

# Therapeutic alternatives in Behçet's syndrome

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## Summary

Behçet's Syndrome (BD) is a chronic, relapsing, recurrent systemic vasculitis with an unknown cause. The disease affects all organs of the body concurrently or consecutively. Its various clinical manifestations result from ubiquitous small-vessel vasculitis, which is the underlying pathology. An Italian study has reported an increased association of the extended haplotype B51-DR5-DQw3. Without a known etiology BD syndrome has no uniformly acceptable therapy.

Our study addresses therapeutic alternatives for the treatment of BD, with the systemic use of interferon  $\alpha$ -2a., which has antiviral, immunomodulatory, antiproliferative, and antitumoral properties. Ten patients diagnosed with BD were referred from September 2002 to September 2005 to the Department of Gynaecology, Obstetrics and Reproduction of the Second University of Naples. The International Study Group (ISG) Criteria for Behçet's Disease (27) was applied.

Patients were treated with oral prednisone; sulfasalazine; clobetasol; and interferon  $\alpha$ -2a. Every month all patients had a complete blood count, platelet count, and liver function test. Biopsies of genital ulcerations identified small vessel vasculitis with mononuclear cell and lymphocytic infiltrates. HLA-B27 and B5 were positive in three subjects.

The pathergy test was positive in all patients. Today the therapy is still ongoing, and none of the patients in therapy with our protocol present clinical symptoms of BD or intolerance. Laboratory findings are in a normal range and none have had neurological failure.

Our findings may be attributable to less severe disease in a patients, to our smaller number of patients, or to other unknown factors. Nonetheless, these findings remain to be confirmed in a larger number of patients.

**Key words:** Behçet's syndrome; Systemic vasculitis.

## Introduction

Behçet's Syndrome (BD) is a chronic, relapsing, recurrent systemic vasculitis with an unknown cause. The disease affects all organs of the body concurrently or consecutively. Its various clinical manifestations result from ubiquitous small-vessel vasculitis, which is the underlying pathology [1, 2].

The syndrome bears the name of the Turkish dermatologist Hülûsi Behçet, who between 1937 and 1940 described recurrent oral and genital ulcerations and iridocyclitis in two patients [3]. However the first description of the symptomatology of the typical clinical triad was observed by Hippocrates, 5th century BC, in his third book of epidemiology [4].

BD has a worldwide distribution, however, the prevalence, is higher in Japan and eastern Mediterranean countries than in Europe or North America [5]. The incidence of BD has increased over the last 40 years, mainly in Japan, where in some parts of the country 8.5 of 100,000 people are afflicted [5]. Its regional and familiar distribution, as well as its association with human leukocyte antigen human lymphocyte antigen (HLA) B51, especially observed in endemic areas and in patients with ocular disease, are evidence of a genetic predisposition [6-9].

In an Italian study an increased association of the extended haplotype B51-DR5-DQw3 was reported [10]. However, specific antigen presentation by B51 molecules is not always involved.

Recently, another theory has been postulated that the gene(s) responsible for BD susceptibility are in linkage disequilibrium with the HLA-B locus, which itself could not be

directly involved [11]. In fact in a collaborative study of the British and Turkish populations, Yazici and Chamberlain confirmed frequent HLA B5 presence among Turkish patients with a relative risk of 7.5, but not in British patients [12].

BD appears variable in terms of prevalence rate, clinical and laboratory features from Eastern to Western populations [13]. In fact in northern European and American patients the disease is more prevalent in women, the clinical course is less severe, a lower incidence and severity of uveitis has been observed and there is no HLA restriction, except ocular BD [13].

In a limited number of patients with herpes simplex virus 1 (HSV 1), DNA was found in peripheral blood lymphocytes by polymerase chain reaction (PCR), and HSV DNA was found in biopsy samples taken from genital ulcers applying PCR [14]. The protein 289 bp of HSV DNA was also detected in these lesions, but not in controls.

