Behçet's disease (BD) is a chronic relapsing-remitting inflammatory disorder affecting multiple organs. Oral aphthae and genital ulcerations represent the hallmark lesions, but the disease can also manifest with uveitis and skin lesions (erythema nodosum, pseudofolliculitis). Vasculitis and thrombosis are serious manifestations of BD. In particular, central nervous system involvement is a life-threatening condition and can be characterized by primary parenchymal lesions (neuro-Behçet) or vascular involvement [1]. According to recommendations of EULAR (European League Against Rheumatism), therapy is tailored to disease phase and severity, and includes corticosteroids, azathioprine, interferon, cyclophosphamide, methotrexate, or tumor necrosis factor-alpha (TNFα) blockers [2]. Recently, interleukin (IL)-1 inhibiting agents were also tested as a possible therapeutic alternative in BD [3,4]. With regard to neuro-BD, biological treatment has been proposed in isolated cases and in small groups of patients [5].

We describe here the clinical course of four Caucasian BD patients with different disease manifestations successfully treated with intravenous immunoglobulins (IVIG).

**ABSTRACT:** Behçet's disease (BD) is a multi-systemic disorder of unknown etiology characterized by relapsing oral-genital ulcers, uveitis, and involvement of the articular, gastrointestinal, neurologic, and vascular systems. The choice of treatment is based on the severity of systemic involvement, clinical presentation and the site affected, and includes corticosteroids, azathioprine, interferon, cyclophosphamide, methotrexate or tumor necrosis factor-alpha and interleukin-1 blockers. We present a case series of four refractory BD patients successfully treated with intravenous immunoglobulins (IVIG). All patients fulfilled International Study Group criteria. The patients' mean age was 38.75 ± 12.09 years and mean disease duration 10.25 ± 8.5 years. Human leukocyte antigen B51 was positive in two of four patients. In addition to oral aphthosis, all patients suffered from genital ulcers and cutaneous BD-related manifestations; central nervous system involvement and arthralgia were found in two patients. Peripheral nervous system, gastrointestinal and eye involvement occurred in 25% of cases. In all patients, previously treated according to EULAR recommendations without reaching satisfactory results, IVIG induced immediate and sustained response over time without incurring any side effects. We propose IVIG administration as an additional effective and safe treatment option in patients with severe and resistant BD.

**KEY WORDS:** Behçet's disease (BD), central nervous system, peripheral nervous system, aphthosis, autoimmunity, intravenous immunoglobulins (IVIG)

**PATIENTS AND METHODS**

**PATIENT 1**
In March 2015 a 44 year old woman diagnosed with BD was admitted to our department with a 19 year history of bipolar aphthosis and pseudofolliculitis refractory to standard treatment. Human leukocyte antigen (HLA)-B51 was positive. In the past, she had been treated with oral prednisone (up to 25 mg/day), colchicine and several immunosuppressive agents including cyclosporine, cyclophosphamide and methotrexate without achieving any real benefit. In addition, the anti-TNF agents infliximab (5 mg/kg every 8 weeks) and golimumab (50 mg/month) as well as the IL-1 receptor antagonist anakinra (100 mg/day) had been administered leading to only temporary improvement. Moreover, the humanized anti-IL-6 receptor antibody tocilizumab (8 mg/kg) had also been tried, but it induced disease exacerbation [6]. At admission, the patient complained of painful swallowing in the right laterocervical region and diffuse pseudofolliculitis. A fibrolaryngoscopy showed a volu-
minous ulcer of the right aryepiglottic fold with edema and hyperemia of the epiglottis.

On the basis of preliminary data in the literature [7-10], a course of gammaglobulin 0.5 g/kg for 3 consecutive days per month was started with colchicine (1 mg/day) and methylprednisolone (4 mg/day). The oral aphthosis and cutaneous lesions subsided after the first two gammaglobulin infusions and resolved at the end of the third administration. Nine days later fibrolaryngoscopy demonstrated complete healing of the ulcer. The patient continued on gammaglobulin therapy and no recurrences were recorded at 1 year follow-up.

