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## Journal Pre-proofs

Anti-*Saccharomyces cerevisiae* antibodies in patients with COVID-19

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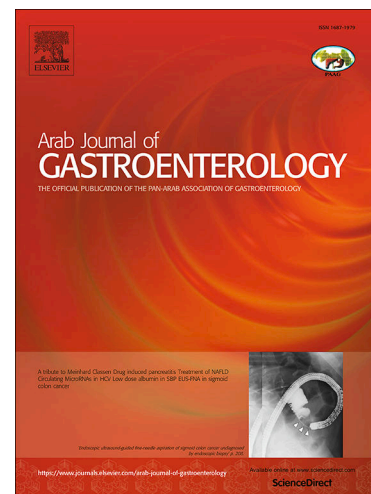
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**Anti-*Saccharomyces cerevisiae* antibodies in patients with COVID-19**

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**Running title:** ASCA and COVID-19

**Anti-*Saccharomyces cerevisiae* antibodies in patients with COVID-19**

**Abstract**

**Background and study aim:** Anti-*Saccharomyces cerevisiae* antibodies (ASCA) have been described in many autoimmune diseases (AIDs). Coronavirus disease 2019 (COVID-19) could trigger AIDs. This study aimed to determine the frequency of ASCA in patients with COVID-19.

**Patients and methods:** This study included 88 adult patients with severe COVID-19, 51 mild COVID-19, and 160 healthy blood donors. ASCA of isotype immunoglobulin (Ig)G and IgA were detected by enzyme-linked immunosorbent assay.

**Results:** The frequency of ASCA (IgG or IgA) was significantly higher in patients with severe COVID-19 (21.6% vs. 3.7%,  $p < 10^{-3}$ ) and in patients with mild COVID-19 than in the healthy controls (13.7% vs. 3.7%,  $p = 0.03$ ). ASCA-IgA was significantly more frequent in patients with severe COVID-19 than in healthy controls (15.9% vs. 0.6%,  $p < 10^{-3}$ ). ASCA-IgG was significantly more frequent in patients with mild COVID-19 than in healthy controls (13.7% vs. 3.1%,  $p = 0.02$ ). ASCA (IgG or IgA) were more frequent in severe than in mild COVID-19, but the difference was not statistically significant (21.6% vs. 13.7%). ASCA-IgA was significantly more frequent in patients with severe than those with mild COVID-19 (15.9% vs. 0%,  $p = 0.003$ ). The mean ASCA-IgG and ASCA-IgA levels were significantly higher in patients with severe COVID-19 than in healthy controls ( $5.8 \text{ U/mL} \pm 11.8$  vs.  $2.3 \text{ U/mL} \pm 2.8$ ,  $p < 10^{-3}$  and  $9.2 \text{ U/mL} \pm 21.5$  vs.  $3.4 \text{ U/mL} \pm 1.7$ , respectively,  $p < 10^{-3}$ ). The mean ASCA-IgG levels were significantly higher in patients with mild COVID-19 than in healthy controls ( $6.2 \text{ U/mL} \pm 12.9$  vs.  $2.3 \text{ U/mL} \pm 2.8$ ,  $p < 10^{-3}$ ). The mean ASCA-IgA levels were significantly higher in patients with severe than in those with mild COVID-19 ( $9.2 \text{ U/mL} \pm 21.5$  vs.  $2.6 \text{ U/mL} \pm 1.2$ ,  $p = 0.03$ ).

**Conclusion:** ASCA was more frequent in patients with COVID-19 than in healthy controls.

## KEY WORDS

Anti-*Saccharomyces cerevisiae* antibodies, severe COVID-19, mild COVID-19, adults

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## Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. COVID-19 is now recognized as a multisystem disease with a broad spectrum of manifestations [2-4]. The pathophysiology of COVID-19 remained unclear; however, much evidence supports the hypothesis that SARS-CoV2 could stimulate autoimmunity in predisposed patients. Histopathological signs of autoimmune reactions have been demonstrated in many organ systems of deceased patients from COVID-19. CD8 T lymphocyte-infiltrated lungs, adrenals, liver, intestine, and other organs confirm an autoimmune process [5]. SARS-CoV-2 can break immunological tolerance by molecular mimicry, standard activation, and epitope spreading and therefore, induce autoimmune diseases (AIDs) [6]. Moreover, the incidence of many AIDs has increased since the beginning of the COVID-19 pandemic [7-9].

