The Effects of Adalimumab in Behçet’s Disease Patients on Clinical Manifestations and on Pro-Inflammatory Cytokines Milieu: Long-Term Follow-Up

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**ABSTRACT**

**Background:** Behçet’s disease is a multi-systemic chronic relapsing inflammatory disease, classified among the vasculitides. The heterogeneity of clinical manifestations challenges the disease management.

**Objectives:** To assess efficacy and safety of adalimumab in patients with active persistent Behçet’s arthritis who did not respond to disease-modifying anti-rheumatic drugs and to assess the impact of treatment on the cytokine milieu.

**Methods:** Our cohort comprised 10 patients with active arthritis who received adalimumab in a 24-week investigator-initiated prospective open-label study. Patients who relapsed within 12 weeks following adalimumab discontinuation could enter a 3-year extension study. The patients underwent a comprehensive assessment including questionnaires and measurement of inflammatory cytokines, adalimumab serum levels, and anti-drug antibodies.

**Results:** A significant improvement was observed in arthritis, disease activity visual analogue scales, Behçet’s disease current activity form, and interleukin-6 (IL-6) levels, but not in health assessment questionnaire and functional assessment of chronic illness therapy fatigue scale questionnaire. Resolution of oral and urogenital ulcers was achieved in all patients. Significant reduction of pain was reported by 40% of patients. The disease relapsed in 9 of 10 patients, within 2–6 weeks following adalimumab discontinuation. Of the 7 patients who continued the study, arthritis was resolved in 5. Two patients with high neutralizing anti-drug antibodies titer relapsed.

**Conclusions:** Adalimumab treatment achieved a significant improvement in arthritis, mucocutaneous manifestations, and IL-6 levels in all study patients but only 40% reported significant pain reduction. The arthritis relapsed in 90% of patients following adalimumab discontinuation and long-term treatment was required.

**KEY WORDS:** adalimumab, Behçet’s arthritis, inflammatory cytokines, interleukin-6 (IL-6)

**BACKGROUND:** Behçet’s disease (more accurately named Behçet’s syndrome) is a multi-systemic chronic relapsing inflammatory disease classified among the vasculitides. The pathogenesis of the disease is unknown. A possible mechanism is that infectious or environmental triggers in an appropriate genetically susceptible individual may eventually lead to an immune mediated process and eventually to a chronic inflammatory multisystem disease [1,2]. The immune process is characterized by a T cell homeostasis perturbation. An expansion of Th1 and Th17 and secretion of pro-inflammatory cytokines with decreased regulation of the immune system is secondary to low levels of T regulatory cells [2,3]. Recently, McGonagle et al. [4] proposed a shared immunopathogenetic basis for Behçet’s syndrome and several spondyloarthropathies, all associated with MHC class I. They coined a unified concept for these diseases: MHC-I-opathy. The disease tissue-localized manifestations are determined by different HLA allele associations, and aberrant innate immune reactions at sites of mechanical stress, which trigger adaptive immunity. Behçet’s syndrome is a heterogeneous disease. The most common manifestations are recurrent oral aphotic ulcers, genital ulcers, and uveitis, but many other systems may be involved (ocular, neurological, vascular, or gastrointestinal) [5]. Arteries and veins of all sizes are involved.

Joint involvement may occur in 40–70% of patients [6-8]. The articular involvement in Behçet’s syndrome is clinically characterized by asymmetric, non-deforming and non-erosive arthritis, usually of medium-large joints, and histological evidence of inflammation in synovial biopsies [9]. Sacroiliitis and spondylitis were described in 7–10% of patients. Although the arthritis usually resolves in several days to weeks, it causes pain and functional limitation similar to those that affect rheumatoid arthritis patients [10]. A minority of patients (2%) will experience chronic erosive arthritis [11]. Those patients will require long-term immunomodulatory treatment. Synthetic disease-modifying anti-inflammatory drugs (DMARDs) are efficient in some of the patients. Several retrospective studies and case series emphasized the role of anti-tumor necrosis factor (TNF)-α therapy mainly for ocular manifestations, but benefits were reported also for extra-ocular manifestations,
including refractory arthritis [12-18].

