

Anti-*Saccharomyces cerevisiae* Antibodies in Rheumatoid Arthritis

Sarra Melayah,^{1,2} Mariem Ghozzi,^{1,2} Malek Jemni,^{1,2} Nabil Sakly,^{2,3} Ibtissem Ghedira,^{1,2} Amani Mankai^{1,4}

¹Laboratory of Immunology, Farhat Hached Hospital, Sousse, Tunisia, ²Department of Immunology, Faculty of Pharmacy, Monastir University, Monastir, Tunisia, ³Laboratory of Immunology, Fattouma Bourguiba Hospital, Monastir, Tunisia, ⁴High School of Sciences and Techniques of Health, Tunis El Manar University, Tunis, Tunisia *To whom correspondence should be addressed. sarra.125@hotmail.fr

Keywords: anti-*Saccharomyces cerevisiae* antibodies, phosphopeptidomannan, rheumatoid arthritis, anti-CCP antibodies, rheumatoid factors, autoimmune disease

Abbreviations: ASCA, anti-*Saccharomyces cerevisiae* antibodies; RA, rheumatoid arthritis; PPM, phosphopeptidomannan; β 2GPI, β 2 glycoprotein I; a β 2GPI, anti- β 2GPI; AID, autoimmune diseases; anti-CCP, anti-cyclic citrullinated antibodies; HBD, healthy blood donors; RF, rheumatoid factors; MBL, mannose binding lectine.

Laboratory Medicine 2022;53:585–589; <https://doi.org/10.1093/labmed/lmac054>

ABSTRACT

Objective: This study was conducted to evaluate the frequency of anti-*Saccharomyces cerevisiae* antibodies (ASCA) in patients with rheumatoid arthritis (RA).

Methods: Eighty-three RA patients with positive anti-cyclic citrullinated antibodies (anti-CCP) and 160 healthy blood donors were included in this study. ASCA IgG and IgA were assessed with enzyme-linked immunosorbent assay.

Results: The frequency of ASCA was significantly higher in RA patients than in healthy subjects (22.9% vs 3.7%, $P < 10^{-3}$). Both ASCA IgG and ASCA IgA were significantly more frequent in RA patients than in the control group (20.5% vs 3.1%, $P < 10^{-3}$ and 9.6% vs 0.6%, $P = .002$, respectively). ASCA IgG and ASCA IgA levels were significantly higher in RA patients than in healthy subjects (7.8 ± 8.4 U/mL vs 2.3 ± 2.8 U/mL, $P < 10^{-6}$ and 6.2 ± 10.9 U/mL vs 3.4 ± 1.7 U/mL, $P = .002$, respectively).

Conclusion: A high frequency of ASCA IgG and ASCA IgA has been found in RA patients.

Rheumatoid arthritis (RA) is an autoimmune disease characterized by persistent synovial inflammation leading potentially to cartilage and bone damage and disability.¹ The etiology of RA is unknown; however, genetic, epigenetic, and environmental factors including gut dysbiosis might be involved in RA.^{2,3} In fact, a gut-joint axis has been incriminated in RA and suggested to contribute to arthritis.⁴ In this context, 2 pathways have been described: intestinal inflammation, which could be responsible for the generation of autoantibodies, and the migration of proinflammatory immune cells, present in the intestine, to the joints.⁴

Anti-*Saccharomyces cerevisiae* (ASCA) are directed against the phosphopeptidomannan (PPM), part of the cell wall of *S cerevisiae*. ASCA have been previously considered as a serological marker of Crohn's disease.⁵ Interestingly, ASCA also have been described in rheumatic diseases such as Behçet's disease,⁶ ankylosing spondylitis,⁷ psoriatic arthritis,⁸ and spondyloarthritis.⁹ Furthermore, ASCA have been described as a valuable test in evaluating patients with RA.¹⁰ Additionally, the frequency of ASCA IgA but not of ASCA IgG was found to be significantly higher in RA patients than in a healthy population in one study,¹¹ whereas other studies failed to show an association between ASCA and RA.^{8,12}

