Prevalence and significance of anti-*saccharomyces cerevisiae* antibodies in primary Sjögren's syndrome

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Received on February 21, 2017; accepted in revised form on April 3, 2017.

Clin Exp Rheumatol 2018; 36 (Suppl. 112): S73-S79.

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Key words: Sjögren's syndrome, antibodies anti-Saccharomyces cerevisiae, anti-Ro52/SSA, anti-Ro60/SSB, Th17 cells

Competing interests: none declared.

ABSTRACT

Objective. *Saccharomyces cerevisiae is a common yeast used in the food industry. IgG and IgA antibodies against the phosphopeptidomannan of the S. cerevisiae cell wall (ASCA) are a well known marker of disease severity in Crohn's disease. Moreover, a number of studies assessed ASCA in several systemic and organ-specific autoimmune diseases postulating molecular mimicry as a possible link between ASCA and autoimmunity. However, since they have never been tested in primary Sjögren's syndrome (pSS), the purpose of this study was to investigate these antibodies in a large cohort of pSS patients, compared to healthy donors (HD), and their significance as potentially helpful biomarker in a clinical setting.*

Methods. *ASCA IgG+IgA were assessed with ASCA screen dot for Blue Diver instrument (Alphadia sa/nv, Belgium). The comparison between the aminoacid sequence of mannan of S. cerevisiae and well characterised auto-antigens peculiar to pSS (52kD and 60kD Ro/SSA, La/SSB) was performed with the Basic Local Alignment Search Tool (BLAST).*

Results. *The prevalence of ASCA in our pSS cohort was 4.8%. We also reported that the ASCA target protein has a high similarity with Ro60/SSA protein further supporting the molecular mimicry hypothesis. Finally, we observed that ASCA positivity is associated with pSS specific clinical and serological features. ASCA$^+$ pSS patients displayed a triple combination of circulating anti-Ro52/SSA, anti-Ro60/SSA and anti-La/SSB antibodies, associated with low complement and cutaneous involvement.*

Conclusion. *Our data suggest a possible pathogenic/prognostic significance of ASCA in pSS.*

Introduction

Saccharomyces cerevisiae is a common yeast used in the food industry. Recently, antibodies against the phosphopeptidomannan, part of the cell wall of *S. cerevisiae* (ASCA), have been assessed in several systemic and organ-specific autoimmune diseases (ADs) (1). Although the pathogenic significance of ASCA is not yet fully understood, the molecular mimicry of self-antigens in several associated ADs has been suggested as a putative mechanism (1-2).

ASCA IgG are a well established biomarker of Crohn's disease (CD), being detectable in 60–70% of patients. In particular, ASCA are more prevalent in adult-onset CD and appear to be linked to a more severe disease. However, their titre remains stable overtime independently of pharmacological treatment (3). ASCA IgG can also be found in patients with ulcerative colitis (UC) but with a lower prevalence, about 10–15% (4). Conversely, ASCA IgA display a higher specificity, but a lower sensitivity, for inflammatory bowel diseases (IBD).

In recent years, the assessment of ASCA has gained growing interest in the rheumatology community in light of the higher prevalence of spondyloarthritis (SpA) in patients with CD and UC and of the lack of reliable biomarkers for SpA. In particular, studies aimed to investigate ASCA in SpA patients showed a higher prevalence of these autoantibodies, both IgG and IgA isotypes, when compared to healthy controls (5-11). Recently, Maillet *et al.* found an association between ASCA positivity in SpA patients and a peculiar clinical phenotype characterised by peripheral arthritis and uveitis (12). A higher prevalence of ASCA has been also observed in patients with rheumatoid arthritis (9-13), systemic lupus

erythematosus (SLE) (14-15), primary antiphospholipid syndrome (16), Behçet's disease (17), autoimmune thyroid diseases (ATDs) (18-19), coeliac disease (20-21), autoimmune hepatitis (22), primary biliary cirrhosis (23-24), primary sclerosing cholangitis (PSC) (24) and type 1 diabetes (25). However, the significance of ASCA in these conditions is still a matter of debate. An intriguing hypothesis may be that exposure to *S. cerevisia*e mannan, according culinary culture, is an environmental trigger that induces autoimmunity in genetically predisposing individuals. This hypothesis appears to be supported also by the evidence of geographic patterns of ASCA distribution (26).