Our primary endpoint was to assess efficacy and safety of adalimumab in a prospective study, in patients with active chronic Behçet’s arthritis not responding to one or more DMARDS. The secondary endpoint was to assess the impact of treatment on other disease manifestations.

PATIENTS AND METHODS

We conducted a single-center, investigator-initiated, prospective, open label study. Adult patients diagnosed with Behçet’s disease as defined by the International Study Group Criteria [19], with active arthritis at screening and inadequate response to treatment with one or more DMARDs were eligible for participation. Active arthritis was defined as tenderness and swelling of at least one medium or large joint or active spondylitis. DMARDs and/or corticosteroids (≤ 10 mg/day prednisone) were permitted if stable for at least 4 weeks prior to screening. The exclusion criteria included other rheumatic autoimmune diseases, significant uncontrolled concomitant diseases, known active infection, history of malignancy in the past 5 years, pregnancy or breastfeeding, history of demyelinating disease, or active tuberculosis.

STUDY DESIGN

• Phase 1: Eligible patients with active arthritis were enrolled in a 24-week open-label, investigator-initiated study with adalimumab treatment that was discontinued at the end of phase 1

• Phase 2: Patients who relapsed within 12 weeks following adalimumab discontinuation could enter a 3-year open-label extension study

The study was approved by the ethics committee of Rambam Health Care Campus (0488-09RMB, ClinicalTrials.gov Identifier: NCT01497717) and all subjects provided informed consent according to the declaration of Helsinki.

TREATMENT

All patients were treated with 40 mg subcutaneous adalimumab every 2 weeks. In patients with inadequate disease control after 12 weeks of treatment, adalimumab was administered weekly. Concomitant DMARDs or corticosteroids, at a stable dose, could be continued throughout the study.

ASSESSMENT

Study visits were at screening, baseline, month 1, and then every 2 months up to 6 months. Patients entering the phase 2 extension study were assessed every 3 months. Questionnaires, disease assessment indices, and safety evaluation were performed. Physical examination included 68 tender and 66 swollen joint counts. Questionnaires included Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), patient Visual Analogue Scale (VAS) for pain, physician overall disease activity VAS, health assessment questionnaire (HAQ), and Behçet’s Disease Current Activity Form (BDCAF) [20]. Functional Assessment of Chronic Illness Therapy (FACIT) fatigue scale questionnaire and SF-36 questionnaire were completed at baseline, weeks 24, week 48, and at the final visit of the patient.

The following laboratory tests were performed at each visit: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), complete blood count, and kidney and liver function tests. Adalimumab trough levels and antibodies were examined at week 12, week 24 and then every 3 months. Adalimumab levels were measured by using the ELISA assay [21] (Abbot Laboratories, Princeton, NJ, USA). Measurement of adalimumab antibodies was done by adaptation of previously developed anti-human λ-chain detection ELISA assay as described [21], comprising of pre-plating the TNF-coated wells with 0.05 mg/ml of adalimumab.

The levels of TNF-α, interleukin (IL)-1β, IL-6, IL-10, IL-17a, and interferon (IFN)-γ were assessed at baseline, at week 24, and at week 48. The cytokines levels were measured in plasma using the ProcartaPlex™ Human High Sensitivity Immunoassay kit (eBioscience Vienna, Austria) and MAGPIX® (Luminex, USA) according to the manufacturer instructions. The immunoassay kit performs quantitative, multiplexed protein measurements from serum, plasma samples using magnetic beads technology from Luminex.

The primary endpoints of this study were to determine the effect of the TNF-α blocker adalimumab, on the articular manifestations of patients with Behçet’s disease who failed one or more DMARDs. Complete response was defined as being free of arthritis; partial response was defined as 50% improvement in swollen joint counts and/or dose reduction/discontinuation of corticosteroids. The secondary endpoint was to determine additional effects of the therapy with adalimumab on the mucocutaneous and other extra-articular manifestations of patients with Behçet’s disease. Complete response was considered the resolution of extra-articular disease manifestations, present at the screening or baseline visits and partial response was defined as 50% improvement of extra-articular manifestations.