The pathogenic role of ASCA remains unknown, and one of the hypotheses of their synthesis is molecular mimicry with self-antigens.¹³ The β 2 glycoprotein I (β 2GPI), a phospholipid-binding glycoprotein, is normally present in plasma at the concentration of 50 μ g/mL to 500 μ g/mL and is a member of the complement control family.¹⁴ Interestingly, a structural similarity between the PPM of the yeast and β 2GPI has been reported.¹⁵ Anti- β 2GPI (a β 2GPI) antibodies have been found in antiphospholipid syndrome but also in other autoimmune diseases (AID).¹⁶ We have previously described a β 2GPI in patients with RA¹⁷; in the present study, our aim was to determine whether ASCA are also frequent in RA.

Materials and Methods

Study Population

We included 83 RA patients (51 females and 32 males). The mean age of our study population was 52 ± 15 years (range, 17–84 years). Sera samples were collected between 2017 and 2018 from 4 hospitals in the center of Tunisia. All patients fulfilled the American College of Rheumatology/European League Against Rheumatism¹⁸ criteria for RA. Patients

with RA and Crohn's disease were excluded from our study. The inclusion criterion of our study was anti-cyclic citrullinated peptide antibodies (anti-CCP) positive.

Sera from 160 healthy blood donors (HBD) (128 females and 32 males) were included as control group (mean age, 21 ± 4 years; range, 17–45 years). Samples of 4 mL venous blood were received in our laboratory for anti-CCP and rheumatoid factors (RF) assay. Sera were collected by centrifugation at 3000 rpm for 15 minutes and an enzyme-linked immunosorbent assay (ELISA) was carried out for anti-CCP and RF. A total of 400 μ L positive anti-CCP sera was aliquoted and stored at -80°C until use for analysis. The ethics committee of Farhat Hached Hospital gave approval for this study.

ASCA Assays

ASCA IgG and IgA were assessed by ELISA (Orgentec Diagnostika). Highly purified mannan from *S cerevisiae* was bound to microwells and served as an antigen. Sera of patients were diluted at 1:100 before the assay and 100 μ L was added to the reaction wells. Specific antibodies in the patient sample bound to the antigen. After incubation, a washing step was performed and subsequently added enzyme conjugate bound to the immobilized antibody-antigen complexes. After incubation, a second washing step removed unbound enzyme conjugate. After the addition of substrate solution, the bound enzyme conjugate hydrolyzed the substrate, forming a blue product. Addition of an acid stopped the reaction, generating a yellow end product. The intensity of the yellow color correlates with the concentration of the antibody-antigen complex and can be measured photometrically at 450 nm. Results were expressed as arbitrary units with a cutoff for positivity of 10 U/mL.

TABLE 1. Characteristics of RA Patients and the Control Group

	RA Patients (n = 83)	Control Group (n = 160)	P
Sex ratio (F/M)	1.6 (51/32)	4 (128/32)	.002
Mean age (y) $\pm$ SD	52 ± 15	21 ± 4	$<10^{-6}$
Age range (y)	17–84	17–45	—
Positive anti-CCP, No. (%)	83 (100)	—	—
Positive IgG-RF, No. (%)	65 (78.3)	—	—
Positive IgA-RF, No. (%)	64 (77.1)	—	—
Positive IgM-RF, No. (%)	71 (85.5)	—	—

anti-CCP, cyclic citrullinated peptide antibodies; RA, rheumatoid arthritis; RF, rheumatoid factors.