*Saccharomyces cerevisiae* (*S. cerevisiae*) is a yeast that is used for bread baking and is one of the predominant yeast in our mycobiota [10]. Anti-*S. cerevisiae* antibodies (ASCA) are directed against the phosphopeptidomannan, a part of the cell wall of *S. cerevisiae*. ASCA is considered one of the serological markers of Crohn's disease [11-12]. Combined with perinuclear anti-neutrophil cytoplasmic antibodies, ASCA has been reported as a valuable marker to discriminate between Crohn's disease and ulcerative colitis [13]. Additionally, ASCA has been detected in many AIDs [14-19]. However, to our knowledge, only one study has determined ASCA in COVID-19 [20]. Therefore, the present study aimed to determine the frequency of ASCA in this viral disease, which could induce AIDs.

## Patients and methods

### Patients

We enrolled two groups of patients with a confirmed COVID-19 diagnosis by real-time polymerase chain reaction test (RT-PCR) of an oropharyngeal swab sample from December 2020 to February 2021. The first group consisted of 88 adult patients with severe COVID-19 and the second group was composed of 51 adults with mild COVID-19. Group classification was based on clinical symptoms, respiratory rate, and oxygen saturation. COVID-19 severity was categorized under the World Health Organization Clinical Progression Scale [21]. All sera samples in the first group were collected after hospital admission, whereas patients in the second group were not hospitalized and were given health care at home, and their sera

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samples were collected at least 14 days after RT-PCR. Medical criteria for hospital admission were severe dyspnea, oxygen saturation on room air of  $\leq 94\%$  or increase respiratory rate ( $\geq 30$  breaths per min), confusion or altered mental status or comorbid condition with worsening shortness of breath, fever, and/or increasing cough. Patients with Crohn's disease or autoimmune disease were excluded from our study.

Sera of 160 blood donors were included as normal controls. All sera were stored at  $-80^{\circ}\text{C}$  until use. The study was approved by the local ethics committee and all patients gave their informed consent.

### Laboratory measurements

ASCA immunoglobulin (Ig)G and IgA were detected by a commercial enzyme-linked immunosorbent assay (ELISA) kit (Orgentec Mainz<sup>®</sup> Germany). The antigen consisted of highly purified mannan from *S. cerevisiae*. Results were expressed as arbitrary units with a cut-off for positivity of 10 U/mL following the manufacturer's instructions.

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### Statistical analysis

The comparison of frequencies of ASCA was performed using Chi-square or Fisher's test. We used a parametric Student's t-test to compare the mean titer of ASCA. A Pearson correlation test was used to show the relationship between variables. A  $p$ -value of  $<0.05$  was considered significant.

### Results

**Table 1** shows the epidemiologic characteristics of patients and healthy controls.

### Frequency of ASCA in patients with COVID-19 and healthy controls

The frequency of ASCA (IgG or IgA) was significantly higher in patients with severe COVID-19 (21.6% vs. 3.7%,  $p < 10^{-3}$ ) and those with mild COVID-19 than in healthy controls (13.7% vs. 3.7%,  $p = 0.03$ ). ASCA-IgA were significantly more frequent in patients with severe COVID-19 than in healthy controls (15.9% vs. 0.6%,  $p < 10^{-3}$ ). ASCA-IgG were significantly more frequent in patients with mild COVID-19 than in healthy controls (13.7% vs. 3.1%,  $p = 0.01$ ) (**Table 2**).

### Comparison of the frequency of ASCA between patients with severe and mild COVID-19

ASCA (IgG or IgA) were more frequent in severe than in mild COVID-19, but the difference was not statistically significant (21.6% vs. 13.7%). ASCA-IgA was significantly more frequent in patients with severe than in mild COVID-19 (15.9% vs. 0%,  $p = 0.003$ ) (Table 2).

### Levels of ASCA

The mean ASCA-IgG levels were significantly higher in both patients with severe and mild COVID-19 than in healthy controls (5.8 U/ml  $\pm$  11.8 vs. 2.3 U/ml  $\pm$  2.8,  $p < 10^{-3}$ ; 6.2 U/ml  $\pm$  12.9 vs. 2.3 U/ml  $\pm$  2.8,  $p < 10^{-3}$  respectively). The difference in the mean ASCA-IgG levels between patients with severe and mild COVID-19 was not significant (5.8 U/ml  $\pm$  11.8 vs. 6.2 U/ml  $\pm$  12.9;  $p = 0.85$ ) (Figure 1).