PATIENT 2
This 53 year old woman was diagnosed with BD according to International Study Group (ISG) criteria [1]. She had suffered from recurrent oral aphthosis and genital ulcers, arthralgia of wrists and hands, and erythema nodosum of the legs since 1999. HLA-B51 was negative. She had already been treated with corticosteroids (up to prednisone 25 mg/day) and colchicine 1 mg/day (interrupted early due to gastrointestinal discomfort), with incomplete control of symptoms. Later, the patient had undergone cyclosporine treatment (3 mg/kg/day) with complete resolution of all symptoms. However, cyclosporine was stopped after 6 months because of an increase in serum creatinine levels, and azathioprine (2 mg/kg/day) combined with prednisone (5 mg/day) was started, which led to good disease control for almost 2 years. At this point in her clinical history she started to complain of severe asthenia, abdominal pain with diarrhea, and paraesthesia of the lower limbs bilaterally. Azathioprine was discontinued, leading to relief of the gastrointestinal manifestations, but paraesthesia and other BD-related manifestations continued. Laboratory investigations showed an increase in erythrocyte sedimentation rate (ESR) (49 mm/hour), while cerebral magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) were normal while diagnostic testing for infectious diseases and immune system disorders were negative. The HLA-B51 allele was positive. A colonoscopy performed on admission did not show macroscopic lesions, while a marked non-specific chronic inflammation was found on histological analysis. A brain MRI showed several bilateral focal lesions particularly evident at the semi-oval centers, periventricular regions, frontal and temporal subcortical white matter and cerebellar peduncles. These lesions were not enhanced after injection of gadolinium. A previous brain MRI had highlighted a focal lesion in the right optic nerve, and a spinal cord MRI identified lesions in the cervical, thoracic and lumbar tracts. To rule out multiple sclerosis (MS) a CSF examination was performed, demonstrating a mild lymphomonocytic pleocytosis and the presence of few intrathecal oligoclonal IgG bands. Despite some diagnostic doubts, a diagnosis of BD was established since the patient fulfilled the ISG criteria for this disorder [4]. She had previously been treated with high dose oral prednisone (1–1.5 mg/kg/day), mesalazine (2400 mg/day), cyclosporine (3 mg/kg/day) and azathioprine (3–4 mg/kg/day) without reaching either an adequate clinical remission of disease or a normalization of acute-phase reactants, which were persistently elevated. The clinical course of the patient progressively worsened with severe weakness and episodes of fecal and urinary incontinence.

At this time, we started therapy with IVIG at a dose of 400 mg/kg/day for 5 days per month. Soon after the first treatment, the patient showed an almost complete disease remission with the disappearance of all neurologic, gastrointestinal, mucocutaneous and systemic disease manifestations. After 14 months of therapy, the patient was already symptom free. Inflammatory markers, checked monthly, remained stable within the normal range.

PATIENT 4
This patient was a 32 year old woman who had experienced four acute neurological attacks in the previous 24 months: two episodes of bilateral blurred vision with ocular pain and headache, a subsequent severe episode of left pyramidal syndrome with hemiplegia, and an episode of bilateral partial visual loss. The CSF examination showed normal biochemistry and cell counts, but numerous oligoclonal IgG bands were identified. Thus, a diagnosis of MS was made and the patient was treated with intravenous methylprednisolone (1 g/day for 5 days) without significant clinical improvement. A few months later, she was admitted for the first time to our institution due to acute painful right visual loss and severe headache. An accurate anamnesis revealed the presence of recurrent oral and genital ulcers and pseudofolliculitis. At that time, neurological examination showed a moderate left hemiparesis with hyper-reflexia and
positive Babinski sign on the same side of the body. She also experienced profound asthenia. Ophthalmologic examination confirmed the acute reduction in her right-side visual acuity and the visual evoked potentials revealed impaired neural conduction in crossed visual pathways. Brain MRI showed diffuse white matter lesions involving the centrum semiovale and the corona radiata of both hemispheres [Figure 1A]. The patient therefore met the ISG diagnostic criteria for BD. In addition, HLA-B51 allele was positive.

