

# ANTI-SACCHAROMYCES CEREVISIAE [ASCA] AND ANTI-NEUTROPHIL CYTOPLASMIC [ANCA] ANTIBODIES ARE NOT INCREASED IN TAKAYASU ARTERITIS

Fatma Ozbakir\*, Serdal Ugurlu\*\*, Aykut F. Celik\*\*\*, Emire Seyahi\*\*

## Abstract

**Objectives:** Takayasu arteritis (TA) may be associated with inflammatory bowel disease (IBD). As there is such an overlap and since both diseases show granulomatous histopathological lesions we reasoned similar biological pathways might be implicated in both conditions. Therefore, we investigated the prevalence of anti-*Saccharomyces cerevisiae* (ASCA) and anti-neutrophil cytoplasmic (ANCA) antibodies - serological markers for IBD - among patients with TA.

**Methods:** Thirty-two patients with TA, 21 with Crohn's disease (CD), 17 with ulcerative colitis (UC) and 34 healthy controls were studied. Among 32 patients with TA, 2 had CD and one had UC concomitantly. ASCA Ig A and Ig G antibodies were analyzed using a commercial ELISA kit. Immune fluorescence analysis (IFA) was used to assess the presence of ANCA antibodies.

**Results:** Only patients with CD had significantly higher levels of both ASCA Ig A and Ig G, compared to patients with TA and healthy controls. Similarly, the frequency of ASCA positive patients was higher only among patients with CD. ASCA Ig A and Ig G antibodies were found in 16 % (5/32) of patients with TA. Among 3 patients, in whom TA and IBD co-existed, only one (one with CD) had positive ASCA Ig G and A antibodies. The p-ANCA antibodies were present among patients with UC (35 %) and CD (10 %).

**Conclusion:** ASCA positivity in TA was similar to that found in UC and healthy controls.

No ANCA antibodies were detected among patients with TA.

**Keywords:** Takayasu Arteritis; Anti-*Saccharomyces Cerevisiae* Antibodies; Inflammatory Bowel Disease; Crohn's Disease; Ulcerative Colitis; Anti-neutrophil Cytoplasmic Antibodies.

## Introduction

Takayasu's arteritis (TA) is a chronic inflammatory disease of large arteries<sup>1</sup>. It involves primarily the aorta and its major branches, causing thickening of the vessel wall and narrowing or occlusion in the lumen. The disease is seen mostly in females and seems to be more prevalent in Asia, compared to other parts of the world.

The association of TA with inflammatory bowel diseases (IBD), although rare, is well known based on several case reports<sup>2-9</sup>. Both TA and IBD have unknown etiology and are reported to share similar chronic granulomatous histopathology<sup>10-11</sup>.

The anti-*Saccharomyces cerevisiae* (ASCA) and anti-neutrophil cytoplasmic (ANCA) antibodies are used as serological markers for IBD. While ASCA is reported in 50 to 80% of patients with CD and 2-14% of patients with UC, ANCA (especially p-ANCA) is found in 40-80 % of patients with UC and 5-25 % of patients with CD<sup>12-15</sup>.

As TA and IBD may coexist and have a common histopathology, we thought that these diseases may have common pathogenesis. In this study, we investigated the frequency of ASCA and ANCA antibodies in TA patients comparing with patients with CD and UC and healthy controls.

## Patients and methods

We studied 32 (all female) consecutive patients with TA, diagnosed according to the criteria defined by the ACR 1990 and seen in the rheumatology out-

\*Central Research Laboratory, Cerrahpasa Medical Faculty, University of Istanbul, Istanbul, Turkey

\*\*Division of Rheumatology, Department of Medicine, Cerrahpasa Medical Faculty, University of Istanbul, Istanbul, Turkey

\*\*\*Division of Gastroenterology, Department of Medicine, Cerrahpasa Medical Faculty, University of Istanbul, Istanbul, Turkey

patient clinic of Cerrahpasa Medical Faculty in Istanbul<sup>16</sup>. We also studied 17 patients with UC and 21 patients with CD, who were followed by the gastroenterology outpatient clinic of the same hospital, as diseased control groups. Patients with UC and CD were all females. Thirty four (7 M/ 27 F) apparently healthy individuals, chosen among the hospital staff, constituted our healthy controls. All participants gave informed consent and the local ethic committee of Cerrahpasa Medical School approved the study.