The immunologic aspects of BD have been described and the main microscopic finding at most sites of active BD is an immune-mediated occlusive vasculitis [15, 16].

In a recent study an increased number of circulating  $\gamma\delta$ T cells and natural killer cells was reported [17, 18]. Their role in the pathogenesis of the disease remains to be elucidated.

Without any known etiology BD syndrome has no uniformly acceptable therapy. There have been many and varied approaches including topical and systemic corticosteroids, immunosuppressive agents, like cyclosporin A (CsA), azathioprine (AZA), cyclophosphamide (CTX), chlorambucil (CHL) and methotrexate (MTX) [19-26].

Our study addresses the a therapeutic alternatives for the treatment of BD with the systemic use of interferon  $\alpha$ -2a. which has antiviral immunomodulatory, antiproliferative, and antitumoral properties.

## Material and Methods

Ten patients from September 2002 to September 2005 were referred to the Department of Gynaecology, Obstetrics and Reproduction of the Second University of Naples. The age ranged from 22 to 31 years (mean,  $25.5 \pm 1.7$ ) and weight between 60 and 83 kg (mean,  $71.5 \pm 0.8$ ). Each subject presented genital ulceration localized in the vulva and vagina which was diffused, painful with shallow craters and a white or yellow purulent base, and erythematous rims. In all cases these ulcers were related chronologically to the menstrual cycle. The past histories revealed diagnoses of recurrent oral lesions and vaginal ulcerations, but the latter less frequently than the oral lesions (three times more in a 12-month period). At the time of the examination the patients presented oval mucous lesions 3–10 mm in diameter with a red rim and without scars. All subjects presented aspecific arthralgia, mono- or non-symmetrical oligoarthritis affecting the large joints in short attacks lasting from several days to two weeks.

Laboratory studies performed in all patients included a complete blood count, erythrocyte sedimentation rate (ESR), liver and kidney function tests, calcium, phosphorus, uric acid and urine analyses, complement C3/C4, rheumatoid factors (RF), antinuclear antibodies (ANA), chest, lumbar and pelvic radiograms and HLA-typing which was studied only in patients with joint manifestations, or when there was a positive history for familial incidence. The subjects with several rheumatological manifestations were examined by a specialist in oral medicine and by a rheumatologist.

All patients were submitted to a pathergy test. All tests were read at 48 hours and the results were considered positive if a sterile erythematous papule of more than 2 mm formed.

Suspected genital lesions were examined by biopsy. Positive clinical and laboratory data suggested patients were suffering from Behçet's Disease. The International Study Group (ISG) Criteria for Behçet's Disease [27] was applied. Patients diagnosed with BD were treated with:

- 20 mg oral prednisone bid for three weeks;
- 2 mg sulfasalazine os bid per three weeks
- clobetasol four topical applications for one week
- interferon  $\alpha$ -2a, 3 MU subcutaneously three times per week for one month, 9 MU three times per week for three months, and 3 MU one time per week for 6-month periods for two years.

Every month all patients performed a complete blood count, platelet count, and liver function test.

## Results

Biopsy of genital ulcerations identified small vessel vasculitis with mononuclear cell and lymphocytic infiltrates. Laboratory abnormalities included increased ESR of 76 mm (in the first hour), C-reactive protein, and total white cell count, as well as anemia.

In one patient the examination was performed during an acute episode of arthritis. The patients were afebrile and had a swollen, warm, tender, red joint. A family history dertermined joint disease was present in four of the ten patients. RF and ANA were present in five patients; two had normal results and three had low titer ANA but no anti-DNA antibodies. Lumbar and pelvic radiograms were within normal limits in all patients.

HLA-B27 and B5 were positive in three subjects while the pathergy test was positive in all patients.

All symptoms disappeared within the first three weeks of therapy. The patients' general condition improved, all

subjects responded well to treatment, there was no active inflammation in any mucocutaneous or genital area and the ulcerations had completely resolved. No new lesions appeared thereafter.

