Anti-*Saccharomyces cerevisiae* antibodies - A novel serologic marker for Behçet's disease

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Received on February 8, 2002; accepted in revised form on June 4, 2002.

Clin Exp Rheumatol 2002; 20 (Suppl. 26); S21-S24.

Key words: Behcet's disease, anti-*Saccharomyces cerevisiae* antibodies, aphthous stomatitis, severity, Crohn's disease.

ABSTRACT

Objective

To evaluate the prevalence and clinical correlations of antibodies against Saccharomyces cerevisiae *(ASCA) among patients with BD.*

Methods

Twenty-seven BD patients were studied. Data from medical files and from patients' interviews was collected, regarding the entire spectrum of disease manifestations, and a severity score was calculated for each patient. IgA- and IgG-ASCA levels, determined by ELISA, were studied in all BD patients and in three control groups: patients with recurrent aphthous stomatitis (RAS), systemic lupus erythematosus (SLE) and healthy volunteers.

Results

Thirteen BD patients (48.1%) were ASCA-positive, compared to one patient in each control group (10%, $p = 0.01$). The mean value of IgG-ASCA in the BD patients was 20.7 ± 12.3 units, significantly higher than in patients with RAS (10.0 ± 5.5, $p < 0.001$), SLE (11.8 ± 9.3, $p < 0.03$) or healthy volunteers (10.8 ± 9.8, $p < 0.02$). Mean IgA-ASCA level was 16.8 ± 8.8 units in the BD patients, significantly higher compared to healthy volunteers (11.0 ± 5.0, $p = 0.02$) but similar to patients with RAS (17.0 ± 5.3). No correlation was found between ASCA and any BD-associated clinical manifestation nor the presence of HLA-B5. No difference was found in the rate of major oral ulcers nor in the systemic disease severity score between positive- and negative-ASCA patients (27.3% vs. 30.8%, and 7.31 ± 1.80 vs. 7.28 ± 2.27 respectively, NS).

Conclusion

The results of our study associate, for the first time, the presence of a distinct antibody, i.e. ASCA, with BD. ASCA were not linked to a specific clinical manifestation of the disease and probably do not pose an increased risk for a more severe disease course.

Introduction

Over the past few years, antibodies against *Saccharomyces cerevisiae* (ASCA), a yeast commonly used in the food industry, were reported in patients with Crohn's disease (CD) with a prevalence varying from 39% to 76% (1-3). ASCA are considered specific for CD and their major clinical use has been to differentiate CD from ulcerative colitis (UC) in both adult and pediatric populations (1, 4). A positive ASCA test with negative perinuclear anti-neutrophilic cytoplasmic antibody (p-ANCA) is strongly associated with CD, and the reverse is true for UC (4). ASCA are also found in healthy first-degree relatives of patients with CD, suggesting a geneticorigin of the antibodies (5,6). The pathogenic significance of these antibodies is unknown, and their exact origin, as well as the epitope against which they are directed, is unclear. They are not thought to be autoantibodies, although molecular mimicry to self-antigens remains a possibility.

Behcet's disease (BD) is a multi-system disorder, the clinical expression of which may be dominated by mucocutaneous, articular, neurologic, urogenital, vascular, intestinal or pulmonary manifestations (7). BD and CD share various clinical similarities, including mucocutaneous manifestations (recurrent oral ulcers, erythema nodosum), gastrointestinal disease favoring the terminal ileum, recurrent arthritis as well as uveitis, thus raising the possibility of certain etiologic and pathogenic factors common to both diseases. Hence, we evaluated the prevalence of ASCA in patients with BD, and looked for possible associations between positive ASCA and various BD-related manifestations as well as disease severity.