TABLE 2. Comparison of the Frequency of ASCA between Female and Male Patients and between RA Patients and the Control Group^a

	RA Females (n = 51)	RA Males (n = 32)	P Value	RA Patients (n = 83)	Control Group (n = 160)	P Value
ASCA IgG or IgA	15 (29.4)	4 (12.5)	.07	19 (22.9)	6 (3.7)	$<10^{-3}$
ASCA IgG and IgA	3 (5.9)	3 (9.4)	.85	6 (7.2)	0	.003
ASCA IgG	3 (25.5)	4 (12.5)	.51	17 (20.5) ^b	5 (3.1)	$<10^{-3}$
ASCA IgA	5 (9.8)	3 (9.4)	.99	8 (9.6) ^b	1 (0.6)	.002

ASCA, anti-Saccharomyces cerevisiae antibodies; RA, rheumatoid arthritis.

^aData are given as No. (%).

^bComparison between ASCA IgG and ASCA IgA ($P = .05$).

RF and Anti-CCP Assays

Serum samples were evaluated for IgG, IgA, and IgM RF by using an ELISA (Orgentec Diagnostika) according to the manufacturer's instructions. Anti-CCP were detected by using an available second generation ELISA (Euroimmun). Optimal cutoff values were determined by plotting sensitivity against $1 - \text{specificity}$ to receiver operating characteristic curve as we have described in our previous studies.^{19,20}

Statistical Analysis

The comparison of frequencies of ASCA was performed using χ^2 or Fisher exact test. The variables were tested for normality using the Kolmogorov-Smirnov test with Lilliefors adjustment. To compare mean levels of ASCA, we used the parametric Student *t*-test. Correlation was assessed using the Spearman test. A *P* value less than .05 was considered statistically significant. SPSS software, version 22, was used for statistical analysis.

Results

TABLE 1 shows patients and HBD characteristics.

Frequency of ASCA in RA Patients and the Control Group

Out of 83 RA patients, 19 (22.9%) had ASCA (IgG or IgA). The frequency of ASCA was significantly higher in RA patients than in healthy subjects (22.9% vs 3.7%, $P < 10^{-3}$). Both ASCA IgG and ASCA IgA were significantly more frequent in RA patients than in the control group (20.5% vs 3.1%, $P < 10^{-3}$; 9.6% vs 0.6%, $P = .002$, respectively) (**TABLE 2**).

Frequency of ASCA According to Gender

Among the 19 patients who had ASCA (IgG or IgA), 15 were females. ASCA (IgG or IgA) was more frequent in females (15/51, 29.4%) than in males (4/32, 12.5%), but the difference was not statistically significant. In RA patients, the frequencies of ASCA IgG and ASCA IgA were not statistically different between males and females (**TABLE 2**).

Comparison between ASCA IgG and ASCA IgA

The frequency of ASCA IgG was higher than that of ASCA IgA in RA patients but the difference was of borderline significance (20.5% vs 9.6%, $P = .05$) (**TABLE 2**).

ASCA IgG and ASCA IgA levels were significantly higher in RA patients than in healthy subjects ($7.8 \pm 8.4 \text{ U/mL}$ vs $2.3 \pm 2.8 \text{ U/mL}$, $P < 10^{-6}$ and $6.2 \pm 10.9 \text{ U/mL}$ vs $3.4 \pm 1.7 \text{ U/mL}$, $P = .002$, respectively) (**FIGURE 1**). In RA patients, no difference was detected between the mean levels of ASCA IgG and ASCA IgA.

FIGURE 1. Comparison of ASCA IgG (A) and ASCA IgA (B) levels in RA patients and control group. ASCA, anti-*Saccharomyces cerevisiae* antibodies; RA, rheumatoid arthritis.