The mean ASCA-IgA levels were significantly higher in patients with severe COVID-19 than in healthy controls (9.2 U/ml  $\pm$  21.5 vs. 3.4 U/ml  $\pm$  1.7,  $p < 10^{-3}$ ). The mean ASCA-IgA levels were significantly higher in healthy controls than in patients with mild COVID-19 (3.4 U/ml  $\pm$  1.7 vs. 2.6 U/ml  $\pm$  1.2,  $p = 0.002$ ). The mean ASCA-IgA levels were significantly higher in patients with severe COVID-19 than those with mild COVID-19 (9.2 U/ml  $\pm$  21.5 vs. 2.6 U/ml  $\pm$  1.2;  $p = 0.03$ ) (Figure 2).

### Association of ASCA with age, sex, and clinical characteristics in patients with severe and mild COVID-19

No correlation was detected between ASCA-IgG or ASCA-IgA and sex, age, or clinical characteristics (fever, cough, and fatigue) in patients with severe COVID-19. A positive correlation was detected between ASCA-IgA and age ( $r = 0.35$ ,  $p = 0.01$ ) and ASCA-IgG and sex ( $r = 0.3$ ,  $p = 0.03$ ) in patients with mild COVID-19. No correlation was detected between ASCA-IgG or ASCA-IgA and clinical characteristics in patients with mild COVID-19.

### Discussion

The present study demonstrated a significantly higher ASCA frequency in both patients with mild and severe COVID-19 than in healthy controls. These results confirm those of Sacchi et al. [20] who determined ASCA in a group of symptomatic and hospitalized patients with COVID-19. We found that IgA was the predominant isotype of ASCA in patients with severe COVID-19 (15.9% vs. 9%). ASCA-IgA was more frequent than ASCA-

IgG (25% vs. 17.5%) even in the study of Sacchi et al. [20]. However, in all AIDs which we previously detected in ASCA [14-19] and inflammatory bowel disease [22], IgG was the predominant isotype (**Table 3**). The discrepancy between AIDs and this severe viral infection could be because SARS-CoV-2-induced mucosal damage preferentially stimulates the IgA immune response [5]. Severe COVID-19 was associated with elevated serum IgA [23].

The frequency of ASCA-IgA in our patients with severe COVID-19 is lower than that of Sacchi et al. [20] (15.9% vs. 25%). The difference between these results could be explained by both the method used for ASCA assay and the number of patients studied. We used ELISA to determine ASCA and Sacchi et al. [20] used fluorescent enzyme immunoassay. Moreover, we included 88 patients in our study but Sacchi et al [20] had only 40 patients.

Only the IgG isotype of ASCA was detected in our patients with mild COVID-19 in contrast to those with severe COVID-19. This is because serum sampling has been done at the hospital admission for patients with severe COVID-19 and at least 14 days after RT-PCR analysis for those with mild COVID-19. IgA has been demonstrated to dominate the early neutralizing antibody response to SARS-CoV-2 [24], while IgG could be detected by day 14 after the onset of symptoms [25]. Additionally, severely SARS-Cov-2-infected patients sustained increased IgA SARS-CoV-2 antibodies compared with those with mild COVID-19 who demonstrated an immunodominant IgG [26]. The present study determined ASCA but not anti-SARS-CoV-2 antibodies; however, interestingly, cross-reactive epitopes exist between *S. cerevisiae* and SARS-CoV-2. The Nucleic Acid Binding Domain of SARS-CoV non-structural protein 3 (nsp3) particularly shows close similarity to the RNA-binding site of the sterile alpha motif of the *S. cerevisiae* Vts1p protein [27].

The present study revealed that ASCA were slightly more frequent in patients with severe COVID-19 than in those with mild COVID-19 (21.6% vs. 13.7%). We hypothesized that ASCA could neutralize *S. cerevisiae* when produced by the immune system, which is a beneficial yeast for our health [28, 29]. Beta-glucan, derived from *S. cerevisiae*, primes the immune system to respond better to any viral infection [30]. Additionally, the use of oral beta-glucan was hypothesized to boost immune responses and abrogate symptoms in COVID-19 [31]. Beta-glucan is a polysaccharide that is abundantly found in the cell wall of *S. cerevisiae* [31], and ASCA is directed against the peptidomannan of the cell wall of *S. cerevisiae*.

