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# Serum soluble CD30 levels in Behçet's disease

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## ABSTRACT

*Behçet's disease is associated with the inflammatory response. Several reports indicate the presence of primarily CD4+ T cells of the Th1 subtype in the inflammation process of the disease. Serum soluble CD30 (sCD30) is reported to be released from CD4+ Th2 type cells and has been suggested to be a marker of Th2 activity. In this study, serum sCD30 levels were measured in active and inactive patients with Behçet's disease, healthy controls and a group of patients with rheumatoid arthritis, typical Th1 disorder using enzyme immunoassay kit. Mean sCD30 value of 54 active patients were found significantly higher than in those of 17 inactive patients ( $p=0.027$ ), 20 healthy controls ( $p=0.040$ ) and 25 patients with rheumatoid arthritis ( $p<0.001$ ). There was a significant correlation between increased sCD30 levels and clinical activity index in active patients with Behçet's disease. High serum levels of sCD30 may reflect the activation of CD4+ T cells or a subset of them in active BD patients. In addition to serum sCD30 levels, measurements of the Th2 cytokines may be a helpful tool for the evaluation of Th2 activity in Behçet's disease.*

## Introduction

CD4+ helper T cells can be divided into subpopulations termed Th1 and Th2 by their ability to release different sets of cytokines in response to antigen and to drive either the cellular or humoral immune response. Th1 cells preferentially produce IL-2, IFN- $\gamma$  and TNF- $\alpha$ , and induce the activation of delayed-type hypersensitivity reactions, while Th2 cells preferentially produce IL-4, IL-5 and IL-10, and induce B cell responses (1).

Behçet's disease (BD) is a chronic multi-system inflammatory disorder of unknown etiology, characterised by recurrent oro-genital aphthous ulcerations, uveitis and skin lesions (2). The com-

mon pathological feature in all of the organ systems affected by the disease is vasculitis, characterized by a perivascular and intramural infiltrate of lymphomononuclear cells. Recent findings have better defined the nature of the inflammation in BD. The predominant inflammatory cell has been demonstrated to be of T cell lineage in immunohistopathological studies of active inflammatory sites (3,4). The perivascular infiltration of the CD4+ T lymphocyte subset together with IL-2R surface markers indicate the Th1 cell response in BD (5). Increased Th1-type cytokine levels were reported in sera from BD patients (6-8). Serum levels of Th2-type cytokines such as IL-10 (9, 10) and IL-4 were also showed to be increased.

CD30 molecule, a member of the TNF superfamily of membrane cytokine receptors, is reported to be expressed on activated CD4+ T cells producing Th2 cytokines. These cells release a soluble molecule (sCD30) (11) that is considered to be Th 2 activity.

The aim of this study was to determine serum levels of sCD30 for the evaluation of the Th2 cell response and to investigate its relationship with the clinical manifestations and disease activity of BD.

## Materials and methods

### Study population

The study group consisted of 71 patients with BD (32 male, 39 female; mean age  $\pm$ SD was  $31 \pm 8$ ; range 18-45), all fulfilling the International Study Group Criteria for the diagnosis of BD (12), and 20 healthy controls (8 male, 12 female; mean age  $\pm$ SD was  $28 \pm 7$ ; range 18-43) and 25 patients with rheumatoid arthritis (RA) as disease controls (8 male, 17 female; mean age  $\pm$ SD was  $37 \pm 6$ ; range 24-47).

At the time of the study, 54 patients with BD had clinically active disease with at least two clinical manifestations: oral ulcers, genital ulcers, skin

**Table I.** Clinical symptoms of patients with active Behçet's disease (n = 54)

Symptom	n	%
Oral ulcers	54	100
Genital ulcers	31	57.3
Eye lesions	15	27.7
Skin lesions	35	64.8
Vascular involvement	7	12.7

lesions, eye lesion, arthritis and vascular lesions. During the study, all active patients had recurrent oral aphthous ulceration, 31 patients had genital ulcers, 35 had skin lesions such as erythema nodosum and/or papulo-pustular lesions, 15 had active uveitis and 7 patients had vascular lesions. The clinical findings in the patients with active BD are shown in Table I. The clinical activity index was calculated in accordance with Yazici *et al.* (13).

Seventeen patients who did not exhibit any symptoms one month prior to examination were accepted as inactive. Eight patients were on immunosuppressive therapy (cyclophosphamide 3, azathioprine 2, cyclosporine 3). Five patients were on corticosteroid.