STATISTICAL ANALYSIS

Summary statistics were calculated including mean and median values, ranges, standard deviations, and frequency distributions. Comparisons between pre- and post-adalimumab covariates were analyzed using the Mann-Whitney U test for quantitative variables and Fisher’s exact test for categorical data. Statistical significance was defined as P < 0.05. Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software, version 25 (SPSS, IBM Corp, Armonk, NY, USA).
RESULTS

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS

Ten of 16 patients who were screened entered the trial. Of these, six were women. Two patients failed to fulfill the screening criteria and four others withdrew their agreement to participate. Most of the patients had a long-lasting disease (median disease duration 7.5 years, range 2–26 years). All had chronic peripheral arthritis refractory to at least two DMARDs, one patient also had sacroiliitis. The most frequent joints involved were wrists, knees, ankles, and shoulders. The main extra-articular manifestations included: recurrent oral and genital aphthosis (all patients), cutaneous lesions (erythema nodosum in 3 patients, psuedofolliculitis in 2), past history of uveitis (1 patient), and deep venous thrombosis (1 patient).

Previous immunomodulatory treatments included colchicine (all patients), methotrexate (7 patients), azathioprine (4 patients), salazopyrine (6 patients), leflunomide (2 patients), hydroxychloroquine (3 patients), minocycline (1 patient), and prednisone (7 patients). Concomitant DMARDs were continued by 5 patients throughout the trial (methotrexate 1 patient, azathioprine 1 patient, methotrexate and azathioprine 2 patients, methotrexate and leflunomide 1 patient). Prednisone (10 mg) was prescribed for 1 patient and 4 patients received colchicine. The demographics and clinical characteristics are described in Table 1.

The first phase response to the treatment is described in Table 2. Resolution of arthritis occurred within 4–8 weeks from adalimumab initiation in 9 of 10 patients. The remaining patient with active synovitis had low adalimumab trough levels without neutralizing antibodies. A substantial amelioration was achieved in his condition, following weekly administration of adalimumab.

Table 1. The demographics and clinical characteristics of the study patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>10</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>6</td>
</tr>
<tr>
<td>Age in years, mean ± SD</td>
<td>43 ± 8.4</td>
</tr>
<tr>
<td>Disease duration in years, mean ± SD</td>
<td>11.6 ± 10</td>
</tr>
<tr>
<td>Oral and genital ulcers, patients number</td>
<td>10</td>
</tr>
<tr>
<td>Erythema nodosum, patients number</td>
<td>3</td>
</tr>
<tr>
<td>Other skin manifestations, patients number</td>
<td>3</td>
</tr>
<tr>
<td>Uveitis, patients number</td>
<td>1</td>
</tr>
<tr>
<td>Thrombotic complications, patients number</td>
<td>1</td>
</tr>
<tr>
<td>Tender joint count, mean ± SD</td>
<td>19 ± 13</td>
</tr>
<tr>
<td>Swollen joint count, mean ± SD</td>
<td>4.6 ± 4.2</td>
</tr>
<tr>
<td>CRP mg/dl, mean ± SD</td>
<td>1.9 ± 3.3</td>
</tr>
<tr>
<td>ESR mm/h, mean ± SD</td>
<td>21.6 ± 16.5</td>
</tr>
</tbody>
</table>

CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, SD = standard deviation

The physician global disease activity index was significantly improved (from 51.5 vs. 24.5, *P* = 0.002), and so was the BDCAF (5.4 vs. 2.1, *P* = 0.001).

Although 40% of patients reported a significant decrease in pain, the change in the patient’s VAS pain did not reach statistical significance at week 24. Complete resolution of oral and genital aphthae was achieved in all patients. No cases of uveitis, cutaneous manifestations, or vascular complications occurred during the study period (including the extension study). There was no change in HAQ assessment and FACIT after 24 weeks. The health change domain in SF36 survey was the only one that improved significantly [Table 2].

