Anti-*Saccharomyces cerevisiae* antibodies (ASCA) in Behçet's syndrome

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ABSTRACT

Objectives. *Anti-*Saccharomyces cerevisiae *antibodies (ASCA) are found in 50-60% of patients with Crohn's disease. Increased as well as normal levels have been reported in Behçet's syndrome (BS). We reassessed the level of IgG and IgA ASCA antibodies in BS and in a group of diseased and healthy controls.*

Methods. *Eighty-five patients with BS were studied along with 20 patients with ankylosing spondylitis (AS), 24 with Crohn's disease (CD), 25 with ulcerative colitis (UC) and 21 healthy volunteers. A commercial ELISA kit was used (Inova Diagnostics).*

Results. *It was only the patients with CD who had significantly higher levels of antibodies compared with the rest of the group (ANOVA: ASCA IgG, p = 0.0001; ASCA IgA, p = 0.0001). 42% of CD, 4% of BS, 4% of UC and 15% of AS patients had a positive IgG+IgA ASCA. There was a significant trend for patients with gastrointestinal (GI) involvement with BS (n = 8) to be more positive for IgG and IgG+IgA ASCA compared to the rest of the patients with BS (n = 77) (Chi-square, IgG, p = 0.02, IgG+IgA, p = 0.001).*

Conclusion. *The rate of positivity of ASCA in BS is comparable to that observed among patients with UC and AS. Patients with BS who have GI involvement may have higher levels of ASCA and this needs to be further studied.*

Introduction

Saccharomyces cerevisiae is a yeast used in food industry. Antibodies against this yeast (ASCA) are present in patients with inflammatory bowel diseases (IBD) and their serum levels have proven useful in the differentiation of Crohn's disease (CD) from ulcerative colitis (UC) in the adult and pediatric populations (1, 2). A positive ASCA favors CD whereas a negative ASCA is usually found in UC (3). The antigenic specificities of these antibodies as well as their pathogenic significance are yet unknown. Their presence in the first degree relatives of patients with CD who do not have the disease suggests a genetic component (4).

CD and Behçet's syndrome (BS) have certain similarities. Oral and gastrointestinal ulcerations, erythema nodosum, arthritis and uveitis are seen in both conditions. Although there is wide variation in the prevalence of gastrointestinal (GI) involvement in BS in different populations (0-60%) (5), the clinical and radiological findings of GI BS and CD share common features (6,7). One study has found an increased prevalence of ASCA in BS (8) whereas another did not (9).

We looked at IgG and IgA ASCA levels in a large group of patients with BS, having a rather wide clinical spectrum, along with appropriate diseased and healthy controls.

Material and methods

Eighty-five patients with BS-17 with predominant mucocutaneous manifestations, 18 with uveitis, 10 with acute and 8 with chronic deep vein thrombosis, 9 with arterial aneurysms, 8 with gastrointestinal involvement and 15 with arthritis-all fulfilling the International Study Group Criteria (10), were studied in addition to 20 patients with ankylosing spondylitis (AS), 24 with CD, 25 with UC and 21 healthy volunteers.

The patients with BS were the regular attendees of the Behçet's syndrome outpatient clinic of Cerrahpasa Medical Faculty, Istanbul that currently enrolls around 5500 patients. They were consecutive patients judged to have active mucocutaneous and/or ocular and/or vascular and/or gastrointestinal lesions during venipuncture.

Table I. The demographic characteristics of the patients and the controls and the rates of IgG and IgAASCApositivity.

	AGE (yr), (95%CI), SEX(M/F)	ASCAIgG (U) (95%CI)	ASCAIgA(U) (95%CI)	ASCA IgG + (%)	ASCAIgA + (%)	ASCA Total + (%)	Any ASCA + (%)
BS (n=85)	34.3 (32.0-36.7) (65/20)	13.5 (11.7-15.4)	17.4 (15.4-19.5)	5/85 (5.9)	16/85(18.9)	3/85 (3.5)	18/85(21.2)
AS (n=20)	33.7 (30.3-37.0) (18/2)	22.7 (10.6-34.7)	18.6 (12.3-24.8)	4/20 (20.0)	4/20 (20.0)	3/20 (15.0)	5/20 (25.0)
CD (n=24)	38.9 (34.6-43.2) (15/10)	36.9 (22.4-51.5)	71.9 (25.3-118.6)	11/24 (45.8)	14/24(58.3)	10/24 (41.7)	15/24(62.5)
UC (n=25)	35.6 (33.0-38.3) (15/10)	20.5 (10.1-30.9)	16.2 (11.7-20.8)	4/25 (16.0)	4/25 (16.0)	1/25 (4.0)	5/25 (20.0)
HC (n=21)	33.7 (30.4-37.0) (12/9)	11.7 (7.9-15.4)	13.4 (10.7-16.0)	2/21 (9.5)	2/21 (9.5)	1/21 (4.8)	3/21 (14.3)