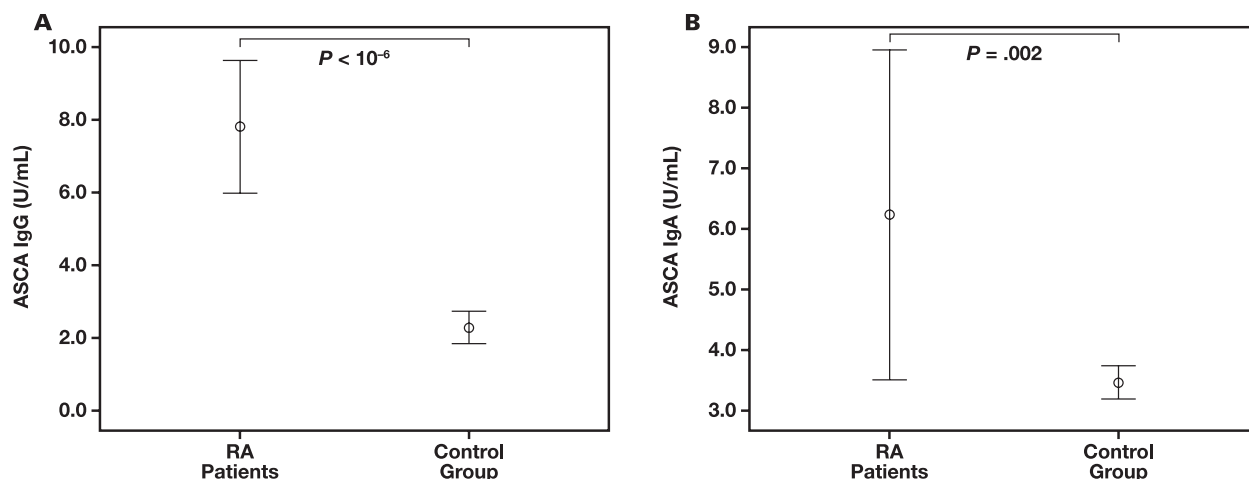


TABLE 3. Titers of ASCA in Patients with Positive Results

Patients	ASCA IgG Titer (U/mL)	ASCA IgA Titer (U/mL)
1	24.1	3.5
2	25.8	4.2
3	19	4
4	13.6	13.2
5	31.4	19.1
6	13.3	3.2
7	11.5	3.1
8	29.9	11.3
9	15.5	11.1
10	9.8	11
11	36.1	100
12	11	3.6
13	19.8	4.7
14	20.3	3.1
15	13.6	4.9
16	11.2	1.6
17	14.8	1.6
18	47.8	12
19	7.3	59.2

ASCA, anti-*Saccharomyces cerevisiae* antibodies.

Geometric mean titer of ASCA IgG was 5.5 U/mL in RA patients and 1.5 U/mL in the control group. Geometric mean titer of ASCA IgA was 4.1 U/mL and 3.2 U/mL in patients and controls, respectively. **TABLE 3** shows titers of ASCA in patients with positive results.

Association between ASCA and anti-CCP

There was no correlation between ASCA IgG or ASCA IgA levels and anti-CCP levels in patients with RA ($r = 0.212$, $P = 0.055$; $r = 0.082$, $P = .465$ respectively) (**FIGURE 2**).

Contribution of ASCA in Patients without RF

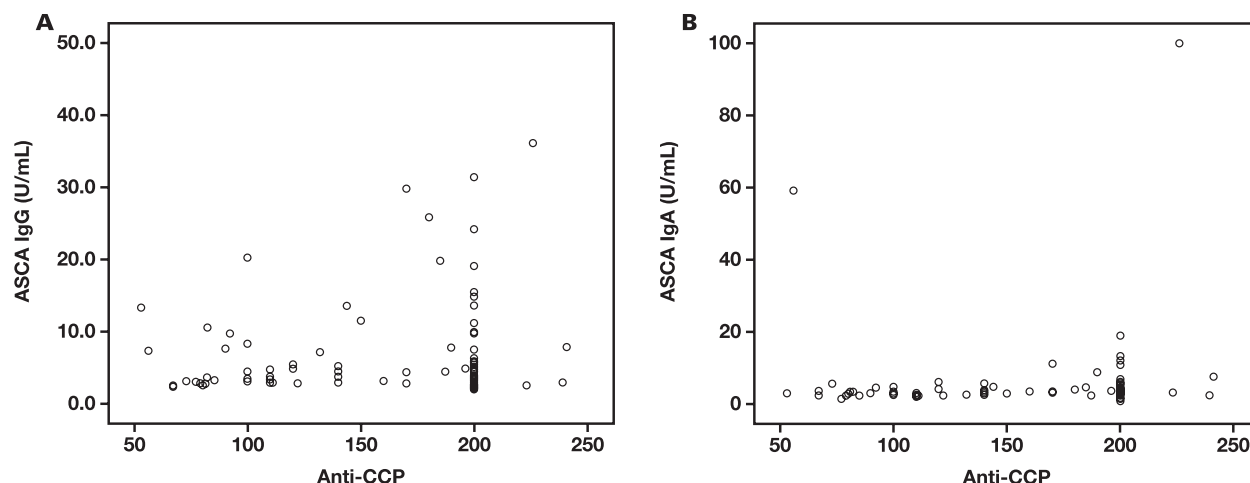
In 19 RA patients with ASCA, 2 (10.5%), 4 (21%), and 3 (15.8%) did not have RF-IgG, RF-IgA and RF-IgM, respectively. Two patients among these 19 (10.5%) did not have any of the 3 isotypes of RF.

