Adalimumab provides long-lasting clinical improvement in refractory mucocutaneous Behçet's disease without formation of antidrug antibodies

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ABSTRACT

Objective. *TNF-blocker adalimumab can be effective in Behçet's disease (BD), a multisystem auto-inflammatory disorder. Unfortunately, the therapeutic efficacy of TNF-blockers can be hampered by the formation of anti-drug antibodies. We present an observational study of adalimumab in refractory BD with measurement of anti-drug antibodies.*

Methods. *The effect of fortnightly 40mg adalimumab in nine patients with therapy refractory mucocutaneous, non-ocular or organ threatening BD was studied up to 60 months. Primary endpoint was a decrease in disease activity, measured by the BD Current Activity Form (BDCAF) within 6 months. Secondary endpoints included serum cytokines and the long-term formation of anti-adalimumab antibodies.*

Results. *BDCAF improved significantly in all nine patients from 5.4 (SD=1.4) to 2.4 (SD=1.4) (p=0.007) within one month up to 6 months and after a prolonged follow-up of 5 years. All patients could either taper or stop concomitant therapy. Symptoms of mucocutaneous lesions, erythema nodosum and joint involvement decreased or disappeared. Serum TNF-alpha levels were elevated in five patients and decreased upon treatment (p=0.017). Adalimumab was safe and none of the patients experienced therapy failure or antibodies against adalimumab.*

Conclusion. *We present an observational study on patients with BD treated with adalimumab and provide a basis for long-term use in refractory mucocutaneous BD. These findings show that adalimumab can safely be administered yielding sustainable clinical effects in refractory BD patients with mucocutaneous disease without formation of anti-adalimumab antibodies, even after a long follow-up.*

Introduction

Behçet's disease (BD) is an auto-inflammatory vasculitis with aphthous lesions, mucocutaneous, joint, neurological or ocular complaints. Symptoms interfere with quality of life and require systemic treatment depending on localisation and severity. An aberrant immunological reaction to an unknown trigger activates T-helper-1 lymphocytes to produce pro-inflammatory cytokines of which tumour necrosis factor alpha (TNF-α) appears most important in BD. Therefore, TNF-inhibition may interrupt the inflammatory process (1, 2). Despite various alternative therapies, TNF-blockers are still key biologics in the treatment of BD (3). EULAR treatment recommendations support a step-up and tailored treatment and a prominent place for TNF-blockers in severe refractory BD (4-6). However, the efficacy can be hampered by formation of anti-drug antibodies (ADA) and subsequent reduced drug levels (7). There are no data on ADA formation in BD so far. The aim of this study is to study the efficacy and long-term use of adalimumab in BD and formation of ADA. Furthermore we linked various cytokines with therapeutic efficacy.

Methods

Inclusion of patients

In this case series 9 treatment refractory BD patients were included based on fulfilling the BD criteria of the International Study Group of Behçet's Disease (8) (Table I and Supplementary Table S1) and treatment with adalimumab because of mucocutaneous disease. Refractory disease was defined as persistent elevated disease activity monitored by BD Current Activity Form (BDCAF) after ≥2 different immunosuppressive agents (9). Immunosuppressive intensification was indicated,

Table I. Effect of adalimumab on BDCAF score and symptoms in Behçet's disease.