Disease activity was assessed according to the number of tender and swollen joints (total 28 joints) (14) in the disease controls, consisting of 25 patients with RA who fulfilled the 1987 ACR criteria (15). The disease activity score (DAS28) was calculated from the number of tender and swollen joints, the patient's assessment of his/her general health on a visual analog scale (16) and the erythrocyte sedimentation rate (ESR). The haemoglobin level, leuco-

**Table III.** Mean serum concentrations of sCD30  $\pm$  SD and P values in Behçet's disease patients, rheumatoid arthritis patients, and healthy controls.

sCD30 IU/ml	P	Groups	sCD30 (IU/ml)
Active BD (n = 54)	p = 0.027	Inactive BD (n=17)	32.05 $\pm$ 6.36
41.62 $\pm$ 15.17	p = 0.040	Healthy controls (n = 20)	33.42 $\pm$ 8.22
	p < 0.001	Rheumatoid arthritis (n = 25)	22.45 $\pm$ 13.05

cyte and platelet counts, CRP values and radiographic findings for the hands and feet were recorded. The clinical and laboratory findings for the patients with RA were shown in Table II.

#### sCD30 assay

Serum sCD30 levels of the patients with BD and healthy and disease (RA) controls were measured using an enzyme immunoassay (ELISA) kit (MedSystems Diagnostics GmbH, Vienna, Austria) according to the manufacturer's protocol (mean sCD30 level  $\pm$  SD 38.7  $\pm$  28.0 U/ml according to the manufacturer).

ESR by the Westergren method and C-reactive protein concentrations by nephelometry were determined at the same time.

#### Statistical analysis

The differences among the BD, RA and healthy control groups were evaluated by Kruskal-Wallis variance analysis. When the p-value from this analysis was statistically significant the multiple comparison test was used to determine the differences between the groups (17). The correlation between the clinical activity index and the sCD30 concentration was evaluated by Spearman's rho.

#### Results

The mean serum concentrations of sCD30  $\pm$  SD and the p values in the patients with BD and the controls (disease and healthy) are shown in Table III and Figure 1.

The mean serum levels of sCD30  $\pm$  SD increased significantly in patients with active BD (n = 54; 41.62  $\pm$  15.17 IU/ml, min-max; 23.38 – 99.20 IU/ml) compared with patients with inactive BD (n = 17; 32.05  $\pm$  6.36 IU/ml; min-max: 19.34 – 41.27 IU/ml) (p = 0.027), healthy controls (n=20; 33.42  $\pm$  8.22

IU/ml; min-max: 21.63 – 51.90 IU/ml) (p = 0.040) and disease controls (n = 25 RA; 22.45  $\pm$  13.05 IU/ml; min-max 7.60 – 61.70 IU/ml) (p < 0.001). There was no significant difference was noted between patients with inactive BD and healthy controls (p = 0.811)

The levels of sCD30 were compared to the clinical disease activity index according to previous criteria (13). Serum levels of sCD30 were found to be correlated with disease activity (r = 0.49, p = 0.001) (Fig. 2).

The mean DAS28 score was 4.4  $\pm$  1.2 in patients with RA. These patients had moderately active disease in terms of the clinical, biological and radiographic values.

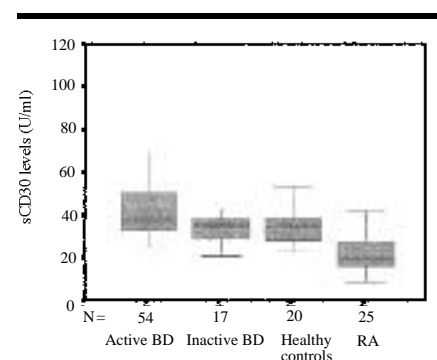
Serum CRP levels were significantly increased in patients with active BD compared to the inactive group (33.2  $\pm$  27.4 vs 4.3  $\pm$  3.9 mg/dl, p < 0.001). The mean ESR was also significantly higher in active than in inactive patients (24  $\pm$  19 vs 14  $\pm$  6 mm/hr, p < 0.05). There was no correlation between CRP and sCD30, nor between ESR and sCD30 values.

