\cite{4}. Mucocutaneous BS is diagnosed when there are typical mucocutaneous involvements, without damage of intestines, eyes, nervous system, etc. Of a cohort of 412 BS patients, 97 patients were newly diagnosed with intestinal BS, and 154 patients were newly diagnosed with mucocutaneous BS. Informed consent was signed by all participants.

Inclusion and exclusion criteria were as follows: (1) All the patients met the new International Criteria for Behçet’s Disease\cite{13}(ICBD); (2) all patients had complete clinical data and laboratory test results; (3) exclude the patients who were younger than 16 years; (4) the selected patients had no other disturbing diseases, such as infective diseases, other autoimmune diseases, endocrinal disorders, or malignancies;
(5) the diagnosis of intestinal BS was made in accordance with previously established criteria based on colonoscopic features and clinical manifestations using a modified Delphi process(4).

**Methods**

**Clinical assessment**

The clinical manifestations of disease first onset and during hospitalization in BS patients with intestinal involvement and mucocutaneous involvement (without intestinal or other organs and systems involvement) were retrospectively extracted from the medical records. The disease activity scores of BS patients were recorded using the simplified Behçet’s Disease Current Activity Form (BDCAF)(14). Patients were interviewed regarding their response to 12 clinical categories over the 4 weeks prior to the day of study enrollment, which composed the frame of BDCAF, and they were then scored from 0 to 12, but only with investigators’ agreements that symptoms were due to BS. Clinical categories were as follows: headache, mouth ulceration, genital ulceration, erythema nodosum, skin pustules, arthralgia, arthritis, nausea or vomiting or abdominal pain, diarrhea or hematochezia, eye involvement, nervous system involvement and major vessel involvement.

**Data collection**

The following information were collected: gender, age, age of disease first onset, disease duration, the score of BDCAF, clinical manifestations of BS, the location of intestinal ulcer, T-SPOT.TB assay (T-SPOT), pathergy test, Hepatitis B core antibody (HBcAb), fever, laboratory indexes (neutrophil-to-lymphocyte ratio (NLR), white blood cell (WBC), red blood cells (RBC), hemoglobin (HGB), platelet (PLT), erythrocyte sedimentation rate (ESR), CRP, serum amyloid A (SAA), complement 3 (C3), complement 4 (C4), CH50, albumin, total cholesterol(TCH), triacylglycerol(TG), glucose, uric acid(UA), low-density lipoprotein(LDL), high-density lipoprotein(HDL), immunoglobulin (IgA, IgG, IgE, IgM), IL-6) and treatment approaches.

**Specific research methods**

Analyze the clinical manifestations and summarize the treatment approaches,
demographic data and laboratory indexes of 97 patients newly diagnosed with intestinal BS and 154 patients newly diagnosed with mucocutaneous BS. Univariate logistic regression analyses were used to analyze gender, age at hospitalization, age of disease first onset, disease duration, the score of BDCAF and laboratory indexes mentioned above whether or not a risk factor of intestinal involvement; get out the meaningful risk factors and turn them into categorical variables according to clinical or laboratory significance; then multivariate logistic regression analyses were used to find out the independent risk factors of intestinal involvement in BS patients.

**Statistical analysis**

The software of SPSS version 23.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Count data were denoted by percentage or ratio. Continuous variables were expressed as mean ± standard deviation (x ± s). Chi-square test or Fisher’s exact test was used for qualitative data analysis. Analysis of variance (ANOVA) was used for quantitative data analysis. Risk factors were analyzed by two classification logistic regression analysis (first by univariate logistic regression analysis to identify significant different variables, then these variables into a multivariate logistic regression analysis selected by forward: LR method) and calculated the odds ratio (OR) and 95% confidence interval (95% CI). Lastly, a Receiving Operating Characteristic (ROC) curve was used to demonstrate the sensitivity and specificity of the selected risk factors. All tests were two-sided. Differences were considered statistically significant when P was less than 0.05 (P < 0.05).