		BT[§]	Day 0	After 1 month	After 3 months	After 6 months	Long term follow-up[¥]
Disease activity (range 0-12)	Mean ± SD	4.2 ± 1.4	5.4 ± 1.4	2.4 ± 1.4	2.6 ± 1.6	2.4 ± 1.5	2.4 ± 1.6
	Range	3-7	3-8	0-4	1-6	0-5	0-4
	P*	0.014	-	0.007	0.007	0.007	0.039
Oral ulcers	Patients (n/total n)	8/8[#]	7/9	2/9	3/9	5/9	
	Mean ± SD	3.4 ± 0.8	3.1 ± 1.8	0.6 ± 1.3	0.7 ± 1.1	1.0 ± 1.3	
	P	0.713	-	0.020	0.016	0.026	
Genital ulcers	Patients	2/8	6/9	0/9	2/9	1/9	
	Mean ± SD	0.5 ± 1.1	1.7 ± 1.6	0 ± 0	0.6 ± 1.1	0.3 ± 1.0	
	P	0.167	-	0.026	0.023	0.026	
Headache	Patients	7/8	7/9	7/9	6/9	6/9	
	Mean ± SD	2.8 ± 1.4	2.6 ± 1.6	1.7 ± 1.4	1.0 ± 1.2	2.0 ± 1.7	
	P	0.731	-	0.066	0.034	0.416	
Erythema Nodosum	Patients	2/8	4/9	0/9	3/9	0/9	
	Mean ± SD	0.8 ± 1.5	1.6 ± 1.9	0 ± 0	0.7 ± 1.1	0 ± 0	
	P	0.102	-	0.059	0.176	0.059	
Pustules	Patients	5/8	7/9	6/9	3/9	4/9	
	Mean ± SD	2 ± 1.9	2.9 ± 1.8	1.0 ± 0.9	0.8 ± 1.4	1.3 ± 1.7	
	P	0.083	-	0.017	0.025	0.068	
Arthralgia	Patients	5/8	8/9	5/9	5/9	4/9	
	Mean ± SD	1.5 ± 1.5	2.7 ± 1.5	1.3 ± 1.7	1.1 ± 1.4	1.4 ± 1.9	
	P	0.066	-	0.496	0.023	0.041	
Arthritis	Patients	0/8	3/9	0/9	0/9	0/9	
	Mean ± SD	0 ± 0	0.9 ± 1.5	0 ± 0	0 ± 0	0 ± 0	
	P	0.102	-	0.102	0.102	0.102	
Abdominal Symptoms	Patients	4/8	4/9	2/9	2/9	1/9	
	Mean ± SD	1.1± 1.4	0.8 ± 1.0	0.6 ± 1.3	0.3 ± 0.7	0.1 ± 0.3	
	P	0.581	-	0.496	0.334	0.098	
Diarrhea	Patients	1/8	3/9	0/9	0/9	1/9	
	Mean ± SD	0.1 ± 0.3	0.8 ± 1.4	0 ± 0	0 ± 0	0.1 ± 0.3	
	P	0.102	-	0.317	0.317	1.00	
Self-rating	Mean ± SD	3.1 ± 1.4	4.1 ± 1.1	2.7 ± 0.5	2.7 ± 0.7	2.4 ± 1.4	
	P	0.066	-	0.010	0.006	0.031	

Clinical manifestations of patients with Behçet's disease treated with adalimumab at various time points before and after initiation. Clinical symptoms are scored (range 0-4) according the BDCAF (9). $p<0.05$ is considered significant.
BT: before treatment (> 21 days; mean of 2.8 months; range 0.7-5.6 months); BDCAF: Behçet's disease current activity form; SD: standard deviation.
*All *p*-values are based on the mean score at the different time points compared to day 0.
[§]*p*-values compared to BT were p=0.016, 0.006 and 0.028 for month 1, 3 and 6, respectively.
[¥]Long term follow-up (n=8, mean 5.0 years, range 3.6-6.1).
[#]One patient was left out of the analysis of clinical symptoms because these were registered longer than 8 months BT, BDCAF, however, could be determined closer to BT.

however, TNF-blockers were not registered for BD in the study period. Therefore, adalimumab was kindly provided by Abbot BV, the Netherlands.
The study protocol was approved by the Medical Ethical Committee of Erasmus MC.

Administration of adalimumab (Humira®)
The patients were instructed to inject adalimumab themselves. Adalimumab was given fortnightly subcutaneously 40 mg/0.8 mL. When symptoms recurred within those two weeks, dose frequency could be increased up to weekly 40 mg. All patients were treated according to running treatment protocols (including hepatitis (serology) and (latent) tuberculosis (chest x-ray, and an IGRA-test (Quantiferon GOLD)) from July 2010 until December 2011. Adalimumab was started on patient care basis and patients were followed for six months. Hereafter, therapy could be continued and was monitored every 3–4 months.

Clinical effect
The primary endpoint of the study was a >30% decrease in BDCAF. Time points included last clinical visit before inclusion (hereafter named "before treatment", BT; >21 days; mean of 2.8 months; range 0.7–5.6 months), day 0, month 1, 3 and 6. Given the normal BD activity fluctuations, time point BT was included to reduce bias. The same clinician filled out the form during follow-up. Liver enzymes, double stranded DNA antibodies and non-specific inflam-

matory parameters (C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)) were monitored. Adverse effects were registered, therapy was adjusted if applicable. Concomitant (immunosuppressive) therapy was tapered according to opinion of the treating physician.