Sjögren's syndrome is a chronic, slowly progressive autoimmune disorder affecting exocrine glands and leading to the sicca complex, a combination of dry eyes (keratoconjunctivitis sicca) and dry mouth (xerostomia) (27, 28). The disease may occur in a primary form, or in a secondary form complicating other autoimmune conditions. Primary Sjögren's syndrome (pSS) is one of the most prevalent autoimmune disorders (29), and the clinical spectrum varies from a benign autoimmune exocrinopathy to a heterogeneous potentially life-threatening systemic disorder, with an increased risk for non-Hodgkin's lymphoma development. Although the presence, and sometimes predominance, of T cells in salivary gland infiltrates underscores their contribution to the pathogenesis of pSS (30-32), growing evidence suggests a central role of B cells in the development of the disease (33-34). Saprophytic microbial flora is likely to maintain the delicate T helper (h) 17-T regulatory (Treg) cell balance in gut-associated lymphoid tissue. Furthermore, data concerning the effect that dietary intake of *S. cerevisiae* may exert on Th17-Treg cell balance are lacking. Currently, abnormalities of Th17/Treg cell balance is deemed to play a role in the development and relapse of many autoimmune diseases, including pSS (32, 35-36) where we recently reported a pathogenic role exerted by a peculiar interleukin (IL)-17-producing T-cell subset lacking the expression of CD4 and CD8 on the cell surface (double negative, DN) (30-31).

To the best of our knowledge, although ASCA have been assessed in several systemic and organ specific ADs, they have never been tested in pSS. The purpose of this study was to investigate these antibodies in a large cohort of pSS patients, compared to healthy donors (HD), and their significance as potentially helpful biomarker in a clinical setting.

Materials and methods

Study cohort and ASCA assessment

One hundred and four patients with pSS according to the American European Consensus criteria were enrolled (37). None of the patients had personal of familiar history of IBD or other autoimmune conditions that could account for ASCA positivity (*e.g.* autoimmune liver diseases, ATDs). Thirty sex- and age-matched healthy subjects acted as controls. Serum samples were collected and stored at -20°C until use. ASCA IgG+IgA were assessed with ASCA screen dot for Blue Diver instrument (Alphadia sa/nv, Belgium). Briefly, the test is based on the principle of an enzyme immunoassay. The test strips are composed of a membrane fixed on a specific plastic support. During the automated test procedure, the BlueDiver instrument sequentially incubates the strips in the wells of ready-to-use reagent cartridges. The strips are first incubated with patients' sera and ASCA, if present, bind to the corresponding specific antigen on the membrane. Upon further incubation with alkaline phosphatase goat antibodies against human IgG+IgA, the enzyme conjugate binds to the antigen-antibody complexes. The strips are finally incubated with a substrate solution. Enzyme activity, if present, leads to the development of purple dots on the membrane pads and the intensity of the colouration is directly proportional to the amount of ASCA present in the sample. Values over 10 AU were considered positive according to manufacturer instructions. Each strip also has a positive and negative internal control.

Flow cytometry and ELISA

Serum and heparinised venous blood samples were obtained from the 5 $ASCA^+$ patients and 30 randomly selected $ASCA^-$ patients. Peripheral blood mononuclear cells were isolated by gradient separation and cells were stimulated in *vitro* with 25 ng/ml phorbol myristate acetate (Sigma Aldrich, St Louis, MO, USA), 1 mg/ml ionomicyn and 0.1 mg/ml brefeldin in complete medium (Roswell Park Memorial Institute 1640, 10% foetal calf serum, 2 mM L-glutamine, 100 U penicillin and 100 mg/ml streptomycin) for 6 h at 37°C. For surface staining, phycoerythrin (Pe), fluoresceine isothiocianate and Pe-Cy7-labelled anti-human CD3, CD4, CD8, CD25 antibodies and respective isotypes were used (BD, San Jose, CA, USA). Subsequently, cells were permeabilised with 0.1% saponin blocking buffer and AlexaFluor-647 labelled anti-human IL- 17 Ab and its isotype were employed for intracellular staining (BD). When required, cells were permeabilised with commercially available FoxP3 staining buffer set (BD) and AlexaFluor-647 labelled anti-human FoxP3 and the respective isotype were used for intracellular staining.

