Clinical characteristics and treatment of “cytopenia phenotype” in Behçet syndrome: A Retrospective Cohort Study using a large data set from a referral center in China

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Abstract

Objectives:

This study aimed to describe the characteristics, treatment, and outcome of hematological involvement and assess possible association to define a “cytopenia phenotype” in BS patients.

Methods:

This was a retrospective study of BS patients in Shanghai Behçet syndrome database who were diagnosed with hematological involvement between October 2012 and December 2021.

Results:

A total of 40 BS patients with cytopenia were included (2.1%, 40/1950), the median follow-up duration was 35 (3-92) months. They presented high rate of trisomy 8 in the bone marrow (n=33, 82.5%). BS patients with cytopenia were more likely to be older age, female and have fewer skin lesions. Gastrointestinal (GI) ulceration, fever and high level of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were more frequent in BS patients with cytopenia. Ulcers in the ileocecal, small intestine and colon region and symptoms such as abdominal pain, weight loss and fever were more frequently seen in intestinal BS patients with cytopenia. All these cytopenia BS patients received standard guideline treatment regimens. In the follow-up, eight BS patients with cytopenia (8/40, 20.0%) died, 9 (22.5%) had not improved. As compared to alive BS patients, died BS patients were more likely to be older age and have high level of ESR.

Conclusions:

BS patients with cytopenia displayed a high rate of somatic mutations trisomy 8, GI involvement and high risk of death. Management of cytopenia was highly heterogeneous and many of the current treatment options are highly variable and suboptimal.

1. Introduction

Behçet syndrome (BS) is a variant of vasculitis. Its basic clinical feature is recurrent oral ulcers as an initial symptom, which is gradually associated with genital ulcers, nodular erythema, and other skin and mucous membrane lesions. It may be selective for ophthalmitis, intestinal ulcer, aortic regurgitation, venous thrombosis, arterial stenosis, aneurysm, arthritis, or cytopenia[1]. BS was first comprehensively described by the dermatologist Hulusi Behçet in 1937. The disease has a high prevalence in countries along the ancient Silk Road, a route of travel and commerce extending from the eastern Mediterranean to East Asia[2].

Many Rheumatic diseases have hematological manifestations. The mechanisms responsible for cytopenia are unclear, but it is thought to be related to marrow suppression by T lymphocytes, peripheral destruction by multi-organ involvement along with the production of pathogenic autoantibodies[3]. The pathogenesis of BS is different from other rheumatic diseases. BS is a vascular inflammatory disease, mainly affecting blood vessels throughout the body, and no pathogenic autoantibodies have been found so far. Few research pay attention to the hematological involvement in BS. There has been a recent increase in the number of case reports showing an association between intestinal BS and myelodysplastic syndrome (MDS)[4, 5]. MDS is a blood disorder characterized by impaired generation and maturation of hematopoietic cells in the bone marrow, leading to peripheral blood cytopenia[6]. It may also progress into acute leukemia. Previously, BS and MDS were considered two different diseases. However, there may be some connection between them since patients with MDS can develop autoimmune diseases such as BS, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), relapsing polychondritis, and vasculitis[7, 8]. It is also unclear whether immunosuppressive agents might affect this association[9]. Our team reported 16 patients with BS with MDS and found that gastrointestinal (GI) ulceration and trisomy 8 were more common in these patients [10].

In this study, based on a large cohort of consecutive BS patients from Shanghai Behçet Syndrome database, we aimed to describe the characteristics, management, and outcome of BS patients with cytopenia.

2. Materials And Methods

2.1. Study Design

This was a retrospective follow-up study of patients with BS in the Shanghai Behçet Syndrome database who had cytopenia and was diagnosed between October 2012 and December 2021. This work was approved by the medical ethics committee of Huadong Hospital affiliated to Fudan University with the following reference numbers: 2016K044 and 2018K031.

2.2. Patients

During the study period, 1950 patients with BS (with complete clinical and laboratory data) received medical care at Fudan University Huadong Hospital. All patients had active BS and were diagnosed according to the International Criteria for Behçet Disease (ICBD)[11] (the 1990 criteria were used because the 2014 criteria[12] were established during the study period). This retrospective study also included a cohort of 54 patients with MDS. MDS was diagnosed and classified according to the WHO classification[13], which takes into consideration morphologic, immunophenotypic, cytogenetic, fluorescence in situ hybridization (FISH), and molecular data. In order to consistent with the time when MDS patients were enrolled, we selected 944 BD patients from January 2017 to December 2021 for control analysis.

2.3. Endoscopy

Two experienced gastroenterologists performed endoscopic examinations using GIF H260 and CF-H260A1 endoscopes (Olympus, Tokyo, Japan) for upper and lower GI tract examinations, respectively. During endoscopy, two biopsies were taken from the gastric antrum for rapid urease tests and histological examination.

2.4 Diagnostic criteria for cytopenia

The presence of anemia (hemoglobin level two standard deviations below the mean for age), leukopenia (white blood cell count less than 4000/mm3), thrombocytopenia (platelet less than 100,000/mm3), and pancytopenia (decrease in all three blood cell types) were investigated. Patients whose hematological findings were attributed to other reasons such as drug toxicity, decreased organ function and infections were excluded from the study.