**Results**

**Clinical manifestations and intestinal lesion sites of intestinal BD patients**

A total of 412 BS patients were recruited in this study. Of the cohort of 412 BS patients, 97 (23.54%) patients were newly diagnosed with intestinal BS, and 154 (37.38%) patients were newly diagnosed with mucocutaneous BS. Of 97 intestinal BS patients, 13 (13.40%) patients underwent the surgical treatment of bowel resection. The clinical manifestations of patients with intestinal BS and mucocutaneous BS were shown in Table 1. The most common clinical manifestations of disease first onset in
intestinal BS patients were oral ulceration (100.00%), followed by genital ulcers (62.89%) and erythema nodule (28.87%), gastrointestinal lesions (28.87%), pseudofolliculitis (25.77%), fever (17.53%), arthritis (16.49%), ocular involvement (5.15%), while the least common were vascular involvement (2.06%) and blood system involvement (2.06%). The clinical manifestations during hospitalization in intestinal BS patients were nausea/vomiting/abdominal pain (50.52%), followed by oral ulceration (48.45%), diarrhea/hematochezia (23.71%), genital ulcers (16.49%), erythema nodule (14.43%), arthralgia (7.72%), blood system involvement (6.19%), ocular involvement (3.09%), major vessel involvement (2.06%), arthritis (2.06%), nervous system involvement (1.03%), headache (1.03%), while the least common was skin pustules (0.00%). The intestinal lesion sites of 97 patients with intestinal BS were shown in Table 2 and Figure 1. The most common intestinal segment involved in intestinal BS patients was terminal ileum (30.9%), followed by ileocecal (18.6%), colon (15.5%), ileocecal and terminal ileum (9.3%), cecum (4.1%), small intestine and ileocecal (3.1%), small intestine and terminal ileum (3.1%), terminal ileum and colon (3.1%), cecum and terminal ileum (2.1%), ileocecal and colon (2.1%), cecum and ileocecal (2.1%), while the least common were small intestine (1.0%), rectum and colon (1.0%), small intestine, colon and cecum (1.0%), small intestine, colon and ileocecal (1.0%), small intestine, terminal ileum and ileocecal (1.0%), ileocecal, colon and rectum (1.0%).

**Basic characteristics of 251 BD patients**

The demographic variables and laboratory results of 251 BS patients (including 97 intestinal BS and 154 mucocutaneous BS) were shown in Table 3. 49.48% of 97 intestinal BS patients were male, while only 29.22% of 154 mucocutaneous BS patients were male ($P=0.00$). There was statistically significant difference in terms of gender, age at hospitalization, age of disease onset, BDCAF, T-SPOT, fever, ESR, CRP, RBC, WBC, HGB, NLR, SAA, C3, albumin, TCH, HDL, IL-6 between intestinal BS and mucocutaneous BS (all $P<0.05$). While there was no statistically significant difference ($P>0.05$) between two groups in terms of disease duration, pathergy test, HBCaAb, PLT, IgA, IgE, IgG, IgM, C4, CH50, TG, glucose, UA and
**Treatment approaches**

Our study found that all intestinal BS patients used intravenous steroid during hospitalization while only 18.83% of mucocutaneous BS patients used oral steroid. Most BS patients used thalidomide, and the frequency of mucocutaneous BS patients was higher than that of intestinal BS patients. However, 89.69% of intestinal BS patients used cyclosporine, 69.07% used sulfasalazine, 65.98% used biologics and 10.31% used mesalazine, which were higher than those of mucocutaneous BS patients. Most intestinal BS patients (89.7%) used ≥ 3 immunosuppressants to control disease while most mucocutaneous BS patients (92.9%) used 1 or 2 immunosuppressant. The information of comparison of treatment approaches between intestinal BS and mucocutaneous BS patients were shown in Table 4.