Up to four different fluorochromes were used in the same vial and debris were excluded by back-gating to $CD3^+$ T cells in FSC/SSC plots. Samples were analysed using FACScalibur flow cytometer and CellQuestPro™ software (BD). IL-17 concentration was assessed with commercially available ELISA kit (R&D Systems Minneapolis, MN, USA) in serum samples.

Database search methods

To compare the aminoacid sequence of mannan of *S. cerevisiae* and well characterised auto-antigens peculiar to pSS (52kD and 60kD Ro/SSA, La/SSB) we browsed the protein database of the National Center for Biotechnology Information (NCBI) and run the Basic Local Alignment Search Tool (BLAST). We selected the alignment with highest identity/positivity and lowest expect (E) value. The E value represents the number of different alignments with scores equivalent to or better than is expected to occur in a database search by chance. Therefore, low E values account for more significant alignments. E values for our results ranged between

0.29 and 9.90 showing therefore a high reliability index.

Statistical analysis
Data were analysed with SPSS 21.0 software. Chi square, Mann Whitney U-test and binary logistic regression analysis were calculated and *p*-values less than 0.05 were considered significant.

Results

ASCA prevalence in pSS and clinical/serological features of ASCA-positive (ASCA+) pSS patients
ASCA IgG+IgA were detected in 5 out of 104 pSS patients (Table I) therefore the prevalence in our cohort was 4.8%. None of the ASCA^{positive} (ASCA^{+}) pSS patients displayed IBD or other autoimmune conditions that could account for ASCA positivity. The median value of ASCA in pSS patients was 12 arbitrary units (AU) (range 12–19). In our cohort, as none of the 30 HD displayed ASCA IgG+IgA, the specificity of the test resulted 100%, despite an extremely low sensitivity. Therefore, the positive predictive value was 100% and the negative predictive value was 23%.
In order to identify possible differences in serological and/or clinical picture, we subdivided our patients according to ASCA positivity. As displayed in Table I, among ASCA^{+} patients, a reduction of C3 and C4 complement fractions resulted significantly more prevalent compared to ASCA^{negative} (ASCA^{-}) patients (both $p<0.05$). Interestingly, all ASCA^{+} patients, but only 39% of ASCA^{-} patients, displayed anti-Ro52/SSA, anti-Ro60/SSA and anti-La/SSB autoantibodies *together* (p=0.01). Conversely, no differences between ASCA^{+} and ASCA^{-} patients could be observed regarding prevalence of other serological features such as rheumatoid factor, leukopenia, hypergammaglobulinaemia or demographic data such as age, age at diagnosis, disease duration. As far as the clinical picture was concerned, ASCA^{+} pSS patients displayed more frequently pulmonary, articular and cutaneous involvement, as proven by the presence of purpura, when compared to ASCA^{-} pSS patients (p=0.04, p=0.01 and p=0.05 respectively). Of interest, binary logistic regression revealed that ASCA^{+} pSS patients display an odds ratio of 14 (95% CI=2.1–97.4; p=0.006) to have cutaneous manifestations of pSS. On the contrary, other extraglandular manifestations, low C3 and low C4 did not result significantly associated with ASCA at binary logistic regression analysis. Finally, in a subgroup of our patient cohort, we analysed circulating Th17, DN and Treg cells as well as serum levels of IL-17, but we failed to observe any differences between ASCA^{+} and ASCA^{-} pSS patients.

Table I. Demographic, clinical and serological features of pSS patient cohort according to ASCA status.