2.5. Cytogenetics

Bone marrow chromosome analysis was performed using chromosome-banding procedures. At least 20 metaphases were analyzed. Abnormal clones were described according to the 2006 International System for Human Cytogenetic Nomenclature (ISCN)[14], and aberrations were counted following the International Working Group on MDS Cytogenetics (IWGMC) consensus guidelines[15]. FISH analysis was performed on short-term cultured bone marrow. Sample preparations and hybridizations were performed according to the manufacturer's recommendations, using commercially available probes (Vysis Inc., Downers Grove, IL, USA). At our hospital, systematic screening for trisomy 8 was performed on patients with BS with cytopenia. Karyotyping was performed on these patients based on clinical suspicion of trisomy 8. Blood marrow aspiration was done to perform karyotyping, and a minimum of 500 interphase cells were analyzed. If the cells with an abnormal signal were < 5%, 1,000 interphase cells were screened. Normal control values were previously established using five normal samples of bone marrow donors and 15 bone marrow samples of patients with iron deficiency anemia but with normal karyotype.

2.6. Clinical Evaluation and Laboratory Findings

The presence of GI symptoms, such as abdominal pain, melena/hematochezia, diarrhea, weight loss, perforation, ileus, or bleeding, and extra-GI symptoms, such as genital ulcers, uveitis, dermatological lesions, neurological involvement, peripheral vasculitis, and joint involvement, were recorded at the time of diagnosis. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, and peripheral blood counts were also recorded at the time of endoscopy.

2.7. Statistical Analysis

The software of SPSS version 25.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Continuous data were tested for normal distribution using the Kolmogorov–Smirnov test. Normally distributed continuous data were presented using mean ± standard deviation and analyzed using Student's *t*-test. Nonnormally distributed data were presented as a median (range) and analyzed using the Mann–Whitney U test. Categorical data were presented as frequencies and analyzed using the chi-squared test or Fisher's exact test, as appropriate. Factors found to be significantly different between the groups were integrated into a logistic regression model to identify independently associated factors for cytopenia development. Two-sided p-values < 0.05 were considered statistically significant.

3. Results

3.1. Patients Characteristics

There were 40 (2.1%) BS patients with cytopenia from 1950 BS patients in the database (Fig. 1 and Table 1). The age of all these patients were at the time of first diagnosed with BS. Table 2 shows the demographic and clinical features of BS patients, BS with cytopenia patients, and MDS patients. Among the 40 BS patients with cytopenia, the majority were female, which was different from BS and MDS patients (67.5% vs 48.9% vs 29.6%, $p < 0.05$). The mean age at diagnosis was 44.3 ± 12.2 years for BS with cytopenia which was older than BS patients without cytopenia (37.9 ± 12.7 years) and younger than MDS patients (67.2 ± 12.4 years) ($p < 0.05$).

Table 1
Clinical characteristics of 40 patients with Behçet syndrome and cytopenia.

Case No.	Age/sex	BS symptoms	cytopenia type	MDS	Karyotype and FISH	Endoscopic findings	Distribution pattern	BS duration (years)	cytopenia duration (years)	Cytotc to cyt
1	47/F	IOGA	Leucopenia + anemia	MDS-RCMD	47XX + 8	Terminal ileum, rectum, CU	Multi-segmental	20	20	-
2	35/F	IOGS	Leucopenia + thrombocytopenia + anemia		47XX + 8	Terminal ileum	Multi-segmental	6	10	-
3	35/M	IOG	Leucopenia	MDS-U	47XX + 8	Terminal ileum	Single	5	1	Cyclos Thalid
4	68/F	IOSA	Anemia	-	47XX + 8	Terminal ileum, ileocecal valve	Multi-segmental	40	1	MMF Thalid
5	46/F	IOGPA	Leucopenia	-	47XX + 8	Ileocecal valve	Single	10	10	-
6	65/M	IOG	Anemia	MDS-RAEB	48,XY,+8,+19,del(20)(q11.2)	Terminal ileum, CU	Multi-segmental	5	0.5	-
7	56/F	IOPS	Leucopenia + anemia	MDS-RCUD-RN	47XX + 8	Ileocecal valve	Single	12	7	-
8	58/F	IOGS	Leucopenia + thrombocytopenia + anemia	-	47XX + 8	Terminal ileum, CU, small intestine	Multi-segmental	36	20	Thalid
9	48/F	IOGP	Leucopenia + thrombocytopenia + anemia	MDS-RCMD	47XX + 8	Ileocecal valve	Single	8	7	-
10	34/F	IOG	Leucopenia + thrombocytopenia + anemia	MDS-RCUD/AA	47XX + 8,-20,add(21)(q21)	Terminal ileum, CU	Multi-segmental	4	4	-
11	36/F	OG	Leucopenia	AA	47XX + 8	Terminal ileum	Single	4	10	-
12	44/F	OGP	Leucopenia	-	47XX + 8	-	-	10	20	-
13	40/F	OG	Leucopenia + thrombocytopenia		47XX + 8	-	-	10	1	-
14	59/M	OGA	thrombocytopenia + anemia	MDS-U	47XX + 8	-	-	10	1	-
15	30/F	IOGS	Leucopenia + thrombocytopenia + anemia	-	47XX + 8	Terminal ileum	Single	5	5	Thalid
16	42/F	OGS	Leucopenia + thrombocytopenia	MDS-U	47XX + 8	-	-	3	5	antitul drugs
17	46/F	OG	Leucopenia	-	47XX + 8	-	-	10	20	-
18	25/F	IOG	Leucopenia	-	47XX + 8	-	-	10	10	-
19	52/M	IOG + Ocular	thrombocytopenia	-	47XY + 8	Terminal ileum, CU	Multi-segmental	7	1	Thalid
20	56/F	IOA	Leucopenia + thrombocytopenia + anemia	MDS-RCMD	48XX + 8 + 9	Terminal ileum, small intestine	Multi-segmental	5	0.5	-
21	45/F	IOP	Leucopenia	-	47XX + 8	intestinal perforation, CU	Multi-segmental	6	2	

I: Intestinal involvement; O: oral ulcer; G: genital ulcer; S: skin lesion; A: arthritis; V: Vascular involvement; P: pathergy. MDS-U: MDS-unclassifiable, RCMD: refra multilineage dysplasia, RAEB: refractory anemia with excess of blasts, RCUD: refractory cytopenia with unilineage dysplasia. CU: colonic ulcers. MMF: mycop bone marrow transplantation. CHM: Chinese herbal medicine. MSC: mesenchymal stem cell.