The presence of ASCA in patients with COVID-19 could be explained by three mechanisms. First, gut microbiota dysbiosis has been demonstrated in patients with COVID-



19 [32-34]. The consequent barrier dysfunction [35] will allow *S. cerevisiae* to reach the intestinal mucosa. The immune mucosa is therefore stimulated and ASCA is synthesized. Second, during COVID-19, the lectin pathway of complement activation is triggered by the binding of mannose-binding lectin (MBL) with SARS-CoV spike protein [36]. Elevated MBL plasma levels have been reported in patients with COVID-19, and it is associated with thrombosis and coagulopathy in patients with severe COVID-19 [37]. Furthermore, the transmembrane spike glycoprotein of SARS-CoV-2 is covered with N-linked glycan having oligomannose and complex sugars [38]. Thus, could we explain the high frequency of ASCA in patients with COVID-19 by an eventual molecular mimicry between mannan of the yeast cell surface of *S. cerevisiae* and mannan of MBL or mannose of SARS-CoV-2? Fascinatingly, plasma MBL levels are also high in AIDs, in which we have previously detected ASCA, particularly T1D, celiac disease, primary biliary cholangitis, and Grave's disease [18]. Third, a common epitope between *S. cerevisiae* and *Candida albicans* has been demonstrated [39]. Furthermore, a high factor for candidemia was found in patients with severe COVID-19 [40]. Therefore, a high-risk factor having ASCA in patients with severe COVID-19 could also be explained by the high incidence of candidemia among these patients.

ASCA could explain some extrapulmonary manifestations of COVID-19. *S. cerevisiae* has structural similarities with many human autoantigens [41]. Remarkably, SARS-CoV-2 has also cross-reactive epitopes with many human proteins [5, 42]. Thus, both ASCA and anti-SARS-CoV-2 antibodies could bind to self-antigens and initiate a complement activation, which has a multiorgan impact on COVID-19 [36]. Cross-reactive epitopes between SARS-CoV and *S. cerevisiae* have been reported [27]. Hence, both anti-SARS-CoV-2 antibodies and ASCA could bind to SARS-CoV-2 or *S. cerevisiae* which translocates from the gut to other organs via the vascular compartment because of a leaky intestinal wall [35] secondary to gut microbiota dysbiosis.

In conclusion, we have demonstrated a higher frequency of ASCA in patients with COVID-19 and revealed that IgA was the predominant isotype of ASCA in patients with severe COVID-19 while only IgG isotype of ASCA was detected in patients with mild COVID-19.

#### **Author's contribution**

SM, AM, MJ, MG, and IG contributed to the study concept and design. SM, AM, MJ, ABC, ABA, KA, NG, MD, WB, MB, SM, and WN contributed to the acquisition of data. SM, AM, MG, ABA, KA, NG, MD, WB, MB, SM, WN, and IG contributed to the analysis and interpretation of data. SM, AM, MJ, MG, and IG contributed to the drafting of the manuscript. SM, AM, MG, ABA, KA, NG, MD, WB, MB, SM, WN, and IG contributed to the critical revision of the manuscript for important intellectual content. All authors identified above approved the final version of this paper, including the authorship statement.

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#### **Conflict of interest**

None of the authors have conflicts of interest to declare.

#### **Ethical approval**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

#### **Informed consent**

Informed consent was obtained from all individual participants included in the study.