	ASCA^{+} (n°5)		ASCA^{-} (n°99)		*p*-value
	n	%	n	%	
Female gender	5	100	99	100	ns
Xerostomia	3	60	80	80	ns
Xerophtalmia	4	80	82	82	ns
Parotid gland swelling	1	20	38	38	ns
Extraglandular manifestations (overall)	5	100	68	68	ns
Arthritis	0	0	51	51	0.05
Raynaud's phenomenon	1	20	22	22	ns
Pulmonary involvement	2	40	6	6	0.04
Cutaneous involvement	4	80	16	16	0.005
Kidney involvement	0	0	1	1	ns
PNS and or CNS involvement	1	20	5	5	ns
Gastrointestinal involvement	0	0	6	6	ns
Cardiac involvement	0	0	5	5	ns
Myositis	0	0	1	1	ns
Lymphoadenopathy	1	20	17	17	ns
Lymphoma	0	0	4	4	ns
Reumatoid Factor	3	60	66	66	ns
Neither Anti-Ro/SSA nor Anti-La/SSB	0	0	32	32.3	ns
Anti-Ro52/SSA only	0	0	8	8.1	ns
Anti-Ro60/SSA only	0	0	0	0	ns
Anti-Ro52/SSA and anti-Ro60/SSA	0	0	9	9.1	ns
Anti-Ro52/SSA and anti-La/SSB	0	0	11	11.1	ns
Anti-Ro60/SSA and anti-La/SSB	0	0	0	0	ns
Anti-Ro52/SSA, anti-Ro60/SSA and anti-La/SSB	5	100	39	39.4	0.01
Low C3	3	60	15	15	0.03
Low C4	3	60	11	11	0.02
Leukopenia	3	60	31	31	ns
Hypergammaglobulinaemia	2	40	53	53	ns
Current smoker	2	40	13	13	ns
Past smoker	0	0	18	18	ns

pSS: primary Sjögren's syndrome; ASCA; anti-S. cerevisiae autoantibody; ASCA+; ASCA^{positive} patient; ASCA-: ASCA^{negative} patient; n: number of patients; PNS: peripheral nervous system; CNS: central nervous system.

Table II. Similarity between pSS-associated autoantigens and *S. cerevisiae* mannan.

Antigens (Homo sapiens)	Comparison to mannan, accession n° EDV13046.1		
	Identities	Positives	E value
52kD Ro/SSA ribonucleoprotein	11/26 (42%)	13/26 (50%)	0.29
60kD Ro/SSA ribonucleoprotein	7/11 (64%)	8/11 (72%)	2.2
La/SSB ribonucleoprotein	6/13 (46%)	9/13 (69%)	1.5

E value: identity/positivity expect value.

Comparison of the mannan from S. cerevisiae wall and pSS autoantigens
Since one putative mechanism to link ASCA and ADs may be molecular mimicry of *S. cerevisiae* mannan and autoantigens, Rinaldi *et al.* recently performed a comparison of the structure of this mannan and a number of autoantigens known to trigger an aber-

Table III. Performance of ASCA assessment in autoimmune disease.