Case No.	Age/sex	BS symptoms	cytopenia type	MDS	Karyotype and FISH	Endoscopic findings	Distribution pattern	BS duration (years)	cytopenia duration (years)	Cytotc to cyt
22	39/M	OGS + Ocular	Leucopenia + thrombocytopenia	-	47XX + 8/46XY	-	-	10	7	-
23	70/F	OV	Anemia	-	47XX + 8 + 9	-	-	0.5	10	-
24	38/M	IOGS	Leucopenia + thrombocytopenia + anemia	MDS-U	48XY + 8 + 9	CU	Longitudinal ulcer	2	2	-
25	44/F	IOGS	Leucopenia + thrombocytopenia	-	47XX + 8	CU	Multi-segmental	10	10	-
26	54/M	IOS	Leucopenia	-	47XY + 8	CU + anastomotic ulcer	Multi-segmental	30	6	-
27	55/F	IO	Leucopenia + anemia	MDS-ED-1	47XX + 8,del(20)	Terminal ileum, CU	Multi-segmental	10	2	-
28	35/F	IOGA	Leucopenia + thrombocytopenia + anemia	-	48XX + 8 + 9	small intestine	Multi-segmental	7	2	-
29	50/M	IOG	Leucopenia + anemia	MDS-U	48XY + 8 + 1	Terminal ileum	Single	10	8	-
30	35/F	IOGA	thrombocytopenia	-	48XX + 8 + 9	CU	Single	6	15	-
31	32/M	IOG	thrombocytopenia	-	48XY + 8 + 9	small intestine	Multi-segmental	10	1	-
32	51/F	IOG + Ocular	Leucopenia + thrombocytopenia + anemia	MDS-U	47XX + 8	Terminal ileum, CU	Multi-segmental	18	3	-
33	49/M	IO	Leucopenia + anemia	MDS-U	46X-Y + 8	Terminal ileum, CU	Multi-segmental Longitudinal ulcer	10	7	antitul drugs
34	30/M	OGS	Leucopenia	-	46XY	-	-	4	6	-
35	58/M	IO	thrombocytopenia + anemia	-	46XY	Small intestinal ulcer and bleeding	Multi-segmental	2	2	-
36	17/F	IOG	Leucopenia	-	46XX	Terminal ileum	Single	1	8	-
37	26/F	IOG	thrombocytopenia		46XX	Terminal ileum	Single	3	3	-
38	47/M	IOGA	Leucopenia + thrombocytopenia + anemia	-	46XY	Terminal ileum, CU	Multi-segmental	6	6	
39	31/F	OGA + Ocular	Leucopenia	-	46XX	-	-	4	1	colchi
40	44/F	IOG	Leucopenia + thrombocytopenia + anemia	-	46XX	Terminal ileum, CU, Small intestinal	Multi-segmental	10	6	Thalid

I: Intestinal involvement; O: oral ulcer; G: genital ulcer; S: skin lesion; A: arthritis; V: Vascular involvement; P: pathergy. MDS-U: MDS-unclassifiable, RCMD: refra multilineage dysplasia, RAEB: refractory anemia with excess of blasts, RCUD: refractory cytopenia with unilineage dysplasia. CU: colonic ulcers. MMF: mycop bone marrow transplantation. CHM: Chinese herbal medicine. MSC: mesenchymal stem cell.

Table 2
Clinical characteristics of BS patients with/without cytopenia and MDS patients.

	BS with cytopenia (n = 40)	BS without cytopenia (n = 904)	MDS (n = 54)	P value
Age, years mean (SD) (at the diagnosis of BS)	44.3 ± 12.2*	37.9 ± 12.7	67.2 ± 12.4**	* 0.002 **<0.001
Male (%)	13 (32.5) *	462 (51.1)	38(70.4)**	* 0.021 **0.001
Oral ulcer	40 (100)	893 (98.8)	-	0.483
Genital ulcer	31 (77.5)	717 (79.3)	-	0.938
Ocular lesion	4 (10.0)	221 (24.4)	-	0.036
Arthritis	9 (22.5)	243 (26.9)	-	0.540
Skin lesions	10 (25.0) *	633 (70.0)	2 (3.7)	0.000
Positive pathergy test	6 (15.0)	55 /125 (44.0)	-	0.001
Central nervous involvement	1 (2.5)	19 (2.1)	-	0.583
Vascular lesions	1 (2.5)	74 (8.2)	-	0.316
GI involvement	31 (77.5) *	110 (12.2)	1 (1.9) **	all < 0.001
Fever	34 (85.0) *	13 (1.4)	10 (18.5) **	all < 0.001
Laboratory findings at diagnosis of BS				
Trisomy 8	33 (82.5)	0/20 (0)	4 (7.4)	all < 0.001
ESR (mm/h)	50.0 ± 33.5*	19.8 ± 20.5		0.000
CRP (mg/L)	26.2 ± 29.9*	12.7 ± 23.6		0.001
WBC (10^9/L)	3.4 ± 2.1*	7.5 ± 3.1		0.000
Hemoglobin (g/L)	95.5 ± 21.7*	130.8 ± 38.6		0.000
Platelets (10^9/L)	131.9 ± 121.0*	240.0 ± 74.9		0.000
* BS with cytopenia compared to BS without cytopenia. ** BS with cytopenia compared to MDS.				
BS: Behçet syndrome; GI, gastrointestinal; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cells.				