**Analysis of risk factors of intestinal involvement in BD patients**

By univariate logistic regression analysis, we found gender, age at hospitalization, age of disease first onset, BDCAF, T-SPOT, fever, ESR, CRP, RBC, WBC, HGB, NLR, SAA, C3, albumin, TCH, HDL and IL-6 were all risk factors of intestinal involvement in BS patients (as seen in Table 5). All the above differences were statistically significant ($P<0.05$ or $P=0.00$). Additionally, disease duration, pathergy test, HBcAb, PLT, IgA, IgE, IgG, IgM, C4, CH50, TG, glucose, UA and LDL were not correlated with intestinal involvement in BS patients ($P>0.05$, shown in Table 5). We then selected the meaningful risk factors and turn them into categorical variables including gender (male), age at hospitalization (<40 years old), age of disease onset (<30 years old), BDCAF (≥2), T-SPOT(+), fever, ESR (≥15mm/H), CRP (≥10mg/L), RBC (<4.3*10^9/L), WBC (>9.5*10^9/L), HGB (<130g/L), NLR (≥2), SAA (≥10mg/L), C3 (<0.8g/L), albumin (<35g/L), TCH (>5.72mmol/L), HDL (<1.29mmol/L) and IL-6 (>7pg/ml) according to clinical or laboratory significance. All those above risk factors were analyzed by multivariate logistic regression analysis. As a result, we found gender (male), BDCAF (≥2), ESR (≥15mm/H), CRP (≥10mg/L), HGB (<130g/L) and IL-6 (>7pg/ml) were the independent risk factors of intestinal involvement in BS patients (as seen in Table 6, all $P<0.05$). The highest risk rate of LDL.
BS patients with intestinal involvement was IL-6 (>7pg/ml), then followed by the HGB (<130g/L), ESR (≥15mm/H), gender (male), CRP (>10mg/L) and BDCAF (≥2) (shown in Table 6). When the concentration of IL-6 increased (>7pg/ml), the risk rate of BS patients with intestinal involvement was 8.23 times more than that with normal level of IL-6. Finally, we performed receiver operating characteristic (ROC) curve analysis, using gender, BDCAF, ESR, CRP, HGB and IL-6 to predict BS patients with intestinal involvement (Figure 2). In the ROC curve analysis, we found ESR (AUC=0.814, 95% CI 0.759-0.869, \( P=0.000 \)), CRP (AUC=0.843, 95% CI 0.790-0.896, \( P=0.000 \)), and IL-6 (AUC=0.754, 95% CI 0.685-0.824, \( P=0.000 \)) could predict whether there was intestinal involvement in mucocutaneous BS patients. However, the gender (AUC=0.399) and HGB (AUC=0.384) could not well predict whether there was intestinal involvement in mucocutaneous BS patients (all AUC<0.5, \( P<0.05 \)).

**Discussion**

Intestinal BS is a rare disease, and the epidemiologic studies of this disease are scarce. There have been several studies that evaluated clinical outcomes and prognosis of intestinal BS(15; 16). However, there are few studies on the risk factors of BS complicated with intestinal involvement. Colonoscopy is still the gold standard for diagnose of intestinal BS, but some patients always refuse to undergo colonoscopy because of its’ invasion and complexity. Therefore, in this study, we focused on the clinical characteristics and laboratory indicators in intestinal BS and mucocutaneous BS patients in China, aiming to find the risk factors of intestinal involvement in BS patients, which may be the early biomarkers that can identify whether BS patients complicated with intestinal involvement. More importantly, our study included newly-onset patients with intestinal BS and mucocutaneous BS, which can better reflect the difference between the two groups.

Moderate frequency (10%) of intestinal involvement in BS patients in China has been reported(5). In our study, of the cohort of 412 BS patients, we found 23.54% of BS patients were newly diagnosed with intestinal BS, which was higher than that reported above and 3.9% reported in Korea(17). Intestinal manifestations in BS
patients usually occur 4.5-6 years after the onset of oral ulcers, and the most frequently involved sites are the terminal ileum(18). In this study, terminal ileum was also found the most frequently involved sites, which was consistent with the results of the above study. In addition, our study found that the small intestine was also frequently involved sites with about 10.31% of intestinal BS patients complicated with small intestinal ulcer. We also found that fever and gastrointestinal lesions were more common clinical manifestations of disease first onset in intestinal BS patients than that in mucocutaneous BS patients.