Disease	Pts n°	HD n°	Assay type	ASCA IgG, n° (%)	Sp %	Se %	ASCA IgA, n° (%)	Sp %	Se %	ASCA IgG or IgA (%)	Ref.
Ankylosing spondylitis	43	78	commercial ELISA	5 (11.6)	89.7	11.6	10 (23.2)	91	23.2	nc	Riente L, 2004 l (9)
Ankylosing spondylitis	79	79	commercial ELISA	3 (3.8)	98.7	3.8	1 (1.3)	100	1.26	nc	Mundwiller ML, 2009 (7)
Ankylosing spondylitis	52	0	commercial ELISA	4 (8)	nc	nc	10 (19)	nc	nc	nc	de Vries M, 2010 (5)
Ankylosing spondylitis	107	103	commercial ELISA	19 (10.9)	94.2	17.8	36 (20.6)	94.2	33.6	47 (26.9)	Aydin SZ, 2008 (6)
Antiphospholipid syndrome	155	40	commercial ELISA	nc	nc	nc	nc	nc	nc	31 (20%)	Krause I, 2007 (16)
Autoimmune hepatitis	67	19	commercial ELISA	11 (16.4)	100	16.4	8 (12)	94.7	11.9	12 (18)	Muratori P, 2003l (24)
Behçet's disease	27	10	commercial ELISA	nc	nc	nc	nc	nc	nc	13 (48.1)	Krause I, 2002 (17)
Graves' disease	24	103	commercial ELISA	3 (12.5)	94	12,5	4 (16.6)	94	16.6	nc	Yazici D, 2010 (18)
Graves' disease	119	160	commercial ELISA	14 (11.8)	98	11,8	1 (0.8)	97	0.9	14 (11.8%)	Mankai A, 2013 (19)
Hashimoto thyroiditis	88	103	commercial ELISA	7 (8)	94	8	12 (13.6)	94	12.6	nc	Yazici D, 2010 (18)
Hashimoto thyroiditis	78	160	commercial ELISA	3 (3.8)	97	3.8	2 (2.6)	97	2.6	5 (6.4)	Mankai A, 2013 (19)
Primary biliary cirrhosis	95	80	commercial ELISA	18 (18.9)	97.5	19	11 (11.6)	98.7	11.5	23 (24.2)	Sakly W, 2008 (23)
Primary biliary cirrhosis	123	19	commercial ELISA	13 (10.6)	100	11.2	23 (18.7)	94.7	18.7	28 (22.8)	Muratori P, 2003 (24)
Primary sclerosing cholangitis	25	19	commercial ELISA	7 (28)	100	28	8 (32)	94.7	28	11 (44)	Muratori P, 2003 (24)
Psoriatic arthritis	75	78	commercial ELISA	4 (5.3)	89.7	5.3	7 (9.3)	91	9.3	nc	Riente L, 2004 (9)
Rheumatoid arthritis	30	152	in-house ELISA	6 (20)	91.4	20	12 (40)	94.7	40	nc	Dai H, 2009 (13)
Rheumatoid arthritis	79	78	commercial ELISA	8 (10.1)	89.7	10.1	14 (17.7)	91	17.7	nc	Riente L, 2004 (9)
Spondyloarthritis	235	0	indirect immunofluorescence	23 (9.8)	nc	nc	51 (21.7)	nc	nc	59 (25.5)	Maillet J, 2016 (12)
Systemic lupus erythematosus	40	152	in-house ELISA	13 (32.5)	91.4	57.5	8 (20)	94.5	7.5	nc	Dai H, 2009 (14)
Systemic lupus erythematosus	116	160	commercial ELISA	34 (29.3)	97	29.3	14 (12)	99.4	12	37 (31.9)	Mankai A, 2013 (15)
Type 1 diabetes	224	157	commercial ELISA	47 (21)	98	21	22 (9.8)	98.7	9.8	55 (24.5)	Sakly W, 2010 (25)
Undifferentiated spondyloartritis	47	103	commercial ELISA	6 (12.8)	94.2	12.8	9 (19.1)	94.2	19	11 (23.4)	Aydin SZ, 2008 (6)

ASCA: anti-S. cerevisiae autoantibody; Pts: patients; HD: healthy donors; Sp: specificity; Se: sensibility; Ref: reference; nc: not calculated.

rant autoimmune response in patients with ADs. They observed a certain degree of similarity between Ro/SSA and *S. cerevisiae* mannan (identities 5/10, 50%; positives 6/10, 60%) (1).
Since two proteins of 60 and 52 kilodalton (kD) encoded by two different genes have been described as targets for anti-Ro/SSA antibodies (38), in our study we assessed the similarity of each of them with *S. cerevisiae* mannan separately. As shown in Table II, the similarity of the 60kD Ro/SSA ribonucleoprotein was higher than that of both 52kD Ro/SSA and La/SSB autoantigens when compared to *S. cerevisiae* mannan (identities 7/11, 64%; positives 8/11, 72%, E value 2.2.).