BS: Behçet's syndrome; AS: ankylosing spondylitis; CD: Crohn's disease; UC: ulcerative colitis; HC: healthy controls; yr: years; CI: confidence interval.
 2 (all groups): ASCAIgG: Pearson = 24.53 p = 0.0001, Crohn excluded: Pearson = 4.86 p = 0.18, ASCAIgA: Pearson = 20.79 p = 0.0001, Crohn excluded: Pearson = 1.16 p = 0.76, ASCAtotal: Pearson = 32.06 p = 0.0001, Crohn excluded: Pearson = 4.38 p = 0.22, Any ASCAtotal: Pearson = 19.48 p = 0.001, Crohn excluded: Pearson = 0.77 p = 0.86.

Patients with CD, UC and AS were recruited from the gastroenterology and rheumatology departments of the same hospital, irrespective of their clinical activity state. Healthy controls were laboratory personnel or medical students. The study was carried out in accordance with the principals of the declaration of Helsinki.

A commercial enzyme-linked immunosorbent assay (ELISA) kit (QUANTA Lite™, INOVA DIAGNOSTICS) were used for both IgG and IgAASCA measurements. The assays were performed by a technician blinded to the diagnoses. Two standard deviations above the mean of the normal values (obtained from our healthy controls) were accepted as the cut off point (28 U for IgG, 25 U for IgA). These values were approximately similar to those of the kit package insert (25 units for both IgG and IgA).

The disease activity of the patients with BS at the time of venipuncture was measured according to the Krause (8) and Yazici (11) indices, both being weighted cumulative scores of the clinical manifestations. A separate analysis was performed with the same indices by analyzing the cumulative organ involvement throughout the disease course with the aim of evaluating damage. The results were correlated with the ASCA titers.

Continuous variables were analyzed by ANOVA and the Bonferroni test and the categorical variables by the chi square tests. The relationship between disease activity and ASCA titers were examined by the Spearman's correlation test. Pvalues below 0.05 were considered significant.

Results

The mean values of the ASCAantibodies are shown in Figure 1.

The demographic characteristics of the patients and controls and the rates of ASCApositivity are shown in Table I. It was only the patients with CD who

Table II. The clinical characteristics of patients with BS who had gastrointestinal involvement.

Patient	Age, sex	ASCA IgG (U)	ASCA IgA(U)	Duration BS (yrs.)	Clinical features	Site of GI involvement	TBC PCR & tissue culture	Current therapy
SK	33,M	22	18	6	OA,GU,OF, Sister with BS	Ileocaecal	(-)	Azathioprine
RD	26,F	9	13	1	OA, GU, EN, Sister with BS	Ileocaecal (Operated)	(-)	Azathioprine
SU	23,M	9	9	2	Path+, OA, GU, OF, EN, ARTH, DVT	Caecal		Interferon
EA	26,M	15	39*	1	OA, GU, OF, EN, UV	Ileocaecal (Operated twice)		Azathioprine
MA	30,M	29*	30*	7	Path+, OA, GU,OF, Uncle's daughter BS	Ileocaecal+colonic		5ASA
KE	28,F	35*	26*	7	OA, GU	Ileocaecal		Azathioprine
ET	25,M	11	12	7	OA, GU, EN, UV	Ileocaecal	(-)	Azathioprine+ prednisolone
HO	24,M	17	20	4	OA, GU, OF, EN, UV,	Colonic (Operated)	(-)	Azathioprine

Duration of disease is defined as the time elapsed since the fulfillment of International Study Group criteria. Path: pathergy; OA: oral aphthae; GU: genital ulcers; EN: erythma nodosum; ARTH: arthritis; DVT: deep vein thrombosis; UV: uveitis. ASA: acetylsalicylic acid *Positive
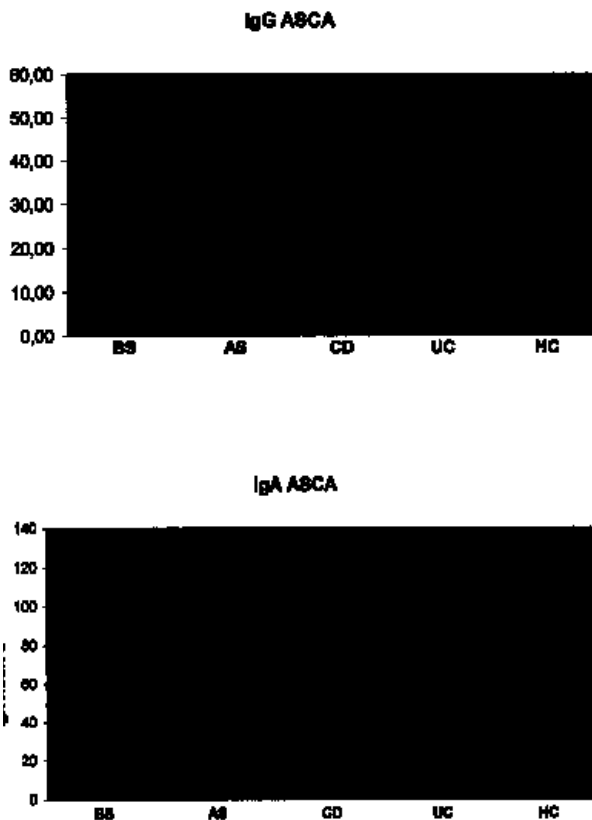
Fig. 1. The levels of IgG and IgA ASCA antibodies in patients and controls.