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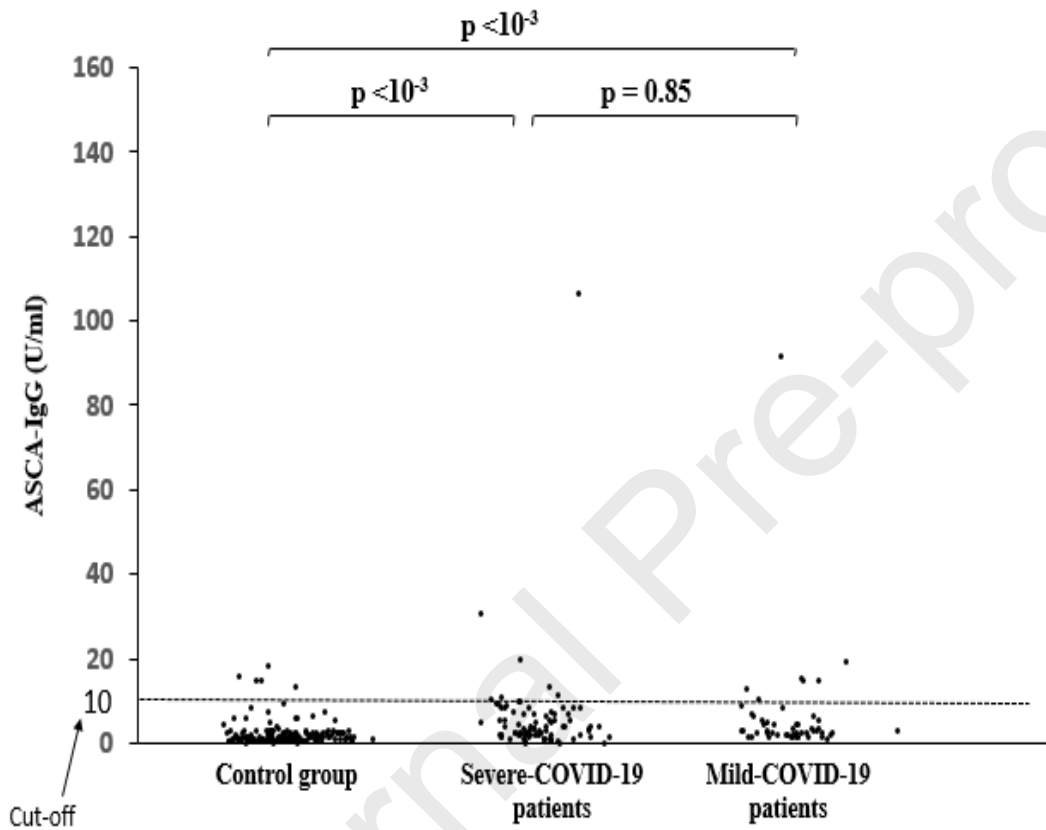
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**Figure 1:** ASCA IgG levels in patients with severe and with mild COVID-19 and healthy