Discussion

In recent years, the possible link between ASCA and autoimmunity has been extensively investigated (1). Here we reported for the first time the prevalence of ASCA in pSS using a highly specific assay. The majority of previous studies assessed ASCA in ADs using ELISA kits, and the studies that observed the highest IgG and IgA ASCA prevalence values, 57.5% in SLE and 40% respectively, were published by the same group using an in-house ELISA kit (13-14). However, the specificity of such assay is consistently lower (91.4%) when compared to commercially available ELISA kits (range: 94–100%). Indeed, when the latter where employed, the prevalence of ASCA IgG ranged between 3.8% in Hashimoto thyroiditis (19) and 29.3 in SLE (15) and the prevalence of ASCA IgA ranged between 0.8% in Graves' disease (19) and 32% in PSC (24) (Table III)
Variability in specificity and, in particular, sensitivity of the available ELISA kits may account for the large difference of ASCA prevalence in different studies focused on the same disease. This variability further complicates the understanding of the meaning of ASCA positivity in ADs. In addition, in the case of concurrent presence of more than one AD, it is difficult to understand which of them may account for ASCA positivity. In this regard, most of the aforementioned studies did not consider that ASCA may be an early marker of coeliac disease (38) while we excluded from our study patients with previous diagnosis of coeliac disease or currently undergoing diagnostic procedures for clinical suspicion of coeliac disease.
On this basis, and keeping in mind that the main purpose of assessing ASCA in patients with ADs is to find a possible pathogenic/prognostic, rather than diagnostic, role, we should ensure to identify only the true ASCA+ patients diagnosed with a single AD and employing a highly specific assay.
In our cohort, we observed for the first time that ASCA positivity seems to identify a peculiar pSS phenotype characterised by anti-Ro52/SSA, anti-Ro60/SSA and anti-La/SSB antibodies, low complement and cutaneous involvement. Since one putative mechanism to link ASCA and ADs may be molecular mimicry of *S. cerevisiae* mannan and autoantigens, Rinaldi *et al.* recently performed a comparison of the structure of this mannan and a number of autoantigens known to trigger an aberrant autoimmune response in patients with ADs (1). In detail, the autoantigen U2 snRNP B″ was found to conserve a superfamily protein domain that shares 83% of the *S. cerevisiae* mannan sequence. As far as anti-Ro/SSA was concerned, they observed a certain degree of similarity between Ro/SSA and *S. cerevisiae* mannan. However, this similarity was much lower than that for other autoantigens such as U2 snRNP B″ and comparable to that of anti-La/SSB. The similarity that we observed between *S. cerevisiae* mannan and 60kD Ro/SSA ribonucleoprotein was higher than that of both 52kD Ro/SSA and La/SSB autoantigens w. This high number of identities and positive substitutions appear to suggest that: i. ASCA may bind autoantigens, including anti-Ro/SSA, as already postulated by Rinaldi *et al.* (1); ii. ASCA may bind more likely the Ro60/SSA autoantigen rather than the Ro52/SSA or La/SSB autoantigens. Interestingly, in our patient cohort, all ASCA+ subjects were autoreactive against Ro52/SSA, Ro60/SSA and La/SSB while among ASCA- patients only 39% displayed the three autoantibodies together.
There is general agreement that while isolated anti-Ro52/SSA antibodies are independently associated with pSS, isolated anti-Ro60/SSB are independently associated with SLE (39-43). In addition, anti-Ro52/SSA positivity, either isolated or with anti-Ro60/SSA positivity, allows to identify a specific subset of patients with more aggressive pSS (44-45). Conversely, anti-Ro60/SSA antibodies correlate positively with hypocomplementaemia in SLE and seem to be more prevalent than anti-Ro52/SSA in cutaneous lupus (46). Taking together the high similarity between *S. cerevisiae* mannan and Ro60/SSA as well as the association of both Ro60/SSA with cutaneous lupus and ASCA with cutaneous manifestations in pSS, it is tempting to speculate a possible cross-reaction of ASCA against Ro60/SSA autoantigen possibly participating in the development of skin manifestations of the disease. Studies are ongoing to try to clarify this hypothesis.
In addition, a recent study demonstrated cross-reactivity between *S. cerevisiae* mannan and epitopes on β2 glicoprotein (GP) 1 (16) and anti-phospholipid antibodies without clinical features of APS have been described in pSS (47). However, none of the ASCA+ pSS patients in our cohort displayed anti-phospholipid antibodies. Interestingly, in the cohort evaluated by Fauchais *et al.,* pSS patients with anti-phospholipid antibodies had a higher prevalence of associated autoimmune diseases such as thyroiditis and primary biliary cirrhosis. Therefore, although these observations about cross reactivity of mannan and β2GP1 are interesting, definitive conclusion cannot be drawn yet.
Commensals such as Saccharomyces are required for nutrition, proper development of Peyer's aggregated lymphoid tissue, and tissue healing. However, it is possible that in the pro-inflammatory pathological microenvironment that characterises many immune-mediated diseases, such as pSS, the alteration of the finely regulated interaction between antigen presenting cells, the non-classically pathogenic microbiota, and Th17-Treg cells in the gut could trigger autoimmunity (48). Moreover, the evidence that patients with gastrointestinal diseases such as CD and coeliac disease