BS: Behçet's syndrome (n = 85), AS: Ankylosing spondylitis (n=20), CD: Crohn's disease (n = 24), UC: Ulcerative colitis (n = 25), HC: Healthy controls (n = 21). Vertical bars represent means and 95% confidence intervals.

ANOVA: ASCA IgG F = 7.74 p = 0.0001 (Bonferroni CD vs BS p = 0.0001, CD vs UC p = 0.03, CD vs HC p = 0.0001)
ASCA IgA F = 9.08 p = 0.0001 (Bonferroni CD vs BS p = 0.0001, CD vs AS p = 0.0001, CD vs UC p = 0.0001, CD vs HC p = 0.0001).

had significantly higher levels of antibodies compared with the rest of the study group (Fig. 1).

It was again the patients with CD who had the highest level of positivity of any ASCA combination (Table I).

A subgroup analysis among patients with BS showed that patients with gastrointestinal involvement had more positive IgG and IgG+IgA ASCA compared to patients with other clinical manifestations (IgG ASCA +[2/8 (25%)] vs [3/77 (4%)], IgG+A [2/8 (25%)] vs [1/77 (1%)]; Chi square IgG, p = 0.02, IgG+A, p = 0.001). There were no significant differences between the other groups.

There was a negative correlation between disease activity and damage with IgA ASCA titers with both the Krause and Yazici indices (r = -0.3 and p = 0.008 for the Krause index and r = -0.2 p = 0.04 for the Yazici index for activity and r = -0.2 and p = 0.04 for the Krause and r= -0.2 p=0.03 for the Yazici indices for damage).

In order to avoid diagnostic overlay the clinical characteristics of the patients with BS who had gastrointestinal in-

volvement and their ASCA levels are given in Table II.

Discussion
Our cross sectional study showed that the levels of ASCA positivity (IgG, IgA, IgG+IgA) in BS were not significantly different than those observed in UC, AS and healthy controls. However, a subgroup analysis among patients with BS revealed that patients with gastrointestinal involvement had higher levels of IgG and IgA ASCA. There was also an inverse correlation between disease activity in BS in general and IgA ASCA titers.

One of the previous studies had found increased IgG and IgA ASCA levels in BS (8). However the study group was not representative of the whole spectrum of BS since it consisted predominantly of females. Moreover BS patients with gastrointestinal involvement and diseased controls with inflammatory bowel disease were absent.

It may be said that our patients who are diagnosed to have gastrointestinal BS are indeed cases of CD since both diseases share common features. Howev-

er, an analysis of the patients shown in Table II clearly shows that all patients included in the GI BS group show the characteristic features of BS.

Gastrointestinal tuberculosis is another condition that may mimic gastrointestinal BS (12). However, four of our patients had negative tissue cultures and polymerase chain reactions for *Mycobacterium tuberculosis.*

The inverse correlation between disease activity, damage and IgA ASCA titers may suggest a protective role of IgA antibodies in BS (13). However our study was not powered to show such a difference and this finding should be interpreted with caution.

The inclusion of patients with CD and UC with differing levels of clinical activity among the control groups, may have made the interpretation of our results somewhat difficult. However the percentage of ASCA positivity among our patients with CD was similar to what has been found previously (3). Furthermore ASCA positivity seems to be a marker of anatomical subtype rather than clinical activity in CD (14).

The patients and controls were not stratified according to the drugs they were currently using , a factor that may have affected the results because of potential effects of immunosuppressives on antibody production. However, patient recruitment mirrored the patterns of general practice and the patterns of drug use were similar among both patient and diseased control groups (data not given). In addition there is no evidence that ASCA production is affected from immunosuppressives (15).

Our study showed that ASCA levels were not appreciably increased among patients with BS to the degreee found in CD. This is useful in differential diagnosis. On the other hand the trend for higher ASCA levels noted among our patients with gastrointestinal BS necessitates a closer look at this group of patients in future studies.

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