One month after therapy the patients presented no joint manifestations or recurrent symptoms related to BD disease.

Three months and one year later the clinical situation was the same.

Today the therapy is still ongoing and none of the patients in therapy with our protocol have presented any clinical symptoms of BD or intolerance. Laboratory findings are in a normal range and none have had neurological failure.

All patients have normal urine sediment and no indication of nephropathy or glomerulonephritis syndrome.

## Discussion and Conclusion

BD may present in many forms, and the original triple symptom complex is usually included. Variable multisystem involvement and absence of disease-specific laboratory markers require a diagnosis of BD to be entirely based on identification of a clinical pattern and fulfillment of definite criteria. In 1990, the International Study Group (ISG) for BD developed new, international diagnostic criteria.

The diagnosis of BD disease is not easy because the cause remains to be elucidated; immune mechanisms, genetic factors, and infectious agents are involved in the etiopathogenesis. The main histological finding in BD is widespread vasculitis which is responsible for organ failure. Although there are disagreements as to whether BD should be classified as an autoimmune disease, a proposed model explaining the etiopathogenesis of BD [28] is that an exogenous factor (virus or bacteria) is involved by macrophages and is recognized by CD4<sup>+</sup> T cells in the context of class II MHC antigens; activated Th 1 T cells produce cytokines IL-2, INF- $\gamma$ , TNF- $\beta$ ) and induce B cell proliferation; INF- $\gamma$  activates macrophages to release TNF- $\alpha$ , IL-1, and IL-8 which then induce the expression of adhesion molecules on endothelial cells. IL-8 also induces chemotaxis and activates neutrophils, both events being responsible for the passage of polymorphonuclear neutrophils and activated T lymphocytes through the endothelium to inflammatory areas. Genetic factors also contribute to the expression and perpetuation of the disease [29].

Autoantibodies are elusive in BD; a HLA association remains controversial. Pathergy testing also can be positive in other autoimmune arthropathy diseases such as spondylarthropathy or myelogenous leukemia treated with INF- $\alpha$ , and therefore is not specific [30, 31] although it is included as one of international criteria of ISG.

Taking the above evidence into consideration it is difficult to propose an international protocol for therapy. Treatment of this syndrome is symptomatic, must be established in accordance with the location of disease and modulated in relation to its clinical severity. In 1960 immunosuppressive agents were applied in BD therapy, and were moderately effective in inducing and maintaining remissions [5].

Immunosuppressive agents include alkylating compounds, purine antagonists, and agents inhibiting IL-2 production. Many complications have been reported during the therapy with these cytotoxic drugs [20].

Cholinesterase inhibitor (CHL) therapy can be complicated by precipitous and persistent pancytopenia, amenorrhea and sterility after 12 months of therapy; infection, elevated risk of leukemia, a secondary malignancy in patients that receive greater than 1300 mg in total. Long-term results with CHL have not been particularly encouraging [19, 32].

Cyclophosphamide (CTX) has been used in cases refractory to CHL toxicities and side-effects include hemorrhagic cystitis, mucosal ulcerations, sterility, kidney toxicity and carcinogenesis [13, 33, 35].

AZA toxic side-effects include bone marrow suppression and hepatotoxicity; AZA is usually used if required by clinical severity [20].

Cyclosporin A (CsA) is non cytotoxic and therefore presumably cannot induce clonal deletion of autoaggressive cells. It inhibits the function of CD4<sup>+</sup> and has a rapid and effective action in severe ocular lesions, however, long-term follow-up has not shown any significant difference from CTX [21, 36]. CsA therapy is generally limited to treatment of acute ocular attacks of BD, but the dose employed has been associated with significant renal toxicity and may lead to rebound phenomena [21, 36].

In our study none of the patients in therapy presented clinical symptoms of BD or intolerance to treatment and none have reported severe side-effects.

Our findings may be attributable to the less severe disease in our patients, to the smaller number of patients, or to other unknown factors. However these findings remain to be confirmed in a larger series of patients.

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