display cutaneous manifestations supports the association between gut alterations and skin disorders (49).

Finally, an altered recruitment and/or function of Treg cells can be an important pathogenic factor in skin diseases, although the exact mechanisms are still unknown (50). However, data concerning the effect that dietary intake of *S. cerevisiae* may exert on Th17-Treg cell balance are lacking and we failed to observe any differences between ASCA$^+$ and ASCA$^-$ pSS patients, regarding circulating Th17, DN and Treg cells as well as serum levels of IL-17.

The majority of studies assessing the role of the microbiome in pSS focused on the effect of hyposalivation on the oral microflora. In particular, hyposalivation in patients with pSS has been associated with a higher number of Candida species on mucosal surfaces (51). Interestingly, infection by *C. albicans* can induce the production of ASCA in humans, while Saccharomyces does not lead to the production of antibodies to *C. albicans*. Moreover, *C. albicans* is an immunogen for ASCA markers of CD (52). Therefore, it would be interesting to clarify whether there is an association between the presence of oral *C. albicans* and ASCA development in pSS.

There is general agreement that while isolated anti-Ro52/SSA antibodies are independently associated with pSS, isolated anti-Ro60/SSB are independently associated with SLE (43-46). In addition, anti-Ro52/SSA positivity, either isolated or with anti-Ro60/SSA positivity, allows to identify a specific subset of patients with more aggressive pSS (43). Conversely, anti-Ro60/SSA antibodies correlate positively with hypocomplementaemia in SLE and seem to be more prevalent than anti-Ro52/SSA in cutaneous lupus (46). Taking together the high similarity between *S. cerevisiae* mannan and Ro60/SSA as well as the association of both Ro60/SSA with cutaneous lupus and ASCA with cutaneous manifestations in pSS, it is tempting to speculate a possible cross-reaction of ASCA against Ro60/SSA autoantigen possibly participating in the development of skin manifestations of the disease. Studies are ongoing to try to clarify this hypothesis.

In conclusion, the wide variability in sensitivity and specificity of available ASCA ELISA kits may explain, at least in part, the different prevalence of ASCA in studies focused on the same disease. This has made difficult the interpretation of the possible role of ASCA in ADs. Our study assessed for the first time ASCA IgG+IgA with a highly specific immune-blot assay in a large cohort of pSS patients, showing that ASCA positivity identifies a peculiar clinical and serological pSS phenotype. In particular, ASCA$^+$ pSS patients displayed a triple combination of circulating anti-Ro52/SSA, anti-Ro60/SSA and anti-La/SSB antibodies, associated with low complement and cutaneous involvement. The high similarity between *S. cerevisiae* mannan and Ro60/SSA autoantigen may suggest a possible pathogenic/prognostic significance of ASCA in pSS. Additional studies also aimed at assessing separately different ASCA isotypes could shed additional light on this intriguing issue.

Key messages

- ASCA positivity identifies a peculiar clinical/serological phenotype of patients with primary Sjögren's syndrome.
- *S. Cerevisiae* mannan displays a high similarity with Ro60/SSA autoantigen.

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