She had previously been treated with standard therapy without achieving remission. The anti-TNF agent etanercept was initiated and led to excellent clinical results, but the occurrence of severe neutropenia required that it be withdrawn. She then began treatment with IVIG at a dose of 400 mg/kg/day for

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**Table 1.** Demographic, clinical and therapeutic features of reported patients

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
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<tbody>
<tr>
<td>Gender</td>
<td>F</td>
<td>F</td>
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<tr>
<td>Age at disease onset (years)</td>
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<td>22</td>
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<tr>
<td>Age at diagnosis (years)</td>
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<td>24</td>
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<tr>
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<td>+</td>
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<tr>
<td>Organ involvement</td>
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<td>Mucocutaneous</td>
<td>Mucocutaneous</td>
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<td>Articular</td>
<td>Peripheral nervous system</td>
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<td>Fever</td>
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<td>Reason for starting IVIG</td>
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<td>Neurological</td>
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<td>Previous therapies</td>
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<td>Prednisone (1.0-1.5 mg/kg/day)</td>
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<td>Colchicine (1 mg/day)</td>
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<td>Azathioprine (3-4 mg/kg/day)</td>
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<td>Tocilizumab (8 mg/kg/every 4 weeks)</td>
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*IVIG = intravenous immunoglobulins*
5 days per month, resulting in an almost complete remission of the visual symptoms after the first treatment period. During the subsequent 6 months of treatment, there were no cutaneous lesions, acute episodes of visual loss, headache or CNS impairment. At 6 months, the IVIG dosage was reduced to 400 mg/kg daily for 2 consecutive days a month. After 12 months of treatment, the patient was clinically stable and no longer complained of diffuse weakness. At that time, a new brain MRI showed a decrease in the size of the white matter lesions [Figure 1B]. At 15 months, a further neurological examination showed significant amelioration of the asthenia, with slightly more strength in the left side of the body as well. At the 2-year follow-up, no further clinical relapses were recorded.

Table 1 summarizes demographic and clinical features of these four patients as well as previous treatment choices.

**DISCUSSION**

During the last few decades IVIGs have been increasingly administered for a wide number of autoimmune and systemic inflammatory diseases, while multiple non-exclusive mechanisms of action have been suggested [11-14]. Rheumatologic immune disorders treated with IVIG include dermatomyositis, polymyositis and Kawasaki disease. However, the increasing use of off-label IVIG shows that several other clinical conditions may benefit from IVIG [11,15,16]. To the best of our knowledge IVIG have so far been evaluated in eight patients with BD. Four patients had a severe and refractory ocular involvement and showed a remarkable clinical response for a period of at least 1 year after IVIG administration [7]. A further patient characterized by bilateral aphthosis, phlebitis and severe cutaneous involvement showed significant improvement soon after treatment with high dose IVIG and low dose aspirin [8]. Finally, a patient with BD-related colitis and a patient with both BD and common variable immunodeficiency also responded well to IVIG administration [9,10]. Nevertheless, IVIG proved to be ineffective in a BD patient with resistant bilateral panuveitis [17].

With regard to BD patients with neurological involvement, treatment with azathioprine, methotrexate and corticosteroids are recommended as first-line treatment [2,18] and intravenous cyclophosphamide and higher doses of corticosteroids are recommended for high risk patients [2]. Treatment with cyclophosphamide may have several side effects and may not be effective [5]. Recently, anti-TNFα agents, in particular the chimeric antibody infliximab, have been used with success in many resistant patients [5,6,17,19]. However, patients resistant to infliximab administration have also been described [20]. Regarding IL-1 inhibition, the receptor antagonist anakinra and the selective anti-IL-1β canakinumab have proven to be an additional BD treatment approach [3,4,21,22], but insufficient data preclude ascertaining the real function of IL-1 inhibition in neuro-BD. Consequently, therapeutic tools at our disposal are relatively limited for this life-threatening disease and expanding treatment opportunities for multi-resistant neuro-BD patients is essential.