Serum cytokine measurement

Cytokine levels of GM-CSF, IFN-γ, IL-10, IL-12(p70), IL-13, IL-1β, IL-2, IL-4, L-5, IL-6, IL-7, IL-8 and TNF-α were measured using a standard 13-plex Luminex kit (Millipore, Beetford, MA, USA) directly before initiation of treatment (day 0) as well as 30 and 180 days thereafter. Levels above 10 pg/ml were considered elevated. Additionally, expression of 11 IFN type I genes were measured using real time quantitative PCR in monocytes from peripheral blood, as described previously (10).

Adalimumab and anti-adalimumab antibody measurement

Adalimumab serum levels and ADA were measured using the Lisa Tracker test (Theradiag, Marne La Vallee, France) according to manufacturer's instructions. The control samples provided by manufacturer (kit controls) were included in the test and were handled in identical manner as patient samples. All measurements were performed in duplicate.

Statistics

Statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Normally distributed data were analysed with Friedman test and not normally distributed data were analysed with the Wilcoxon signed-rank sum test. A p-value of <0.05 was considered significant. Both time point BT and day 0 were compared to the those after 1, 3 and 6 months, respectively.

Results

Patient characteristics

All 9 patients were intensively pretreated and had high activity scores (Suppl. Table S1). Six patients were from Turkish descent. There was no ocular, major vessels or nervous system involvement within 3 months before inclusion. In one patient dose frequency of adalimumab injections was increased to once per ten days after four months because of persistent erythema nodosum. All patients could taper or stop concomitant medication (Suppl. Table S2).

Clinical effects

All patients showed significantly decreased BDCAF scores after starting adalimumab, both as compared to BT (p=0.016) as to day 0 (p=0.007) (Table I, Fig. 1C). Within one month all patients reached the primary endpoint that lasted up to six months in eight of nine patients (80%, p=0.007). Presence of mucocutaneous lesions and arthralgia decreased significantly in duration. If present, erythema nodosum and arthritis disappeared completely within six months. Additionally, seven patients rated their own condition as improved ($p<0.05$). No worsening of clinical symptoms occurred (Table I). One patient was lost to follow-up because of migration. After the first six months of treatment, adalimumab was continued in the remaining eight (mean 5.0 years, range 3.6–6.1) and remained therapeutically effective (Table I, p=0.039). One patient had discontinued after 3.6 years because of radiotherapy for progression of a pre-existing benign meningeoma and restarted hereafter.

Adverse effects

No severe adverse effects occurred. Five patients developed slightly elevated liver enzymes (<3 times the normal values), without the need to stop or adapt dose or frequency of adalimumab. One patient had a mild respiratory infection, swiftly responding to oral antibiotics. After one month one patient had transient abdominal pain and diarrhoea, without need to stop adalimumab. None of the other patients experienced such complaints. Two patients developed anti-dsDNA antibodies in six months, however, no autoimmune disease or therapy failure were observed for the next 60 months.

Non-specific inflammatory parameters

CRP was elevated (mean 16.7 mg/l±20.3, range 1–61 mg/l) in four patients and normalised after six months. Only three patients showed elevated ESR, which decreased during treatment. White blood cell count (data not shown) was normal in most patients.

Serum cytokine levels

TNF-α serum levels were elevated (>10 pg/ml) in five (56%) patients before start of treatment. Levels decreased significantly to normal values in the first month and thereafter (p=0.002; Fig. 1A). Adalimumab levels tended to correlate inversely with TNF-α levels (p=0.057; Fig. 1B). Although TNF-α levels did not correlate with BDCAF score at treatment start, both decreased predominately in the first month of treatment and remained significantly lower compared to baseline during follow-up (p=0.007 and p=0.017, respectively; Fig. 1C). TNF-α serum levels did not correlate with CRP levels (only two overlapping patients). All other cytokines remained stable and within the normal range in all patients at all time-points (with the exception of the 6 month sample in one patient, which showed a mildly elevated TNF-α level of 11 pg/ml) (data not shown). Expression of the 11 IFN type I genes measured, was not increased and did not change over the course of therapy in all five patients measured (data not shown).