In the BS with cytopenia group, oral ulcers were the most common finding (100%), followed by fever (85.0%), genital ulcers (77.5%), GI involvement (77.5%), and skin lesions (25.0%), positive pathergy test (15.0%). Uveitis and vascular lesions, which are common features of BS, only one (2.5%) patient recorded respectively. GI involvement and fever incidence were more common in BS with cytopenia patients ($p < 0.001$), while ocular lesion, skin lesions and positive pathergy test were less common ($p < 0.05$). Trisomy 8 were significantly more common in BS patients with cytopenia (85.0% vs 0% vs 7.4%, $p < 0.001$). Leukocyte count, hemoglobin levels, and platelet count were significantly lower, and ESR and CRP significantly higher in BS with cytopenia patients than in those BS without cytopenia ($p < 0.05$). In terms of the time of cytopenia and BS, our study found that 21(52.5%) cases of BS occurred before cytopenia, 11 (27.5%) cases of cytopenia and BS occurred at the same time, and 8 (20.0%) cases of BS occurred after cytopenia. Through logistic regression analysis, it was found that gender, age, fever, GI involvement, and high level of ESR and CRP were independent risk factors for BS with cytopenia (Supplement Table 1).

3.2 Types of cytopenia in BS patients

Among the 40 BS patients with cytopenia, the most common hematologic abnormality were leukopenia (n = 11, 27.5%) and pancytopenia (n = 11, 27.5%), followed by thrombocytopenia (n = 4, 10.0%), anemia (n = 3, 7.5%). Eleven patients (27.5%) were bi-cytopenia, including leucopenia plus anemia (n = 5, 12.5%), leucopenia plus thrombocytopenia (n = 4, 10.0%) and thrombocytopenia plus anemia (n = 2, 5.0%) (Fig. 1). All of these anemia patients were macrocytic anemia.

25 (62.5%) of the cytopenia BS patients whom lacking morphological criteria in the bone marrow were not considered as patients with MDS or other hematologic disease. 13 (32.5%) were diagnosed with MDS, and two (5.0%) patients were initially diagnosed with MDS and later diagnosed with aplastic anemia (AA). All patients were clinically and cytogenetically evaluated once when BS was active, with or without treatment. Based on the WHO MDS classification, seven (17.5%) patients had MDS-unclassifiable (MDS-U), three (7.5%) had refractory cytopenia with multilineage dysplasia (RCMD), one (2.5%) had refractory anemia with excess of blasts (RAEB), one (2.5%) had refractory cytopenia with unilinear dysplasia (RCUD), and one (2.5%) had refractory cytopenia with unilinear dysplasia and refractory neutropenia (RCUD-RN).

3.3. Characteristics of BS Patients with cytopenia in GI Involvement

Among the 904 patients with BS, 110 had GI without cytopenia, while 31 had GI involvement in BS patients with cytopenia group. Table 3 shows the demographic and clinical features of the two groups. Among the 31 GI BS patients with cytopenia, the majority were female and older age, which was different from GI BS patients ($p < 0.05$). In patients with GI BS with cytopenia, oral ulcers were the most common finding (100%), followed by fever (93.5%), genital ulcers (71.0%), skin lesions (22.6%), arthralgia (22.6%), positive pathergy tests (12.9%), ocular involvement (6.5%), and central nervous involvement (3.2%). GI BS Patients with cytopenia were more likely to have intestinal ulcers in the ileocecal ($p < 0.001$), small intestine ($p = 0.014$) and colon regions ($p = 0.003$), while no esophagus ulcers were found ($p = 0.011$). Figure 2 shows the typical colonoscopy results of BS patients with GI and cytopenia. Abdominal pain, and weight loss were more common in the patients with cytopenia ($p < 0.001$). In addition, we selected 20 patients with intestinal ulcer and anemia for bone marrow chromosomal testing, and no chromosomal abnormalities were found. This is significantly different from BS patients with cytopenia (83.9% vs 0%, $p < 0.001$). GI BS patients with cytopenia were likely to have lower leukocyte count, hemoglobin level, and platelet count, and higher ESR and CRP levels ($p < 0.05$).

Table 3
Clinical characteristics of intestinal BS patients with and without cytopenia.

	Intestinal BS with cytopenia (n = 31)	**Intestinal BS without cytopenia (n = 110)**	***P* value**
Age at diagnosis of intestinal BS (years)	44.6 ± 12.2	36.5 ± 15.1	0.004
Male (%)	9 (29.0)	58 (52.7)	0.020
Duration (years)	10.3 ± 8.9	9.3 ± 9.2	0.129
Systemic signs			
Oral ulcer	31 (100)	108 (98.2)	> 0.99
Genital ulcer	22 (71.0)	69 (62.7)	0.397
Ocular lesion	2 (6.5)	3 (2.7)	0.660
Arthralgia	7 (22.6)	22 (20.0)	0.754
Skin lesions	7 (22.6)	42 (38.2)	0.107
Positive pathergy test	4 (12.9)	20 (18.2)	0.674
Central nervous system	1 (3.2)	1 (0.9)	0.917
Symptoms of GI involvement			
Abdominal pain	28 (90.3)	37 (33.6)	0.000
Melena/hematochezia	10 (32.3)	30 (27.2)	0.587
Diarrhea	3 (9.7)	46 (41.8)	0.002
Weight loss	28 (90.3)	44 (40.0)	0.000
Fever	29 (93.5)	8 (7.3)	0.000
Perforation	4 (12.9)	8 (7.3)	0.530
Ileus	1 (3.2)	8 (7.3)	0.690
Location of intestinal ulcers			
Esophagus	0 (0)	18 (16.4)	0.011
Ileocecal	26 (83.9)	46 (41.8)	0.000
Small intestine	6 (19.4)	6 (5.5)	0.014
Colon	15 (48.4)	24 (21.8)	0.003
Multi-segmental	20 (64.5)	73 (66.4)	0.848
Laboratory findings at intestinal BS diagnosis			
Trisomy 8	26 (83.9)	0/20 (0)	0.000
ESR (mm/h)	58.8 ± 31.19	35.7 ± 14.7	< 0.001
CRP (mg/L)	37.3 ± 33.8	21.2 ± 33.2	0.020
WBC (10^9/L)	3.6 ± 2.3	7.5 ± 3.2	< 0.001
Hemoglobin (g/L)	94.2 ± 20.4	123.9 ± 18.9	< 0.001
Platelets (10^9/L)	129.8 ± 116.9	248.0 ± 84.0	< 0.001