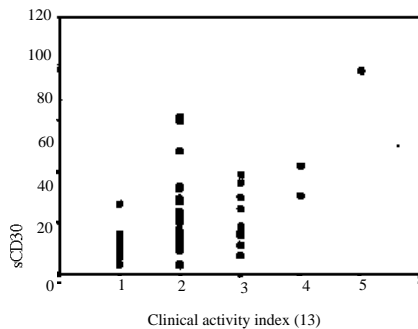
#### Discussion

BD is recognised as a systemic inflam-

**Table II.** Clinical and laboratory findings in patients with rheumatoid arthritis (n = 25).

Age/Sex	37 $\pm$ 6.7	8M/17 F
Disease duration (year)	6.2 $\pm$ 4.9	
Morning stiffness (minute)	55.22 $\pm$ 101.67	
Painful joint count	9.4 $\pm$ 8.1	
Swollen joint count	1.8 $\pm$ 2.1	
WBC/mm <sup>3</sup>	7.700 $\pm$ 2.791	
Platelet/mm <sup>3</sup>	349,000 $\pm$ 137.500	
CRP(mg/dl)	34.33 $\pm$ 50.49	
RF (IU/ml)	167.42 $\pm$ 179.30	
Disease activity score (DAS28)	4.4 $\pm$ 1.2	

**Fig. 1.** Serum sCD30 levels in patients with BD and RA and controls.



**Fig. 2.** Serum sCD30 levels in active Behçet's disease.

matory disease and characterised by immunological abnormalities and activated T cells. Histopathological examination of active inflammatory tissues has shown predominantly perivascular CD4+ T cell infiltration and IL-2R expression, indicating a Th1 cell response (5). T lymphocytes involving a Th1 immune response were found to be increased in peripheral blood and were predominantly distributed in the mucocutaneous lesions in BD (18). A strong polarization of the immune response toward the Th1 pathway correlated with the progression of BD (19). Th1 type cytokines such as IL-2, TNF- and IFN- were found to be high in BD (6-8). Increased TNF- production *in vitro* was also found in BD (20). Hamza *et al.* revealed that BD patients with disease that is either active or in remission have increased serum levels of Th1-type and Th2-type cytokines upon *in vitro* stimulation. The percentages of CD4+ T cells containing IFN-gamma and CD40L were higher in active BD compared to healthy controls (8). On the other hand, active inflammatory ocular tissues showed predominantly CD4+ T cell infiltration as well as B cell and plasma cell aggregation (21) or immunoglobulin and complement deposits (22) suggesting a humoral immune activation were reported in BD. Th2 cytokines (IL-4 and IL-10) inducing B cell immune response were found to be raised in the serum of patients with BD (9, 10). Del Prete *et al.* reported that Th1 clones expressed little or no CD30 mRNA, whereas Th2 clones showed CD30 mRNA (11). Bengsson *et al.* showed

that the CD30 molecule is expressed in activated CD4+ T cells of all three subtypes (Th0, Th1 and Th2) and that this expression is sustained in Th2 cells (23). In another study, CD30 expression was also found in Th0-, Th1- and Th2-type clones (24). We found significantly high levels of sCD30 in patients with active BD compared to inactive patients and healthy controls ( $p = 0.027$ ,  $p = 0.040$ , respectively). High serum levels in patients with active BD may reflect the activation of all CD4 +T cells or a subtype of these. Elevated serum levels of sCD30 have also been found in Th1-driven disorders such as RA (25, 26), Sjögren's syndrome (27) and Hashimoto's thyroiditis (28). In our RA patients with moderately activity, serum levels of sCD30 were significantly low compared to patients with inactive BD and healthy controls ( $p = 0.002$ ,  $p < 0.001$ , respectively).

Determination of CD30+ cell numbers in the circulation and tissue together with serum levels of sCD30 may elucidate clearly the significance of this molecule in several diseases. However, in systemic lupus erythematosus, another Th2 driven disorder, high serum levels of sCD30 are not associated with increased CD30+ cell numbers in the blood (29). Calligaris *et al.* reported that increased sCD30 concentrations in patients with active systemic lupus erythematosus may reflect disease activity (29). Wang *et al.* found that sCD30 was associated with disease activity and severity in generalized Wegener's granulomatosis (30). In our study, serum levels of sCD30 were not only significantly increased, but were also correlated with the clinical activity index (13) in BD patients ( $r = 0.49$ ,  $p = 0.001$ ).

These data suggest that increased sCD30 levels appear to be associated with active BD, which is predominantly involved in cellular immunity and which shows a strong Th1 immune response. Further research concerning Th2 activity in BD is needed.

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