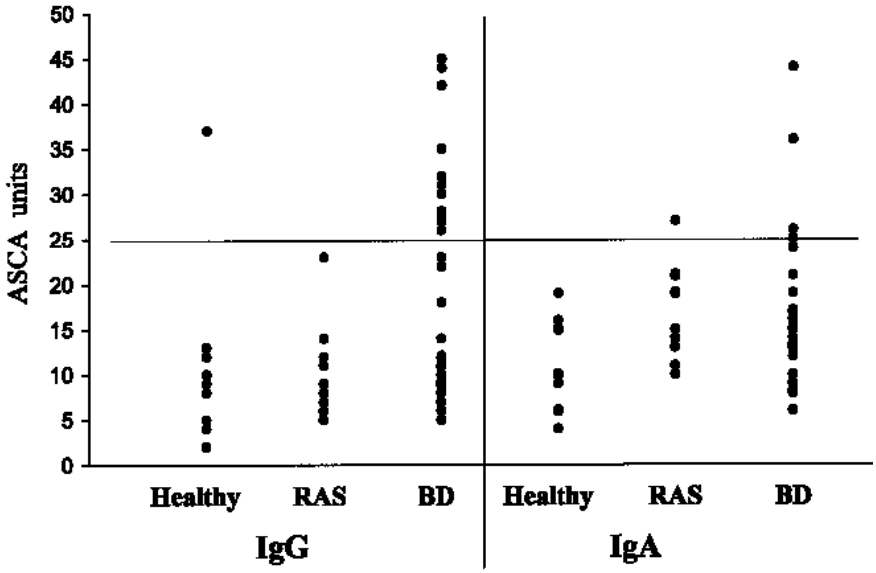

Fig. 1. ASCA levels (IgG and IgA) in patients with Behcet's disease (BD), recurrent aphthous stomatitis (RAS) and healthy controls. Levels equal to or greater than 25 units are considered positive.

Patients and methods

Twenty-seven BD patients were studied, all of them fulfilled the International Study Group (ISG) criteria for BD (8). Data from medical files and from patients' interviews was collected, regarding the entire spectrum of disease manifestations. Major oral ulcers were defined as oral ulcers which are larger than 1 cm in diameter, and/or heal with scarring, minor oral ulcers were defined as smaller than 1 cm, which heal without scar formation (9). None of the BD patients had inflammatory bowel disease (IBD), nor a first-degree family relative with IBD. Furthermore, none of the patients had gastrointestinal manifestation of BD.

The severity score was calculated as the sum of 1 point for each of mild symptoms, 2 points for each of moderate symptoms and 3 points for each of severe disease manifestations, as previously described (10, 11).

ASCA levels were determined by ELISA employing commercial kits for IgG- or IgA-ASCA (QUANTA Lite™, INOVA Diagnostics Inc., San Diego, CA, USA), and following the manufacturer's instructions. Values of 25 or more units were regarded positive according to the assay manufacturer's perception. IgG- and IgA-ASCA levels were measured in the group of BD patients and in two control groups- ten patients with idiopathic recurrent aphthous stomatitis (RAS) with no other clinical feature of BD, and ten healthy volunteers. IgG-ASCA were also measured among ten patients with systemic lupus erythematosus (SLE), all of them with high levels of anti-DNA autoantibodies.

Statistical analysis was performed employing two-tailed student's t-test for mean values, and the chi-square test or Fisher's exact test if appropriate for table analysis.

Results

Twenty-seven BD patients were studied. There were 7 males (25.9%) and 20 females (74.1%), mean disease duration was 13.1 ± 9.1 years. BD-related manifestations in the study population are presented in Table I. All the patients, per definition, had recurrent aphthous stomatitis, the prevalences of other manifestations were as follows: genital ulcers 88.9%, ocular involvement 44.4%, typical cutaneous lesions 81.5%, positive pathergy reaction 58.3% (tested in 12 patients), articular disease 74.1%, central nervous system involvement 11.1%, vascular disease (deep or superficial vein thrombosis or arterial aneurysms) 41.2%. The prevalence of HLA-B5 was 79.2%. Thirteen BD patients were either IgG- or IgA-ASCA positive (48.1%), of whom two patients were positive for both IgA- and IgG-ASCA (Table I). In contrast, only one patient in each control group was positive for ASCA (10%, $p = 0.01$).

Figure 1 shows the results of the IgG- and IgA-ASCA assays. The mean value of IgG-ASCA in the BD patients was 20.7 ± 12.3 (range 6-45) units which was significantly higher than in the patients with RAS (10.0 ± 5.5, $p < 0.001$) or healthy volunteers (10.8 ± 9.8, $p < 0.02$). Mean IgA-ASCA levels were 16.8 ± 8.8 units in the BD patients, significantly higher than in the group of healthy volunteers (11.0 ± 5.0, $p = 0.02$) but similar to the patients with RAS (17.0 ± 5.3). IgG-ASCA levels were also determined among ten SLE patients, one of whom (10%) had positive ASCA value. Alike the results among patients with RAS or healthy control groups, mean ASCA levels were significantly higher in BD patients compared to SLE patients (20.7 ± 12.3 vs. 11.8 ± 9.3, $p < 0.03$).

Regarding the BD patients, no correlation was found between positive ASCA values and the presence of genital ulcers, ocular disease, skin lesions, positive pathergy reaction, deep or superficial vein thrombosis, arterial disease, joint manifestations, neurological involvement or the presence of HLA-B5. There was also no difference in the rate of major oral ulcers between positive- and negative-ASCA patients (27.3% vs. 30.8%, NS), nor in the BD-severity score (7.31 ± 1.80 vs. 7.28 ± 2.27, NS).

Discussion

The clinical diagnosis of BD may pose considerable difficulties. Since the disease is multi-systemic and does not have any pathognomonic symptom or laboratory findings, the diagnosis is made solely on the basis of the criteria proposed by the International Study Group for BD in 1990, based on a cluster of clinical manifestations (7, 8). Nonetheless, the diagnosis might be delayed for several years, until all ISG criteria have been fulfilled. Thus, the presence of a relatively specific labora-

Table I. BD patient's characteristics.