BS: Behçet syndrome; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; WBC: white blood cells. HI: hematologic involvement.

3.4. Treatment of BS with Cytopenia

Overall, 40/40 (100%) BS patients with cytopenia had received treatment, 33 (82.5%) with steroids (prednisone, median dose 30 mg/day [IQR 20–48])(Fig. 3). Along with steroids, the immunosuppressive drugs that were used included thalidomide (n = 38), infliximab (n = 29), cyclosporine (n = 28), adalimumab (n = 4) and colchicine (n = 5). Some patients were switched to other treatments due to poor response to steroids and tumor necrosis factor inhibitors, among which the treatment was changed to azacitidine (n = 2), bone marrow transplantation (n = 2) and Chinese herbal medicine (n = 1).

3.5 Mortality of BS patients with cytopenia

In the follow up, one female presented with MDS and cervical cancer, one female presented with laryngeal carcinoma. (Table 1). BS patients with cytopenia were divided into four categories according to their treatment status: improved, stable, not improved and died (Fig. 3). In a median follow-up of 35 months (range, 3 to 92 months), 9/40 (22.5%) cases showed complete responses to first-cycle therapy (about three months) and achieved clinical improvements for BS and hematologic abnormalities. Meanwhile, 8/40 (20.0%) cases showed no improvement, not only in BS but also in hematologic abnormality. Several cases (15/40; 37.5%) showed stability in BS and cytopenia. In total, 8/40 (mortality rate: 20.0%) patients died during follow-up: two from infectious complications, five from hematological complications, and one with an undetermined cause of death. As compared from the BS with cytopenia alive patients, age were much older in the dyed patients group (53.4 ± 11.4 vs 42.0 ± 11.4, $P = 0.016$) (Table 4). Leucopenia and isolated trisomy 8 were more common in dyed patients but did not have statistical significance. High level of ESR were significantly more frequent among dyed patients ($p = 0.019$).

Table 4
Comparison of clinical characteristics between death and survival of BS patients with cytopenia

	BS with cytopenia alive (n = 32)	BS with cytopenia died (n = 8)	*P* value
Age at diagnosis of BS (years)	42.0 ± 11.4	53.4 ± 11.4	0.016
Male (%)	6 (18.8)	3 (37.5)	0.348
BS Duration (years)	9.9 ± 2.9	5.8 ± 2.9	0.232
Cytopenia Duration (years)	6.9 ± 6.1	5.1 ± 4.1	0.451
Systemic signs			
Oral ulcer	32 (100)	8 (100)	> 0.99
Genital ulcer	26 (81.2)	5 (62.5)	0.348
Ocular lesion	3 (9.4)	1 (12.5)	> 0.99
Arthralgia	8 (25.0)	1 (12.5)	0.655
Skin lesions	8 (25.0)	2 (25.0)	> 0.99
Central nervous system	1 (3.1)	0 (0)	> 0.99
GI involvement	24 (75.0)	7 (87.5)	0.655
Types of cytopenia			
Leucopenia	11 (34.4)	0 (0)	0.080
Anemia	1 (3.1)	2 (25.0)	0.096
thrombocytopenia	3 (9.4)	1 (12.5)	> 0.99
thrombocytopenia + anemia	2 (6.3)	0 (0)	> 0.99
Leucopenia + anemia	3 (9.4)	2 (25.0)	0.257
Leucopenia + thrombocytopenia	3 (9.4)	1 (12.5)	> 0.99
Pancytopenia	9 (28.1)	2 (25.0)	> 0.99
Laboratory findings at BS diagnosis			
abnormal karyotype	25 (78.1)	8 (100)	0.218
Isolated trisomy 8	23 (71.9)	3 (37.5)	0.159
ESR (mm/h)	43.8 ± 27.7	74.4 ± 45.0	0.019
CRP (mg/L)	18.9 ± 20.9	53.0 ± 44.4	0.068
WBC (10^9/L)	3.2 ± 1.9	4.2 ± 2.7	0.225
Hemoglobin (g/L)	97.1 ± 22.7	89.4 ± 16.4	0.376
Platelets (10^9/L)	117.9 ± 82.2	188.1 ± 217.1	0.397

BS: Behçet syndrome; GI: gastrointestinal; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; WBC: white blood cells.

4. Discussion

BS is a complex, multisystemic disorder with unknown etiology and unique geographic distribution. There has been controversy over whether to call it a “disease” or “syndrome.” Many studies have shown that it is composed of multiple phenotypes, such as skin-mucosa, eye, gastrointestinal, vascular, and neurological involvement[16]. The present study we focused on a unique group of BS patients whom with cytopenia. There is no large sample report on BS patients with cytopenia in the literature, and the reports are mainly case reports[17, 18].