Sera were collected by centrifugation of venous blood samples and stored at -20°C, until being analyzed. Serum levels of ASCA IgG and IgA were determined using a commercial Enzyme-linked immunosorbent assay (ELISA) kit (Euroimmun-Germany) and that of Anti-MPO and Anti-PR3 were determined by using a commercial ELISA kit (Trinity-USA). Immune fluorescence analysis (IFA) was used to assess the presence of ANCA antibodies. Cut-off values accepted according to the manufacturer's instructions were  $\geq 20$  U/ml for ASCA and  $\geq 0.91$  EU/ml for anti-MPO and anti-PR3, respectively.

### Statistical analysis

Comparisons of continuous variables between groups were made by one-way analysis of variance using the Bonferroni correction. The categorical variables were compared by the chi-square test or the Fisher exact test. All tests were performed using SPSS for Windows, version 13.0, software (SPSS Inc, Chicago, IL).

### Results

The mean age was similar between the study groups (TA:  $40 \pm 12$  years; CD:  $39 \pm 11$  years; UC:  $38 \pm 11$  years) and healthy controls ( $38 \pm 9$  years), ( $P = 0.890$ ). The mean disease duration of TA patients ( $9 \pm 5$  years) was significantly longer than that of patients with CD ( $4 \pm 5$  years) and UC ( $6 \pm 5$  years), ( $P < 0.001$ ).

Arterial involvement sites were subclavian artery (25/32; 78 %), common carotid artery (18/ 32; 56 %), abdominal aorta (12/ 32; 38 %), thoracic aorta (6/32; 19 %) and renal artery (4/ 32; 13 %). At the time of the study 15 (47 %) of the patients with TA had active disease, according to the criteria defined by Kerr et al<sup>1</sup> and 84 % (27/ 32) were under treatment with immunosuppressives (corticosteroids: 23, azathioprine: 14 and methothrexate: 11).

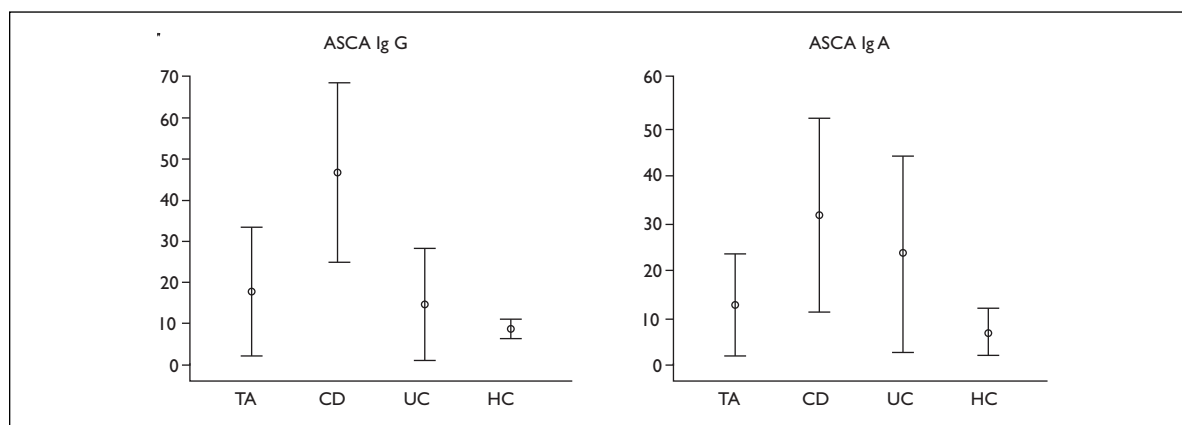
Two patients had concomitant CD, while one had UC. In all 3, the diagnosis of IBD preceded the diagnosis of TA (1, 3 and 5 years ago). All 3 were in remission for both diseases at the time of the study.

As shown in Table I and Figure 1, serum levels of ASCA (Ig G and Ig A) and the frequency of ASCA positivity (Ig G and Ig A) were almost twice increased among patients with TA, compared to that found among healthy controls, however, the difference was not statistically significant. ASCA Ig G and Ig A antibodies were present in 5 patients with TA (15.6 %), whereas, they were only present in 3 (8.8 %) healthy controls ( $P = 0.397$ ). Of the 5 patients with TA, who had positive ASCA Ig G and A antibodies, 2 (40 %) were active in terms of TA. Among 3 patients, in whom TA and IBD co-existed,