Pt.	Age (years)	Sex	Disease duration (years)	OU	GU	Ocular disease	Skin lesions	EN	Path.	Joints	Neuro-Behçet	DVT	SVT	HLA B5	ASCA IgG	ASCA IgA
1	31	F	3	+	+		+		ND	+				ND		
2	21	F	7	+	+	+	+	+	ND	ß				+		+
3	60	F	15	+	+	+	+		+	+		+	+	+		
4	42	F	20	+	+		+	+	+	+		+		+	+	+
5	41	F	11	+	+		+		ND	+			+	+	+	
6	56	F	10	+	+	+			+	+			+	+	+	
7	56	F	15	+	+	+	+	+		+						
8	60	F	41	+		+	+	+	ND				+	ND	+	
9	36	F	6	+	+	+			ND	+				ND	+	
10	47	F	24	+		+	+	+	+	+			+	+		
11	40	M	4	+		+	+	+		+		+		+		
12	29	F	13	+	+		+		ND					+		
13	25	M	20	+	+		+		ND					+		
14	50	M	14	+	+		+	+	+	+	+			+		
15	19	F	2	+	+		+	+		+						
16	42	F	9	+	+		+		ND	+		+		+		
17	64	F	27	+	+		+		ND		+			+	+	
18	37	F	17	+	+		+		ND	+				+	+	+
19	36	M	8	+	+		+		ND	+					+	
20	40	M	6	+	+		+		ND	+		+	+	+		+
21	44	M	2	+	+	+			ND	+				+		
22	37	M	9	+	+	+	+		ND					+		
23	47	F	11	+	+	+				+				+	+	
24	47	F	29	+	+		+	+		+	+			+		
25	37	F	16	+	+		+		+	+					+	
26	30	F	9	+	+		+	+	ND					+	+	
27	48	F	7	+	+	+			+	+		+				

OU: oral ulcers, GU: genital ulcers, Ocular disease- at least one of anterior/posterior uveitis, or retinal vasculitis, Path: positive pathergy test, EN: erythema nodosum, Joint: arthralgia/arthritis, DVT: deep vein thrombosis, SVT: superficial vein thrombosis, ND: not done.

tory marker can substantially facilitate the diagnosis of BD, and possibly support a diagnosis before all disease manifestations have occurred.

The results of our study associate, for the first time, the presence of a distinct antibody with BD. We show that ASCA, until now considered rather specific for CD, appears in BD patients with a prevalence comparable to those reported for CD. Further specificity studies are still needed among patients with inflammatory arthritides and other autoimmune conditions. Nonetheless, the significantly higher ASCA values in BD patients compared with patients with RAS, SLE or in healthy volunteers imply that ASCA, taken in an appropriate clinical context, could become a useful diagnostic tool for BD. Recently, a small control group of 7 patients with intestinal BD was reported to have mean IgG-ASCA levels similar to CD patients (12). The absolute number of ASCA-positive cases, however, was not reported. Moreover, since the expression of intestinal BD may resemble that of CD, it is possible that some of those ASCA-positive patients might actually had CD.

In our study, in order to avoid possible overlap with CD, none of the patients nor any family relative had IBD. Furthermore, none of the patients had chronic gastrointestinal manifestations. It appears, therefore, that the presence of ASCA in BD is an integral part of the disease, not associated with intestinal or other specific target-organ involvement. Interestingly, the results of our study show that the levels of IgA-ASCA (but not of the IgG isotype) were similar in patients with BD and RAS. It is possible that some of the RAS patients actually have BD, as yet undiagnosed, since other clinical manifestations have not yet appeared. IgA-ASCA may also play a role in pathogenic mechanisms associated with gastrointestinal mucosal ulcerations, such as in CD, BD or RAS. Nonetheless, the presence of ASCA might not be associated with the severity of oral ulcerations, since the presence of major oral ulcers, the most severe form of oral aphthosis was similar in BD patients with or without ASCA. Furthermore, the systemic severity score of BD was also similar in the two groups of patients. Thus,it is conceivable that the presence of ASCA do not pose an increased risk for a more severe disease course in BD. Nonetheless, since our BD patients were studied during a routine follow-up, most of them being in a clinical remission, a conclusion can not be drawn about the relation between ASCA and BD activity.

Further prospective studies are needed to evaluate whether ASCA titers are correlated with clinical relapses of the disease. Concerning the diverse clinical expression of BD in various geographical areas, it will also be of interest to evaluate the prevalence of ASCA among BD patients worldwide, and a possible genetic basis of ASCA, as implied by studies among healthy first-degree relatives of patients with CD (5, 6).

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