Discussion

In this study, we assessed the frequency of ASCA IgG and IgA in a series of 83 patients with RA. We found a significantly higher frequency of both ASCA IgG and ASCA IgA in RA patients than in the control group (20.5% vs 3.1%, $P = .001$; 9.6% vs 0.6%, $P = .002$, respectively). In studies by Hoffman et al¹² and Riente et al,⁸ the frequency of ASCA IgG and ASCA IgA was not significantly different between RA patients and healthy subjects. Another study showed a significant difference for ASCA IgA and not IgG in RA patients compared to controls.¹¹ The frequency of ASCA IgG that we found in this study was similar to that of Dai et al¹¹ (20.5% and 20%, respectively); however, the frequency of ASCA IgA is lower than that found in Dai et al¹¹ (9.6% and 40%, respectively). In the Dai et al¹¹ study, IgA was the predominant isotype of ASCA but in our study, IgG was predominant. In fact, in all our previous studies on ASCA in AID,^{21–26} ASCA IgA frequencies were significantly lower than those of ASCA IgG. The frequency of ASCA (IgG or IgA) in our series was lower than that of Lichtenstein et al (22.9% vs 65.6%, respectively).¹⁰ This difference seems to be due to the inclusion criteria selected for the 2 studies. We included only patients who were positive for anti-CCP, whereas 52% of patients in the study of Lichtenstein et al were negative for anti-CCP. Moreover, in their series, 29% of patients with ASCA had neither anti-CCP nor RF.¹⁰ Consequently, it is possible that if we included seronegative patients for anti-CCP, the ASCA frequency could be higher. Interestingly, 10.5% of patients with ASCA in our series had no RF. Thus, when we combine ASCA with the known serological markers of RA (anti-CCP and RF), we increase RA diagnostic sensitivity.

Why are ASCA produced during RA? In RA, an altered intestinal barrier function seems to be secondary to the upregulation of zonulin, an intestinal peptide responsible for the regulation of the integrity of tight junctions.⁴ The increased production of zonulin has been associated with gluten consumption²⁷ and gut microbiota dysbiosis.⁴ Furthermore, it has been demonstrated that treatment with a zonulin antagonist specifically increases intestinal barrier integrity and reduces arthritis

FIGURE 2. Correlation between ASCA IgG (A) and ASCA IgA (B) levels and anti-CCP titers in RA patients. ASCA, anti-*Saccharomyces cerevisiae* antibodies; anti-CCP, anti-cyclic citrullinated peptide antibodies.



onset.²⁸ In RA, the resulting leaky intestinal wall allows passage of environmental antigens and exposes further *S cerevisiae* to the mucosal immune system, explaining the high frequency of ASCA. Moreover, we have previously found a significantly higher frequency of ASCA in patients with other AID in comparison to healthy subjects^{21–26} and in all these AID, gut microbiota dysbiosis and altered intestinal permeability have been described.^{29,30}