Adalimumab levels and anti-adalimumab antibodies

Before treatment in all patients adalimumab levels were below detection limit and no ADA were found. After 1 and 6 months mean adalimumab level were 7.33 μg/ml (range 1.38–11.83) and 8.78 μg/ml (range 1.96–16.00), respectively, without ADA formation in the first six months, or up to 5 years (n=8, mean 5.0 years, range 3.6-6.1). Adalimumab levels remained stable (10.3 μg/ml (range 1.6–17)).

Discussion

In this observational study all therapy refractory BD patients responded significantly to adalimumab without formation of ADA for up to 5 years.

Adalimumab has been shown similarly effective in BD. However, those

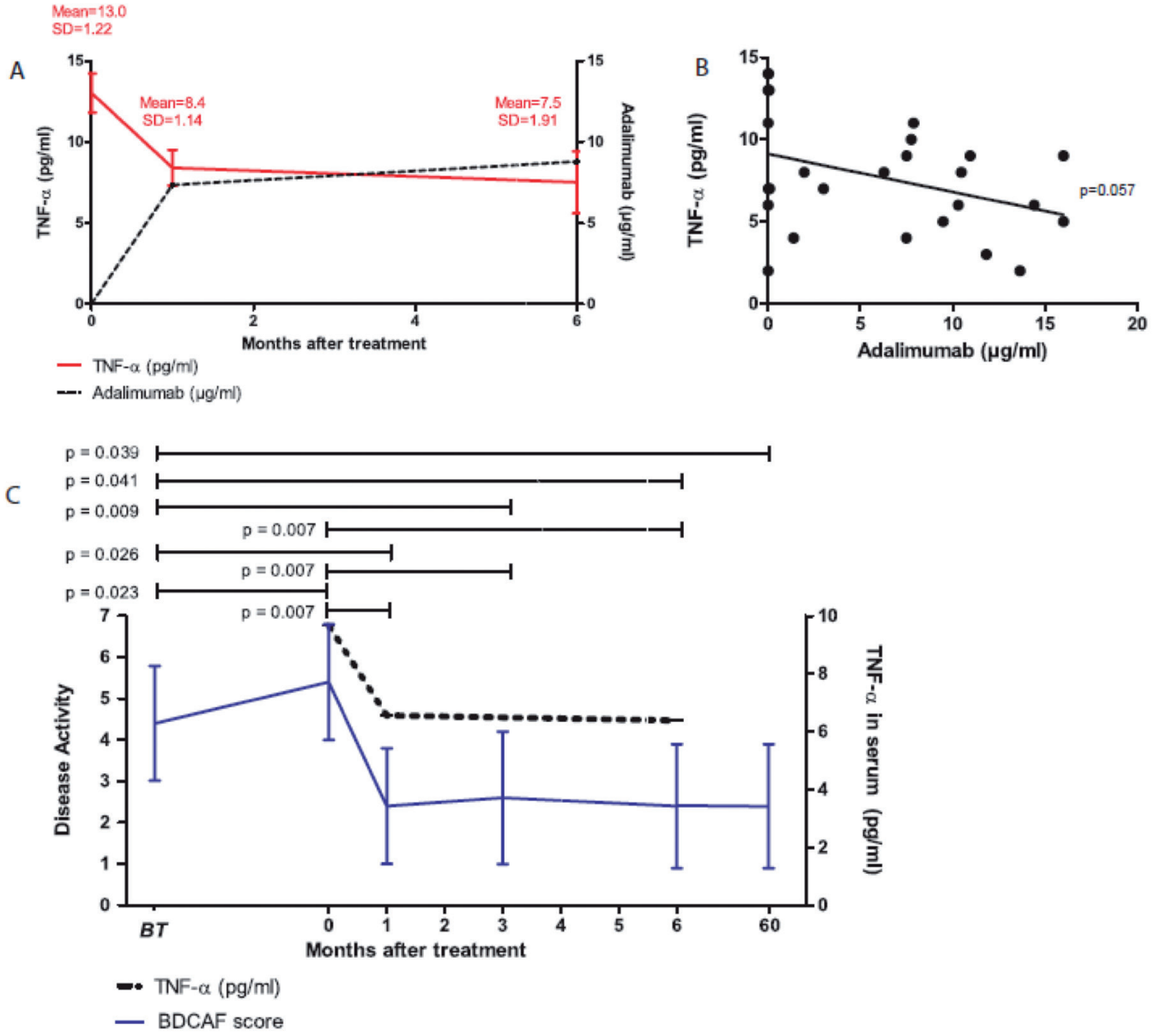

Fig. 1. TNF-α, adalimumab levels and BDCAF levels during treatment.
A: Mean TNF-α levels (pg/ml) with standard deviation during treatment, mean adalimumab levels (µg/ml). Threshold for normal levels of TNF-α is 10 pg/ml.
B: Correlation between TNF-α levels and adalimumab levels. *p*-value represents the correlation, calculated using linear regression.
C: Relative BDCAF score and TNF-α in all patients.
BDCAF: Behçet's disease current activity form; BT: before treatment (>21 days; mean of 2.8 months; range 0.7-5.6 months).
p-values represent the comparison of the BDCAF's between the depicted time points as indicated by the horizontal line.

studies were shorter in the follow-up (11-13). Though the presented study is observationally designed our selection was based on standard patient care. Intensification of immunosuppressive therapy was indicated for these therapy refractory cases. All patients had therapeutic benefit comparable with previous studies with another TNF-blocker, infliximab (13). One of the limitations of this study might be the lack of a control group to exclude the possibility of regression to the mean. This was overcome by measuring an increasing disease activity in the period before selection. Additionally, we consider the successful tapering of anti-inflammatory drugs indicative for a substantial clinical response. This is supported by the, albeit non-significant, inverse correlation between adalimumab levels and TNF-α levels, and clinical improvement compared to time point 0 and BT. In addition none of the patients developed vital organ involvement in the five years being treated with adalimumab. The uniformity of results and comparability with other studies suggest a true beneficial effect of adalimumab.

In the prolonged observational period no therapy resistance or ADA formation was found. In similar studies with RA and CD patients 17%-28% of patients developed ADA within 26 weeks (7, 14, 15). In our study no ADA were detected during the initial 6 months or after prolonged follow-up. The absence of ADA formation as compared to diseases associated with auto-immunity suggests a