**Table I. The mean serum levels and positivity rates of ASCA and ANCA antibodies among patients and controls**

	<b>Takayasu arteritis (n=32)</b>	<b>Crohn's disease (n=21)</b>	<b>Ulcerative Colitis (n=17)</b>	<b>Healthy controls (n= 34)</b>	<b>P</b>
ASCA Ig G, U/ml	17.85 $\pm$ 43.10	46.81 $\pm$ 48.12	14.74 $\pm$ 26.41	8.73 $\pm$ 6.9	0.001*†‡
ASCA Ig A, U/ml	12.82 $\pm$ 32	31.81 $\pm$ 45.42	23.55 $\pm$ 40.38	6.91 $\pm$ 14.73	0.033‡
ASCA Ig G+, n (%)	4 (12.5%)	9 (42.9%)	2 (11.8%)	2 (5.9%)	0.003*†‡
ASCA Ig A+, n (%)	2 (6.3%)	6 (28.6%)	4 (23.5%)	1 (2.9%)	0.013‡
ASCA Ig G and A+, n (%)	5 (15.6%)	10 (47.6%)	6 (35.3%)	3 (8.8%)	0.004‡
p-ANCA +, n (%)	0	2 (10)	6 (35)	0	<0.001
c-ANCA +, n (%)	0	1	0	0	—
Anti-MPO, EU/ml	0.63 $\pm$ 0.24	0.67 $\pm$ 0.11	0.70 $\pm$ 0.11	0.61 $\pm$ 0.17	0.286
Anti-PR3, EU/ml	0.35 $\pm$ 0.11	0.35 $\pm$ 0.07	0.75 $\pm$ 0.95	0.39 $\pm$ 0.16	0.005§¶**

\*: CD vs TA; †: CD vs UC; ‡: CD vs Healthy controls; §: UC vs TA; ¶: UC vs CD; \*\*: UC vs Healthy controls



**Figure 1.** ASCA Ig G and A antibody levels within 95 % confidence intervals

TA: Takayasu arteritis (n = 32), CD: Crohn's disease (n = 21), UC: Ulcerative colitis (n = 17), Healthy controls (n = 34). Vertical bars represent means and 95% confidence intervals.

only one (one with CD) had positive ASCA Ig G and Ig A antibodies. Therefore, results remained the same, even after exclusion of patients, in whom TA and IBD co-existed. Serum levels of ASCA Ig G and A were increased only among patients with CD. ASCA Ig G antibodies were increased only among patients with CD, while ASCA Ig A antibodies were increased among both patients with CD and UC. Positivity for any ASCA (Ig G and Ig A) was increased significantly only among patients with CD.

P-ANCA antibodies were present only among patients with UC (35 %) and CD (10 %). Similarly, only patients with UC had significantly higher levels of anti-PR3. Mean anti-MPO levels did not differ between the study groups. C-ANCA antibodies were positive only in one patient with CD.

## Discussion

In this controlled study, we showed that the frequency of ASCA positivity in TA was similar to that found in UC and healthy controls. ANCA antibodies were not found in patients with TA. As expected, patients with CD had significantly higher levels of ASCA, while patients with UC had significantly increased frequency for p-ANCA positivity.

We are not aware of any published data with regard to ASCA prevalence in TA. The lack of reliable serologic markers in TA often causes difficulties in assessment of disease activity. Few of them are consistently elevated in TA. Anti-endothelial cell antibodies, IL-6, IL-18 and serum amyloid A may

be listed as such examples<sup>17-21</sup>. On the other hand, ANCA, ANA, anti-DNA, and anti-Ro antibodies were often reported to be negative among patients with TA<sup>21</sup>. Similarly, surrogate markers of disease activity; such as soluble vascular adhesion molecules, von Willebrand factor antigen, factor VII, thrombomodulin, and angiotensin converting enzyme were either not increased or were not helpful in monitoring disease activity<sup>22-23</sup>.

The percentage of ASCA positivity among our patients with CD and UC was in the previously reported range<sup>12-15</sup>. ASCA seems to be helpful in detecting patients with CD; nevertheless, its specificity for CD is low, since other diseases - such as intestinal Behçet's syndrome, autoimmune hepatitis and ankylosing spondylitis - also show moderately high ASCA positivity<sup>24-27</sup>.

There were 3 patients with IBD (9 %) in our TA group. A French study reported also a frequency of 9 % (4/ 44) for TA-CD co-existence<sup>2</sup>. Similar granulomatous histopathology and inflammatory pathways may suggest common etiopathogenesis<sup>2,10-11</sup>. More studies are needed to clarify this interesting association.

In conclusion, TA and IBD may co-exist. ASCA and ANCA antibodies do not seem to be useful in the diagnosis of patients with TA.

## Correspondence to

Emire Seyahi,  
Halaskargazi Cad. No: 209-211, Huzur Ap. D: 2,  
Sisli, Istanbul, Turkey, 34360  
Telephone: + 90-533-8184234 / Fax: +90-212-5890808  
E-mail: eseyahi@yahoo.com

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