Notably, in our case series patients 3 and 4 showed CNS findings and MRI results compatible also with MS, thus making the differential diagnosis challenging. However, patient 4 showed a good response to TNF inhibition, as we would expect from a patient with BD. Patient 3 was not given TNF inhibitors because of a doubtful diagnosis. In fact, anti-TNF agents are widely associated with demyelinating side effects [23]. Conversely, IVIG therapy seems to be neither effective nor dangerous in patients suffering from MS [24]. In this context, the marked response to IVIG treatment tipped the balance toward a BD diagnosis in this patient.

With regard to IVIG dosage and treatment duration in BD, no clear indication can be provided since limited published data are available to date. In our case series, we employed 0.4–0.5 g/kg/day for 5 consecutive days a month; however, further studies are necessary to establish their optimal dosage and the duration of treatment.

In conclusion, the present report describes four BD patients with protein organ involvement refractory to standard treatments and responsive to IVIG therapy. In all patients, the response to IVIG therapy was immediate and sustained over time without incurring side effects. We therefore propose IVIG as an additional effective and safe treatment option in patients with severe and refractory BD. Further studies on a larger number of patients are needed to identify the real therapeutic role of IVIGs, their mechanism of action in BD, their optimal dosage and treatment duration.

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**References**


**Capsule**

**Managing metastasis**

Because of the poor prognosis of metastatic cancer, it is critical to determine exactly how various factors contribute to cancer spread. Miecnik et al. examined the impact of tumor-intrinsic, microenvironmental, and immunological factors on tumor metastasis in colorectal cancer patients. A decrease in the presence of lymphatic vessels and a reduction in immune cytotoxicity was associated more strongly with the metastatic process than were tumor-intrinsic factors such as chromosomal instability or cancer-associated mutations. Testing this so-called immunoscore could be used as a biomarker to predict metastasis and guide therapy.

*Sci Transl Med* 2016; 8: 327ra26

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**Capsule**

**Visualizing the beginnings of melanoma**

The 'cancerized field' concept posits that cells in a given tissue sharing an oncogenic mutation are cancer-prone, yet only discreet clones within the field initiate tumors. Studying the process of cancer initiation has remained challenging because of (i) the rarity of these events, (ii) the difficulty of visualizing initiating clones in living organisms, and (iii) the transient nature of a newly transformed clone emerging before it expands to form an early tumor. A more complete understanding of the molecular processes that regulate cancer initiation could provide important prognostic information about which precancerous lesions are most prone to becoming cancer and also implicate druggable molecular pathways that, when inhibited, may prevent the cancer from ever starting. Kaufman and group developed a crestin:EGFP reporter that recapitulates the embryonic neural crest expression pattern of crestin and its expression in melanoma tumors. The authors show through live imaging of transgenic zebrafish crestin reporters that within a cancerized field (BRADF000E-mutant, p53-deficient), a single melanocyte reactivates the NCP state, establishing that a fate change occurs at melanoma initiation in this model. Early crestin+ patches of cells expand and are transplantable in a manner consistent with their possessing tumorigenic activity, and they exhibit a gene expression pattern consistent with the NCP identity readout by the crestin reporter. The crestin element is regulated by NCP transcription factors, including sox10. Forced sox10 overexpression in melanocytes accelerated melanoma formation, whereas CRISPR/Cas9 targeting of sox10 delayed melanoma onset. They also show activation of super-enhancers at NCP genes in both zebrafish and human melanomas, identifying an epigenetic mechanism for control of this NCP signature leading to melanoma.

*Science* 2016; 351: 10.1126/science.aad3867

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