The high frequency of ASCA in our RA patients could also be explained by a possible cross-reactivity between the mannan of *S cerevisiae* and the mannan of mannose binding lectine (MBL). Interestingly, increased MBL levels have been reported in RA, suggesting that MBL could play a differential role in the pathogenesis of RA.^{31,32} In addition, the innate immune complement protein MBLs and their MBL2 genetic variants have been shown to be associated with many AID such as RA.^{33,34} Remarkably, plasma levels of MBL are also elevated in AID in which we have previously detected ASCA and in particular type 1 diabetes, celiac disease, primary biliary cholangitis and Graves disease.²⁵

Could ASCA be implicated in the pathogenesis of RA? We have previously demonstrated a high frequency of $\alpha\beta$ 2GPI in RA patients.¹⁷ Interestingly, Krause et al¹⁵ demonstrated that a subpopulation of $\alpha\beta$ 2GPI is specific to the glycosylated site of the β 2GPI molecule that cross-reacts with the PPM, part of the cell wall of *S cerevisiae*. In fact, a similarity in molecular structures (39%) was found between the mannan of the yeast *S cerevisiae* and the β 2GPI.¹³ Therefore, could we hypothesize that ASCA detected in our RA patients are $\alpha\beta$ 2GPI cross-reactive antibodies? Interestingly, the frequency of ASCA IgG (20.5%) in the present study is similar to that of $\alpha\beta$ 2GPI-IgA (26.7%) in our previous study on RA.¹⁷ Moreover, because β 2GPI is a ubiquitous glycoprotein and is present even in the joint,³⁵ ASCA could bind to β 2GPI in the joint and induce complement activation and inflammation. On the other hand, Rinaldi et al¹³ found that some autoantigens of RA share sequences with mannan expressed by the cell wall of *S cerevisiae*. So, could we imagine that ASCA synthesized during RA could bind to citrullinated peptides in the joint? The binding of those antibodies to citrullinated peptides will result in immune complex formation, complement activation, and a release of proinflammatory cytokines promoting chronic joint inflammation that

is typical for RA.³⁶ Moreover, because *S cerevisiae* could migrate from the gut to the joint because of a leaky gut, so ASCA could bind to *S cerevisiae* in the joint and induce the inflammatory process described above.

In conclusion, in this retrospective study, we found a high frequency of ASCA in patients with RA. This suggests that PPM, a component of the *S cerevisiae* cell wall, might induce chronic arthritis, and ASCA could improve the immunological diagnosis of RA.

Author Contributions

The authors contributed equally to study concept and design. All authors contributed to acquisition of data, analysis and interpretation, drafting of the manuscript and critical revision for important intellectual content. All authors approved the final version of this paper, including the authorship statement.

Funding

This study is supported by Unité de recherche, Auto-immunité et Allergie (03/UR/07-02), Faculté de Pharmacie de Monastir, Université de Monastir, Monastir, Tunisia.

REFERENCES

- Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet*. 2016;388:2023–2038. doi:10.1016/S0140-6736(16)30173-8.
- Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: a review. *JAMA*. 2018;320:1360–1372. doi:10.1001/jama.2018.13103.
- Picchianti-Diamanti A, Panebianco C, Salemi S, et al. Analysis of gut microbiota in rheumatoid arthritis patients: disease-related dysbiosis and modifications induced by etanercept. *Int J Mol Sci*. 2018;19:2938. doi:10.1016/j.jbspin.2015.10.011.
- Zaiss MM, Joyce Wu HJ, Mauro D, Schett G, Ciccia F. The gut-joint axis in rheumatoid arthritis. *Nat Rev Rheumatol*. 2021;17:224–237. doi:10.1038/s41584-021-00585-3.
- Peeters M, Joossens S, Vermeire S, Vlietinck R, Bossuyt X, Rutgeerts P. Diagnostic value of anti-*Saccharomyces cerevisiae* and

- antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease. *Am J Gastroenterol*. 2001;96:730–734. doi:10.1111/j.1572-0241.2001.03613.x.
6. Krause I, Monselise Y, Milo G, Weinberger A. Anti-*Saccharomyces cerevisiae* antibodies—a novel serologic marker for Behçet's disease. *Clin Exp Rheumatol*. 2002;20(4 suppl 26):S21–S24.
 7. Aydin SZ, Atagunduz P, Temel M, Bicakcigil M, Tasan D, Direskeneli H. Anti-*Saccharomyces cerevisiae* antibodies (ASCA) in spondyloarthropathies: a reassessment. *Rheumatology (Oxford)* 2008;47:142–144. doi:10.1093/rheumatology/kem324.
 8. Riente L, Chimenti D, Pratesi F, et al. Antibodies to tissue transglutaminase and *Saccharomyces cerevisiae* in ankylosing spondylitis and psoriatic arthritis. *J Rheumatol*. 2004;31(5):920–924.
 9. Mailliet J, Ottaviani S, Tubach F, et al. Anti-*Saccharomyces cerevisiae* antibodies (ASCA) in spondyloarthritis: prevalence and associated phenotype. *Joint Bone Spine*. 2016;83(6):665–668.
 10. Lichtenstein JR, Epstein AL. Antibodies to brewer's yeast in rheumatoid arthritis. *Cureus*. 2019;11:e4691. doi:10.7759/cureus.4691.
 11. Dai H, Li Z, Zhang Y, Lv P, Gao XM. Elevated levels of serum IgA against *Saccharomyces cerevisiae* mannan in patients with rheumatoid arthritis. *Cell Mol Immunol*. 2009;6:361–366. doi:10.1038/cmi.2009.47.
 12. Hoffman IE, Demetter P, Peeters M, et al. Anti-*Saccharomyces cerevisiae* IgA antibodies are raised in ankylosing spondylitis and undifferentiated spondyloarthropathy. *Ann Rheum Dis*. 2003;62:455–459. doi:10.1136/ard.62.5.455.
 13. Rinaldi M, Perricone R, Blank M, Perricone C, Shoenfeld Y. Anti-*Saccharomyces cerevisiae* autoantibodies in autoimmune diseases: from bread baking to autoimmunity. *Clin Rev Allergy Immunol*. 2013;45:152–161. doi:10.1007/s12016-012-8344-9.
 14. de Groot PG, Meijers JC. $\beta(2)$ -Glycoprotein I: evolution, structure and function. *J Thromb Haemost*. 2011;9:1275–1284. doi:10.1111/j.1538-7836.2011.04327.x.
 15. Krause I, Blank M, Cervera R, et al. Cross-reactive epitopes on beta2-glycoprotein-I and *Saccharomyces cerevisiae* in patients with the antiphospholipid syndrome. *Ann N Y Acad Sci*. 2007;1108:481–488. doi:10.1196/annals.1422.051.
 16. Gómez-Puerta JA, Cervera R. Diagnosis and classification of the antiphospholipid syndrome. *J Autoimmun*. 2014;48–49:20–25.
 17. Melayah S, Changuel M, Mankaï A, Ghedira I. IgA is the predominant isotype of anti- $\beta(2)$ glycoprotein I antibodies in rheumatoid arthritis. *J Clin Lab Anal*. 2020;34:e23217. doi:10.1002/jcla.23217.
 18. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum*. 2010;62:2569–2581. doi:10.1002/art.27584.
 19. Sghiri R, Bouajina E, Bargaoui D, et al. Value of anti-mutated citrullinated vimentin antibodies in diagnosing rheumatoid arthritis. *Rheumatol Int*. 2008;29:59–62. doi:10.1007/s00296-008-0614-8.
 20. Sghiri R, Bouagina E, Zaglaoui H, et al. Diagnostic performances of anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis. *Rheumatol Int*. 2007;27:1125–1130. doi:10.1007/s00296-007-0351-4.