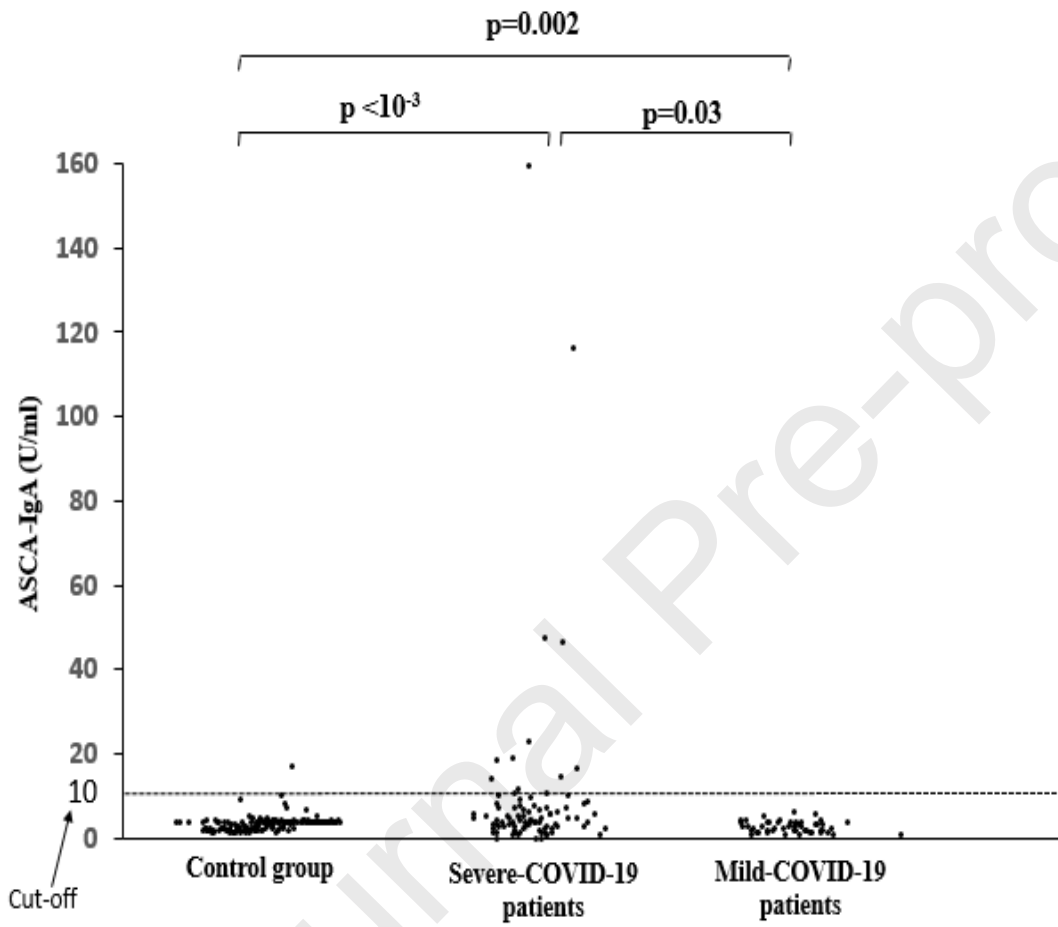
**Commented [Editor6]:** Page: 13

Remark: Please ensure to add a space before and after mathematical operators in all figures. Additionally, change the hyphen symbol to minus sign when indicating negative values.



controls.

ASCA: anti-*Saccharomyces cerevisiae* antibodies



**Figure 2:** ASCA IgA levels in patients with severe and mild COVID-19 and healthy controls.

ASCA: anti-*Saccharomyces cerevisiae* antibodies



**Table 1.** Epidemiologic features of patients with COVID-19 and healthy subjects

	Severe-COVID-19 (n = 88)	Mild-COVID-19 (n = 51)	Control group (n = 160)	<i>p</i> Severe-COVID-19/mild-COVID-19
<b>Sex-ratio (M/F)</b>	1.1 (46/42)	0.2 (9/42)	4 (128/32)	<10 <sup>-3</sup>
<b>Median age (IQR) (Years)</b>	62 (55–70)	34 (22–44)	20	NS
<b>Age range (years)</b>	21–82	19–58	17–45	-

M: male; F: female; IQR: interquartile range

**Commented [Editor7]:** Tip: **En Dash** : The en dash is used in place of a hyphen in cases where the paired elements carry equal weight or represent a parallel relationship such as Carbon–Magnesium bond or Bose–Einstein statistics. Page: 15

**Table 2.** Frequency of ASCA in patients with COVID-19 and healthy controls

	Severe-COVID-19 (n = 88) % (n)	Mild-COVID-19 (n = 51) % (n)	Control group (n = 160) % (n)	p Severe-COVID-19/ control group	p Mild-COVID-19/ control group	p Severe- COVID- 19/mild- COVID-19
ASCA IgG or IgA	21.6 (19)	13.7 (7)	3.7 (6)	<10 <sup>-3</sup>	<b>0.03</b>	NS
ASCA IgG and IgA	3.4 (3)	0	0 (0)	NS	NS	NS
ASCA IgG	9 (8)	13.7 (7)	3.1 (5)	NS	<b>0.02</b>	NS
ASCA IgA	15.9 (14)	0	0.6 (1)	<10 <sup>-3</sup>	NS	<b>0.003</b>

ASCA: anti-*Saccharomyces cerevisiae* antibodies; NS: not significant

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Tip: **Minus:** When presenting negative values a minus sign is used instead of a hyphen.

**Table 3.** Frequency of ASCA in our previous studies on autoimmune diseases and inflammatory bowel disease

<b>Authors</b>	<b>Autoimmune diseases</b>	<b>ASCA IgG (%)</b>	<b>ASCA IgA (%)</b>	<b><i>p</i></b>
<b>Toumi D et al. 2007 (14)</b>	Celiac disease	24.8	8.8	<b>&lt;10<sup>-3</sup></b>
<b>Sakly W et al. 2008 (15)</b>	Primary biliary cholangitis	18.9	11.6	NS
<b>Sakly W et al. 2010 (16)</b>	Type 1 diabetes	21	9.8	<b>&lt;0.002</b>
<b>Mankaï A et al. 2013 (17)</b>	Systemic lupus erythematosus	29.3	12.1	<b>0.001</b>
<b>Mankaï A et al. 2013 (18)</b>	Graves' disease	11.8	0.8	<b>0.001</b>
<b>Mankaï et al. 2016 (19)</b>	Antiphospholipid syndrome	15.6	7.8	<b>0.04</b>
<b>Wang et al. 2017 (22)</b>	Crohn's disease	52.1	33.8	<b>0.03</b>
<b>Wang et al. 2017 (22)</b>	Ulcerative colitis	31.7	17.1	NS

ASCA: anti-*Saccharomyces cerevisiae* antibodies; NS: not significant