In this retrospective study, we assessed the demographic, clinical, laboratory manifestations, and treatment of these patients from Shanghai BS database. Cytopenia was observed in 2.1% of our BS patients. From our large number sample research, BS patients with cytopenia have their own characteristics that are different from BS and MDS patients. Firstly, BS patients with cytopenia were more likely to be female and older than BS patients and much younger than MDS patients. Secondly, gastrointestinal (GI) ulceration and fever were more common, and the level of ESR and CRP significantly higher in BS patients with cytopenia. Thirdly, somatic mutations trisomy 8 were more common in BS patients with cytopenia (82.5%).

GI ulceration was observed in 77.5% of our BS patients with cytopenia. Abdominal pain and weight loss were more common while diarrhea was less common. The location in the ileocecal, small intestine and colon region were more frequently seen in intestinal BS patients with cytopenia. To investigate the role of

trisomy 8 in GI ulceration, we randomly selected 20 BS patients without cytopenia and performed bone marrow karyotype analysis. Trisomy 8 (83.9%) were more common in intestinal BS with cytopenia. In summary, BS with cytopenia were more common in older women, high incidence of intestinal ulcers and trisomy 8.

Some studies have even referred to BS with cytopenia as a “Behçet-like disease”[19, 20]. Analyses of several case reports have shown an association between trisomy 8 and intestinal BS with MDS[4, 21–23]. Indeed, trisomy 8 in BS with MDS has been reported in 87% of patients[4], compared to 5–7% of patients with primary MDS but without BS[24]. Trisomy 8 with BS but without MDS has also been reported[25, 26]. From our research data, we can see that 62.5% of the cytopenia BS patients whom lacking morphological criteria in the bone marrow were not considered as patients with MDS, and 85% with trisomy 8 especially isolated trisomy 8. Although our study also compared the difference analysis between patients with chromosomal abnormalities and those without chromosomal abnormalities, no statistical difference was found, and further follow-up of these patients is needed (Supplement Table 2). Another very prominent feature is that the anemia in these cytopenia patients is macrocytic anemia, unlike microcytic hypochromic anemia caused by intestinal ulcers in BS patients. In our study, no regularity was found in the timing of cytopenia and BS, and the mutation of chromosome 8 may be closely related to it. Trisomy 8 is considered a secondary or late event in the MDS transformation process[27, 28]. The precise mechanisms underlying the tumorigenesis remain unclear, although a relationship has been observed between trisomy 8 and symptoms related to BS, both in the present study and in the literature. Isolated trisomy 8 has been discussed in some studies[29, 30]. It can be a constitutional condition as a constitutional mosaicism (cT8M) in healthy people, and it was not considered a tumor marker in certain studies. Trisomy 8 is a somatic mutation. Unlike germline mutations, somatic mutations occur throughout the lifespan, and may play a causal role in non-heritable rheumatological diseases, especially conditions that start in later life[31]. This also explains why BS patients with trisomy 8 do not have a tendency to familial aggregation and most of them occur in older age.

Management of BS patients with cytopenia can be challenging since there is no consensus- or evidence-based guidelines in the current literature. In the present cohort, our patients have been treated according to the treatment algorithm presented in Fig. 3. As the European League Against Rheumatism (EULAR) recommendations for the management of BS, most cytopenia patients especially GI involvement who received corticosteroid combined with monoclonal anti-TNF antibodies such as infliximab or adalimumab[32]. Twenty patients did not respond to corticosteroids and anti-TNF antibodies. Two of them were then given bone marrow transplantation and one patient was then given Chinese herbal medicine. All of these three patients achieved complete remission of GI findings and cytopenia after treatment. This is consistent with some case reports[33, 34]. However, two of the patients died after switching to azacitidine. This is different from some case reports that azacitidine is effective in these patients[35, 36]. As the follow-up time increased, we found that some patients' cytopenia and intestinal ulcers recurred and became more serious. Only 9 cytopenia BS patients improved, which shows how treatment difficulties for this particular clinical phenotype.

This study has some limitations because of its retrospective design and data from one single center, including some potential selection bias and missing data. Further, we considered that BS with cytopenia to be an independent subtype and have not done further subtyping analysis with other subtypes of BS, only analyzed the intestinal BS. Because the number of BS cytopenia patients without trisomy 8 was very small, it is necessary to compare the clinical data of the with and without trisomy 8 BS patients, so as to separate the BS cytopenia patients with trisomy 8 as a new clinical syndrome.

5. Conclusion

BS with cytopenia patients have mostly oral/genital ulcers, rash, fever and GI ulcerations, while ocular lesions are uncommon, and have unique characteristics in terms of gender, age, high proportion of trisomy 8 and low survival rate. Supporting that this phenotype may be an entity distinct from classical BS. Further studies are needed to determine the role of overexpressed trisomy 8 genes, identify the underlying mechanisms, and determine the best treatment regimen.

Declarations

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The authors had nothing to disclose.

Authors’ contributions

Y S performed the statistical analysis and wrote the paper. J-L G designed the study. L S, H-F M and D L recruited the patients. L-L J, J-F C, and J Z revised the paper. The authors read and approved the final manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Availability of data and materials

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

Ethics approval and consent to participate

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

Consent for publication

All authors and patients agreed to publish this manuscript.

Competing interests

The authors declare that they have no competing interests.