There was statistically significant difference in terms of gender, age at hospitalization, age of disease onset, BDCAF, T-SPOT, fever, ESR, CRP, RBC, WBC, HGB, NLR, SAA, C3, albumin, TCH, HDL, IL-6 between intestinal BD and mucocutaneous BD (all $P<0.05$). By univariate logistic regression analysis, we found gender, age at hospitalization, age of disease onset, BDCAF, T-SPOT, fever, ESR, CRP, RBC, WBC, HGB, NLR, SAA, C3, albumin, TCH, HDL and IL-6 were all risk factors of BS complicated with intestinal involvement. In terms of gender, some studies revealed that male was a risk factor for eye involvement in BS(19; 20). Age difference was investigated in studies with relatively small numbers of BS cases. However, those studies failed to show age dependency in most of the manifestations(21; 22). Our study found age of disease onset and at hospitalization in intestinal BS patients were younger than those in mucocutaneous BS. Fever, ESR, HGB, NLR and albumin had been demonstrated as indicators that can evaluate disease activity of intestinal BS in a few studies(15; 17; 23-26). Some studies have found that elevated CRP levels can predict reactivation and postsurgical relapse of intestinal involvement in BD(1; 8; 25; 27). Additionally, SAA in intestinal BS patients was found significantly higher than that in controls(28), the levels of SAA showed a better correlation with disease activity of intestinal BS patients than CRP(29). According to clinical or laboratory significance, we selected the above meaningful risk factors and turn them into categorical variables. By multivariate logistic regression analysis, we found gender (male), BDCAF ($\geq2$), ESR ($\geq15\text{mm/H}$), CRP ($>10\text{mg/L}$), HGB ($<130\text{g/L}$) and IL-6 ($>7\text{pg/ml}$) were the independent risk
factors of intestinal involvement in BS patients. A few previous studies suggested that there was similar frequency among men and women who complicated with intestinal involvement in BS (20; 30; 31), while several studies showed a slightly increased risk of intestinal BS among males(1.1-1.2:1)(32-34), which was consistent with our finding. This may be related with that some clinical manifestations at initial diagnosis tend to be more severe in male patients and they go to hospital earlier(35). BDCAF reflects the level of disease activity in BS patients, and it is logical that patients with intestinal involvement have a higher score than patients only with mucocutaneous involvement. The elevated levels of ESR and CRP can evaluate disease activity of intestinal BS have been discussed above, and our finding was consistent with the discoveries of other researchers. When ESR ≥15mm/H and CRP >10mg/L were found in a mucocutaneous BS patients, the risk of the patient complicated with intestinal involvement will increase 4.10 and 2.95 times more than that with normal level of ESR and CRP. Ye JF also found that patients with intestinal BS had lower HGB, higher levels of CRP and higher ESR than those patients with non-intestinal BS(36), which was consistent with our finding. Several studies have found that IL-6 was significantly higher in BS patients(9-11), suggesting that it was related to clinical activity of BS. In our study, elevated level of IL-6 (>7pg/ml) suggested the risk of mucocutaneous BS patients complicated with intestinal involvement will increase 8.23 times more than those with normal level of IL-6.

However, our study was conducted in a single center and included a relatively small number of BS patients. Our results may not be completely applicable to the general population. Therefore, multi-center samples are needed for verification in the future.

**Conclusion**

This study investigated the clinical characteristics and laboratory indicators in intestinal BS and mucocutaneous BS patients in China and analyzed the risk factors of intestinal involvement in BS patients. The independent risk factors of intestinal involvement in BS patients consist of gender (male), BDCAF (≥2), ESR (≥15mm/H), CRP (>10mg/L), HGB (<130g/L) and IL-6 (>7pg/ml). When the above factors
happen, they always remind us the presence of intestinal involvement in mucocutaneous BS patients. Therefore, attention should be paid to the risk factors of intestinal involvement and treatment plan should be adjusted immediately to avoid the occurrence of serious clinical symptoms including perforation and bleeding in BS patients.

**Declarations**

**Ethical approval and consent to participate**

This work was approved by the medical ethics committee of Huadong Hospital affiliated with Fudan University with the following reference numbers: 2016K044 and 2018K031.

**Consent for publication**

All patients agreed to publish this manuscript.

**Availability of data and material**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

All authors declare that they have no conflict of interest.

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**Authors' contributions**

Cheng-cheng Hou performed the statistical analysis and wrote the paper. Jian-long Guan designed the study. Jing-fen Ye and Hai-fen Ma followed the patients. All authors read and approved the final manuscript.

**Acknowledgements**

The authors had nothing to disclose.

**Abbreviations**
Behçet’s syndrome, BS; BDCAF, Behcet’s Disease Current Activity Form; NLR, neutrophil-to-lymphocyte ratio; WBC, white blood cells; RBC, red blood cells; PLT, Platelets; HGB, Hemoglobin; SAA, Serum amyloid A; C3, complement 3; C4, complement 4; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; TCH, total cholesterol; TG, triacylglycerol; UA, uric acid; LDL, low-density lipoprotein; HDL, high-density lipoprotein; IL-6, Interleukin 6.

Reference

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Figure 1

Intestinal lesion sites of 97 patients with intestinal BS
Figure 2

ROC curve analysis ESR (AUC=0.814), CRP (AUC=0.843) and IL-6 (AUC=0.754) could predict whether there was intestinal involvement in mucocutaneous BD patients (P=0.000). However, the gender (AUC=0.399) and HGB (AUC=0.384) could not well predict whether there was intestinal involvement in mucocutaneous BD patients (all AUC<0.5, P<0.05). BDCAF, Behcet’s Disease Current Activity Form; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; HGB, Hemoglobin; IL-6, Interleukin6. AUC, area under curve.

Supplementary Files

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- Tables.pdf