 21. Toumi D, Mankaï A, Belhadj R, Ghedira-Besbes L, Jeddi M, Ghedira I. Anti-*Saccharomyces cerevisiae* antibodies in coeliac disease. *Scand J Gastroenterol*. 2007;42:821–826. doi:10.1080/00365520601154996.
 22. Sakly W, Jeddi M, Ghedira I. Anti-*Saccharomyces cerevisiae* antibodies in primary biliary cirrhosis. *Dig Dis Sci*. 2008;53:1983–1987. doi:10.1007/s10620-007-0092-y.
 23. Sakly W, Mankaï A, Sakly N, et al. Anti-*Saccharomyces cerevisiae* antibodies are frequent in type 1 diabetes. *Endocr Pathol*. 2010;21:108–114. doi:10.1007/s12022-010-9118-7.
 24. Mankaï A, Sakly W, Thabet Y, Achour A, Manoubi W, Ghedira I. Anti-*Saccharomyces cerevisiae* antibodies in patients with systemic lupus erythematosus. *Rheumatol Int*. 2013;33:665–669. doi:10.1007/s00296-012-2431-3.
 25. Mankaï A, Thabet Y, Manoubi W, Achour A, Sakly W, Ghedira I. Anti-*Saccharomyces cerevisiae* antibodies are elevated in Graves' disease but not in Hashimoto's thyroiditis. *Endocr Res*. 2013;38:98–104.
 26. Mankaï A, Layouni S, Ghedira I. Anti *Saccharomyces cerevisiae* antibodies in patients with anti- $\beta(2)$ glycoprotein I antibodies. *J Clin Lab Anal*. 2016;30:818–822. doi:10.1002/jcla.21942.
 27. Bruzzese V, Scolieri P, Pepe J. Efficacy of gluten-free diet in patients with rheumatoid arthritis. *Reumatismo*. 2021;72:213–217. doi:10.4081/reumatismo.2020.1296.
 28. Tajik N, Frech M, Schulz O, et al. Targeting zonulin and intestinal epithelial barrier function to prevent onset of arthritis. *Nat Commun*. 2020;24;11:1995.
 29. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system. *Nat Rev Immunol*. 2017;17:219–232. doi:10.1038/nri.2017.7.
 30. Chen B, Sun L, Zhang X. Integration of microbiome and epigenome to decipher the pathogenesis of autoimmune diseases. *J Autoimmun*. 2017;83:31–42. doi:10.1016/j.jaut.2017.03.009.
 31. Saevarsdottir S, Steinsson K, Grondal G, Valdimarsson H. Patients with rheumatoid arthritis have higher levels of mannan-binding lectin than their first-degree relatives and unrelated controls. *J Rheumatol*. 2007;34(8):1692–1695.
 32. Ammitzboll CG, Thiel S, Ellingsen T, et al. Levels of lectin pathway proteins in plasma and synovial fluid of rheumatoid arthritis and osteoarthritis. *Rheumatol Int*. 2012;32:1457–1463. doi:10.1007/s00296-011-1879-x.
 33. Turner MW, Hamvas RM. Mannose-binding lectin: structure, function, genetics and disease associations. *Rev Immunogenet*. 2000;2(3):305–322.
 34. Goeldner I, Skare TL, Utiyama SR, et al. Mannose binding lectin and susceptibility to rheumatoid arthritis in Brazilian patients and their relatives. *PLoS One*. 2014;9:e95519. doi:10.1371/journal.pone.0095519.
 35. Pawlak Z, Mrela A, Kaczmarek M, Cieszko M, Urbaniak W. Natural joints: boundary lubrication and antiphospholipid syndrome (APS). *Biosystems*. 2019;177:44–47. doi:10.1016/j.biosystems.2018.10.018.
 36. De Brito Rocha S, Baldo DC, Andrade LEC. Clinical and pathophysiological relevance of autoantibodies in rheumatoid arthritis. *Adv Rheumatol*. 2019;59:2. doi:10.1186/s42358-018-0042-8.