A significant reduction of IL-6 serum levels was observed in 8 of 10 patients at week 24 [Figure 1]. The delta change in IL-6 levels at week 24 correlated well with clinical improvement, physician global disease activity index, BDCAF, and reduction of ESR. One of the patients with no reduction of IL-6 at week 24 had active synovitis and low adalimumab trough levels. The second one was on clinical remission.

Table 2. Response to treatment

<table>
<thead>
<tr>
<th>Patients</th>
<th>Baseline</th>
<th>24 weeks</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tender joint count mean ± SD</td>
<td>19 ± 13</td>
<td>10.9 ± 12.6</td>
<td>0.14</td>
</tr>
<tr>
<td>Swollen joint count mean ± SD</td>
<td>4.6 ± 4.2</td>
<td>0.6 ± 0.6</td>
<td>0.006</td>
</tr>
<tr>
<td>Physician global disease activity index-mean ± SD</td>
<td>51.5 ± 18.5</td>
<td>24.5 ± 16</td>
<td>0.002</td>
</tr>
<tr>
<td>VAS-pain mean ± SD</td>
<td>72 ± 19.8</td>
<td>56 ± 33</td>
<td>0.18</td>
</tr>
<tr>
<td>BDCAF mean ± SD</td>
<td>5.4 ± 1.6</td>
<td>2.1 ± 1.4</td>
<td>0.001</td>
</tr>
<tr>
<td>HAQ mean ± SD</td>
<td>1.76 ± 0.8</td>
<td>1.59 ± 0.9</td>
<td>0.12</td>
</tr>
<tr>
<td>FACIT Mean ± SD</td>
<td>18 ± 13</td>
<td>21.8 ± 13.2</td>
<td>0.24</td>
</tr>
<tr>
<td>SF36</td>
<td>15.5 ± 21.4</td>
<td>50 ± 31.2</td>
<td>0.009</td>
</tr>
<tr>
<td>IL-6 levels mean ± SD</td>
<td>10.2 ± 13</td>
<td>2 ± 0.8</td>
<td>0.046</td>
</tr>
</tbody>
</table>

BDCAF = Behçet's disease current activity form, FACIT = functional assessment of chronic illness therapy, HAQ = health assessment questionnaire, VAS = visual analogue scales

Figure 1. Delta change in IL-6 levels between baseline and week 24
An increase in serum IL-10 levels was observed in only two patients. There was no correlation between delta change in serum IL-10 levels and VAS pain.

**PHASE 2 EXTENSION STUDY**

Adalimumab was discontinued in all patients after 24 weeks, according to the study protocol. The disease relapsed in 9/10 patients, within 2–6 weeks (median 4). Seven patients agreed to enroll into the extension study. The other two withdrew their consent because of concern from treatment related possible side effects. After 1 year of treatment, the arthritis relapsed in two patients. Both of them developed high antidrug antibodies titer and an adalimumab serum trough level of 0 with a concomitant raise in VAS pain and IL-6 level. Adalimumab was discontinued. Another patient with low adalimumab trough levels and no antibodies improved after providing adalimumab weekly. Five patients completed 2 years of treatment and four completed 3.5 years (24 weeks of study period and 3 years of extension study). Complete remission was achieved in three patients, the other two continued to complain of musculoskeletal pain, but without signs of arthritis or elevation of CRP and ESR. The study drug was discontinued in one of the patients after 2.5 years of treatment because of persistent diffuse musculoskeletal pain without evidence of active arthritis. Resolution of oral and genital ulcers was achieved in all patients during the study. No relapse of other extra-articular disease manifestations occurred. A statistically significant improvement in HAQ (1.76 vs. 0.49, \( P = 0.01 \)) and the health change domain in SF36 survey was observed in those patients who completed the 3 years extension study. No improvement was noticed in FACIT or in the other domains of SF36 results.

Adverse events included upper respiratory tract infections in two patients and pneumococcal pneumonia in one patient. None of the events required treatment discontinuation.