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Figures

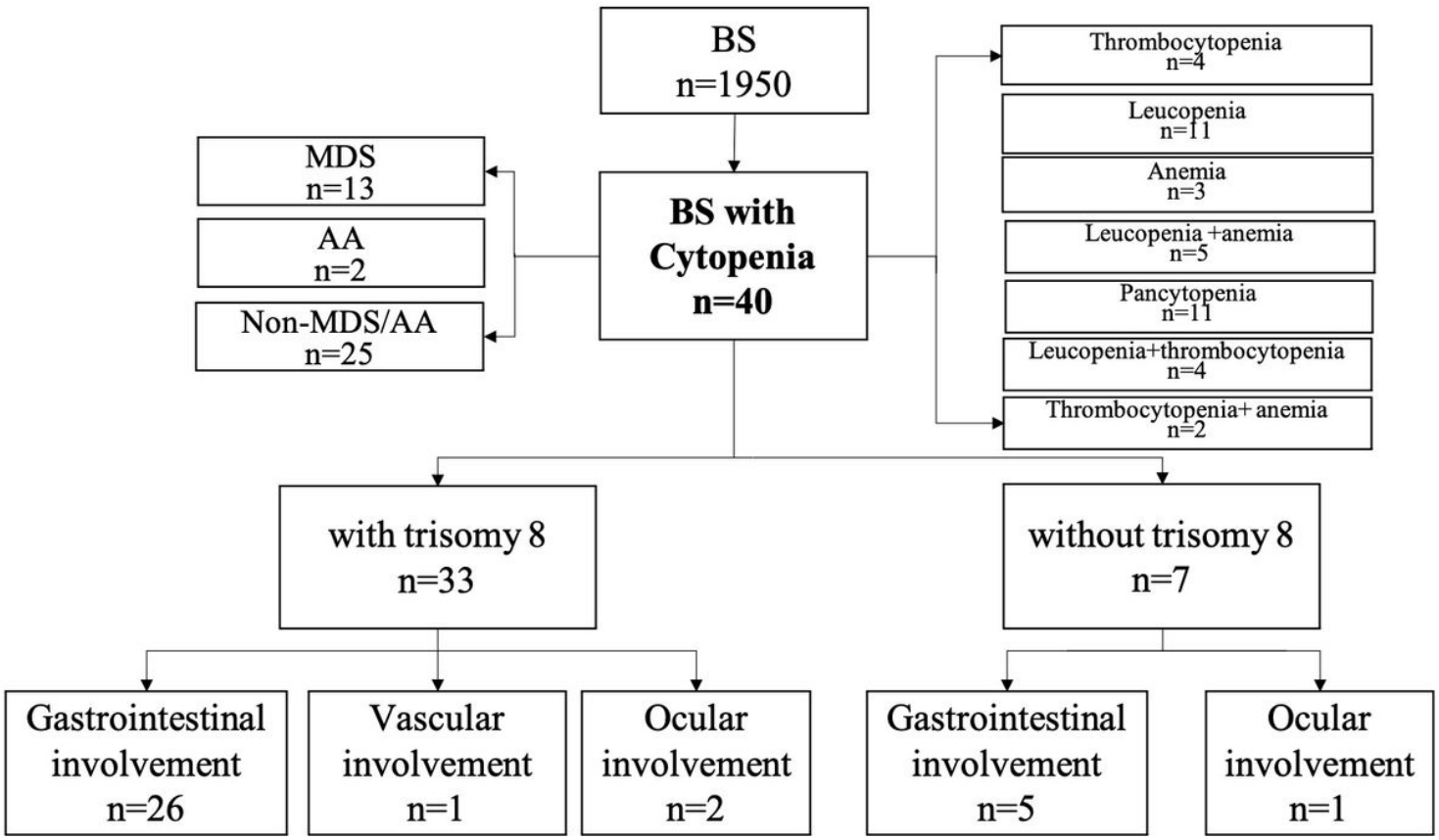

Figure 1

Flow diagram of screening, selection and follow up of the study BS population.