different pathophysiology and might explain the durability of biologics in BD. Another explanation could be the use of concomitant immunosuppressive medication, which might prevent formation of ADA (14, 16). This could partly explain our observations, however in most patients immunosuppressive therapy could be tapered. It remains of further debate if concomitant immunosuppressives is necessary in preventing ADA formation in BD (13). The presented observations could also suggest that ADA formation might be disease specific and not require preventive treatment.

The exact duration of in which TNF-blocking therapy remains effective is unknown. Nevertheless prolonged adalimumab (mean period of 60 months) in this study appears safe and is consistent with other reports on infliximab in BD (6, 13), and suggests similar therapeutic efficacy between adalimumab and infliximab.

This study did not address discontinuation of TNF-blocking treatment after prolonged treatment. Optimal tapering strategies could potentially decrease anti-TNF use without increasing disease burden, and a recent study reported it was feasible to discontinue anti-TNF agents in half of the patients under long-term anti-TNF treatment (17). The optimal timing of discontinuation, identification of patients that remain in remission and identification of patients at high risk for progression of disease should be addressed in future research.

Five patients initially expressed elevated TNF-α serum levels, normalizing within one month after adalimumab, in line with previously described case-reports (18, 19). Importantly, our data also indicates absence of increased serum TNF-α levels does not exclude a clinical response to adalimumab and points out serum cytokine levels do not always accurately reflect the inflammatory environment within affected tissues (20). Although, decrease of initially elevated levels of TNF-α correlates with clinical response, absence of increased serum TNF-α does not exclude possible local effect of blocking TNF-α. Local TNF-α measurements might better reflect true disease activity (20).

In conclusion, we provide a basis for the use of adalimumab in refractory mucocutaneous BD. The long lasting positive clinical results without ADA formation provide a strong signal that adalimumab can be administered safely in refractory BD.

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Key messages

- Adalimumab can safely and effectively be used in refractory mucocutaneous BD patients.
- The long-lasting positive clinical results without ADA formation provide a strong signal that adalimumab can be administered safely in refractory BD.

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