**DISCUSSION**

Our study is an investigator-initiated prospective open label study aimed to assess efficacy and safety of adalimumab in Behçet’s disease patients with active non-remitting arthritis and to assess the impact of treatment on the cytokine milieu. Only patients who failed at least one DMARD for arthritis and in whom the articular involvement was the most dominant clinical manifestation were included in the study. Adalimumab treatment was well tolerated and achieved a significant improvement in arthritis in all of our study patients. Discontinuation of the drug led to arthritis relapse in most of the patients within a short time. Renewal of treatment with adalimumab generated prompt improvement in all seven patients enrolled in the phase 2 extension study and a long-term remission in four. Secondary treatment failure occurred in two patients who developed neutralizing antibodies to adalimumab after 1 year of treatment. Resolution of oral and genital ulcers was achieved in all patients during adalimumab treatment. No other disease manifestations occurred during the study.

Other studies found elevated serum levels of innate-system related cytokines, including IL-1, IL-6, IL-15, TNF-α and also TH17 and TH1 related cytokines including IL-2, IFNγ, IL-17, and IL-23 in Behçet’s disease patients compared to controls, suggesting their role in the pathogenesis of Behçet’s syndrome [4,22]. In all of our patients, the levels of INF-γ, IL-17A, and TNF-α were undetectable, while IL-1β was elevated only in one patient. All study patients reacted well to anti-TNF-α treatment despite the undetectable TNF-α levels in blood serum. The change in IL-6 levels at week 24 correlated well with clinical improvement, physician global disease activity index, BDCAF, and reduction of ESR. We presume that reduction in serum IL-6 levels (a pro-inflammatory cytokine involved in innate immunity) is a more sensitive and reliable marker of arthritis activity than TNF-α serum levels. The correlation between IL-6 levels and disease activity score and clinical activity index in Behçet’s disease patients was described also by Gholijani et al. [22].

There was no correlation between delta change in serum IL-10 levels and VAS pain.

The physician global disease activity index was significantly improved and so was the BDCAF. In spite of the remarkable improvement of arthritis and mucocutaneous involvement and the reduction in IL-6 levels, only 40% of the patients report a considerable amelioration of pain. No significant reduction was achieved in tender joint count. This finding may raise the question about fibromyalgia prevalence among Behçet’s disease patients. Several studies found a relatively high prevalence of fibromyalgia among Behçet’s disease patients [23,24]. A statistically significant improvement in HAQ assessment was observed only in those patients who completed the extension study, but not by week 24. Adalimumab treatment did not ameliorate fatigue in our patients. No improvement was noticed in FACIT or in the other domains of SF36 results, except of the health change domain throughout the entire study period. Fatigue is an important factor with negative impact on quality of life in Behçet’s disease patients [10,25].

**LIMITATIONS**

Our study is a prospective study which assessed the influence of adalimumab treatment on chronic Behçet’s disease arthritis through a comprehensive evaluation of clinical aspects, functional questionnaires, cytokines levels and immunogenicity of drug study and a long-term follow-up. The main limitation of our study is the relatively small number of patients.

**CONCLUSIONS**

Our study demonstrates a substantial improvement in arthritis and mucocutaneous manifestations in all patients, accompanied by a
significant decrease in IL-6 levels. Adalimumab treatment was well tolerated. Only 40% reported significant pain reduction and no improvement of fatigue was observed. We assume that a subset of patients with insufficient improvement in joint tenderness and generalized pain, probably secondary to fibromyalgia, may require comprehensive pain management, besides anti-inflammatory therapy.

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**Capsule**

**Targeting a key enzyme in SARS-CoV-2**

Scientists across the world are working to understand severe acute respiratory syndrome: coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19). Zhang et al. determined the X-ray crystal structure of a key protein in the virus life cycle: the main protease. This enzyme cuts the polyproteins translated from viral RNA to yield functional viral proteins. The authors also developed a lead compound into a potent inhibitor and obtained a structure with the inhibitor bound, work that may provide a basis for development of antiviral drugs.