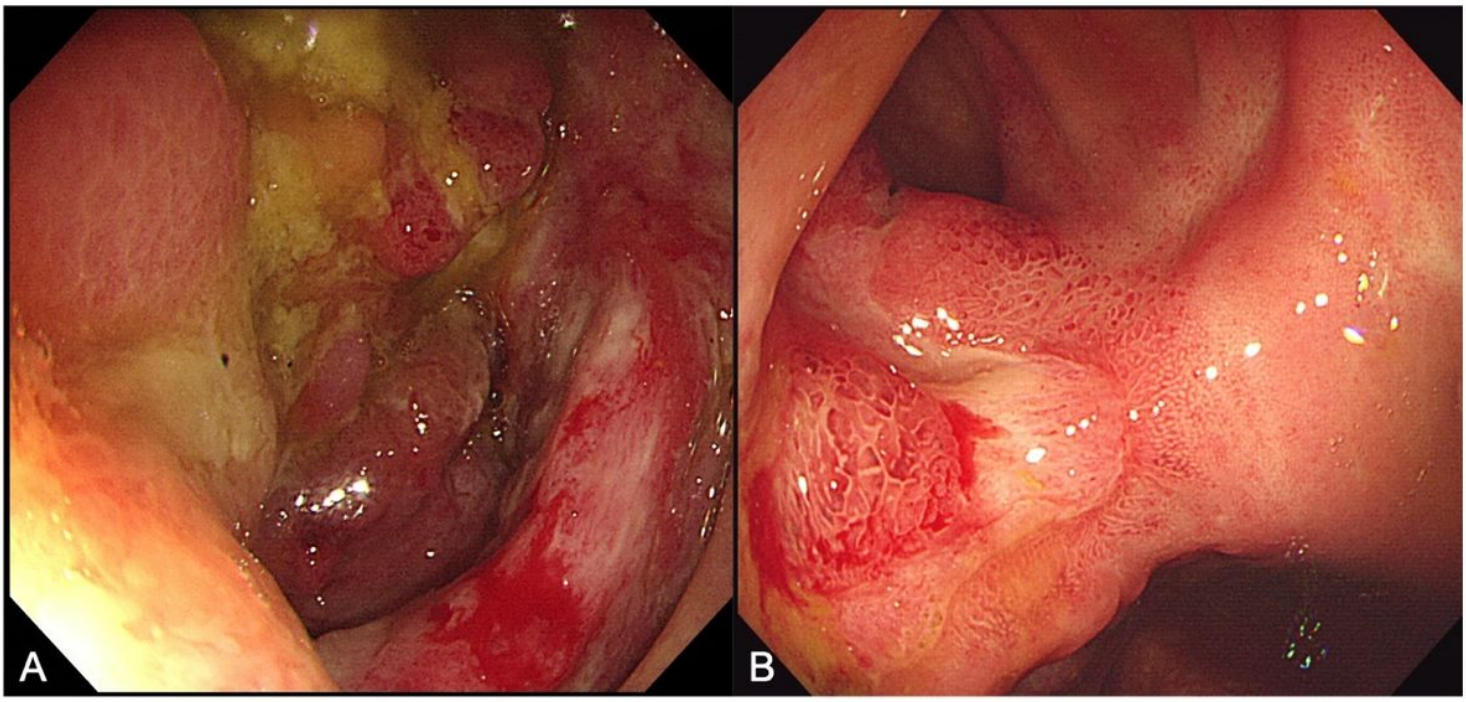

Figure 2

In two BS patients with cytopenia, typical colonoscopy results of gastrointestinal involvement showed severe open ulcers in the ileocecal area (A), huge round edema at the end of the ileum, deep layer and diffuse erosion ulcer (B).

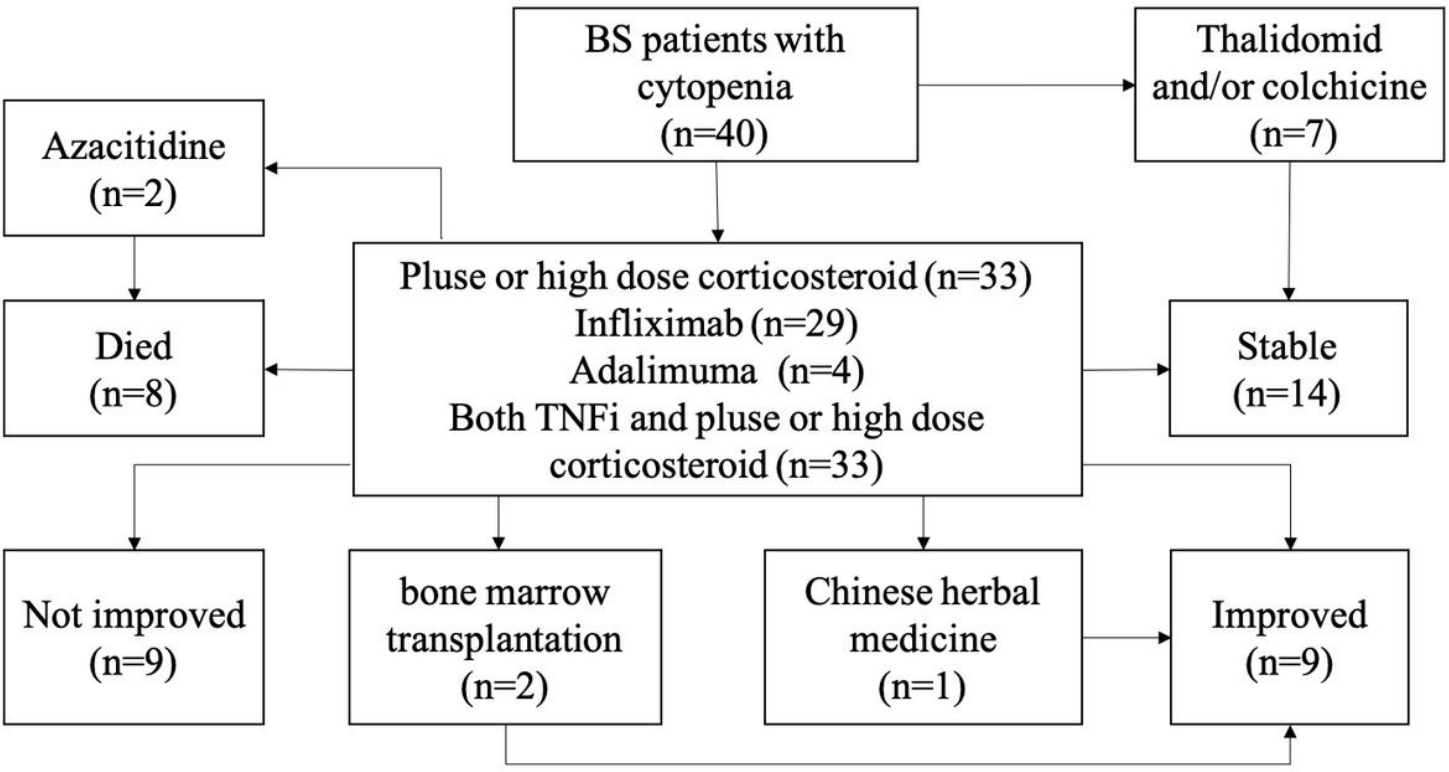

Figure 3

Treatment algorithm of BS patients with cytopenia.

Improved: clinical symptoms improved and peripheral blood cells normal; Stable: clinical symptoms improved and peripheral blood cells maintained at previous levels without significant changes; Not improved: clinical symptoms not improved and peripheral blood cells were lower than before.

Supplementary Files

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- SupplementTable1.docx