INTRODUCTION

Beta 2 Glycoprotein I (β2GPI) is a plasma protein with a circulating concentration of 0.2 mg/ml. This molecule plays a key role in hemostasis, homeostasis, and immunity. Antibodies against β2GPI (aβ2GPI) have been associated with antiphospholipid syndrome (APS) pathogenicity. Antiphospholipid syndrome is formally defined as the association of arterial/venous thrombosis and/or pregnancy losses in patients with persistent antiphospholipid antibodies (aPL). These autoantibodies include not only aβ2GPI but also anticardiolipin antibodies (aCL) and lupus anticoagulant. Although only IgG and IgM isotypes of aCL and aβ2GPI are included in the 2006 APS classification criteria, current evidence suggests that aβ2GPI-IgA may be involved in the pathophysiology of APS. In fact, it has been

RESEARCH ARTICLE

Frequency of serological markers of rheumatoid arthritis in patients with IgA anti-β2 glycoprotein I antibodies

Sarra Melayah1,2,3 | Mariem Ghozzi1,2,4 | Amani Mankaï1,5,6 | Fatma Mechi1,2 | Ibtissem Ghedira1,2

1Laboratory of Immunology, Farhat Hached Hospital, Sousse, Tunisia
2Department of Immunology, Faculty of Pharmacy, Monastir University, Monastir, Tunisia
3LR12SP11, Biochemistry Department, Sahloul University Hospital, Sousse, Tunisia
4Research Unit “Epidemiology and Immunogenetics of Viral Infections, LR14SP02”, Sahloul University Hospital, Sousse, Tunisia
5High School of Sciences and Techniques of Health, Tunis El Manar University, Tunis, Tunisia
6Research Unit “Obesity: Etiopathology and Treatment, UR18ES01”, National Institute of Nutrition and Food Technology, Tunis, Tunisia

Correspondence
Sarra Melayah, Laboratory of Immunology, Farhat Hached Hospital, Rue Ibn El Jazzar, 4000 Sousse, Tunisia.
Email: sarra.125@hotmail.fr

Abstract

Aim: To determine the frequency of serological markers of RA in patients with anti-β2 glycoprotein I antibodies (aβ2GPI) of IgA isotype.

Material and Methods: A retrospective study was conducted on 67 patients with aβ2GPI-IgA. Ninety healthy blood donors (HBD) were used as a control group. IgG anti-cyclic citrullinated peptides antibodies (CCP-Ab) and rheumatoid factors (RF) IgG, IgA, and IgM were detected by enzyme-linked immunosorbent assay (ELISA).

Results: Seventeen patients and eight HBD had CCP-Ab and/or RF (25.4% vs. 8.9%, p = 0.005, CI 95% [14.95; 35.79], odds ratio = 3.5). The frequency of CCP-Ab was significantly higher in patients than in healthy subjects (14.9% vs. 3.3%, p = 0.009). IgA isotype of RF was significantly higher in patients than in controls (7.5% vs. 0%, p = 0.02). In male patients, CCP-Ab and/or RF were more frequent than in healthy male subjects (37.5% vs. 11.8%, p = 0.02). In patients, no correlation was found between the levels of aβ2GPI-IgA and CCP-Ab (r = 0.082, p = 0.51). There was no correlation between the level aβ2GPI-IgA and the level of the isotypes of RF (IgG, IgA, and IgM) in patients (r = 0.1, p = 0.37; r = 0.17, p = 0.17 and r = 0.07, p = 0.59 respectively).

Conclusion: Frequencies of CCP-Ab and RF are high in patients with aβ2GPI-IgA suggesting that these patients are susceptible to developing RA.

KEYWORDS
anti-cyclic citrullinated peptides antibodies, antiphospholipid syndrome, anti-β2 glycoprotein I antibodies, rheumatoid arthritis, rheumatoid factors
shown that isolated α2GPI-IgA positivity (without IgG or IgM aCL/α2GPI and LA) is associated with venous thrombosis and pregnancy morbidity. However, α2GPI-IgA was demonstrated in autoimmune diseases other than APS.

Rheumatoid arthritis (RA) is a chronic and systemic autoimmune disease. It is characterized by synovial inflammation that can lead to irreversible joint damage and disability when not managed. Biomarkers of RA available for routine clinical use are rheumatoid factors (RF) and anti-cyclic citrullinated peptides (CCP-Ab). These autoantibodies are found in up to 80% and 95% of RA patients, respectively.

Common links between RA and APS have been reported. Glycosylation is implicated in the synthesis of highly pathogenic forms of antibodies to citrullinated proteins. Moreover, dysregulation of glycosylation could also generate antibodies of APS. Microparticles are considered mediators of cellular cross-talk inflammatory disease and high levels of microparticles have been found in both APS and RA. Regarding cytokines, tumor necrosis factor (TNF) is of central importance in RA pathogenesis. On the other hand, α2GPI induces monocyte release of TNF. Another main link is the genetic predisposition, which is important in the development of antibodies of APS and plays a role in RA risk, severity, and progression of this disease. Common genes for the two diseases include partly HLA system (HLA-DR4). In addition, an elegant recent study revealed that α2GPI-IgA is associated with the development and progression of coronary atherosclerosis in RA and predicts formation and persistence of high-risk mixed plaques.

Since many similarities exist between APS and RA and we have previously demonstrated that α2GPI-IgA is frequent in patients with RA. The objective of the present study was therefore to know if the vice versa is true by determining the serological markers of RA in patients who have α2GPI-IgA.

2 | PATIENTS AND METHODS

2.1 | Patients and controls

This retrospective study included sera of 67 patients collected from the database of our laboratory. The inclusion criteria of our study were positivity of α2GPI-IgA. Patients with other autoimmune diseases were excluded, particularly systemic lupus erythematosus and Sjögren syndrome. Antinuclear antibodies (ANA) were negative for all patients. Sera were collected between January 2018 and December 2019 from four hospitals in the center of Tunisia. Patients were consulted for suspicion of APS. The diagnosis of APS could not be confirmed because we do not have the second sample for the antiphospholipid antibodies assay. Medical records of the patients were retrospectively reviewed, and clinical events evoking APS were noted (Table 1).

Sera from 90 healthy blood donors (HBD) served as the control group.

| TABLE 1  | Clinical manifestations of patients with α2GPI  |
|----------|-----------------------------------------------|
| Clinical manifestations | Number of patients |
| Cirrhosis | 3 |
| Deep vein thrombosis | 11 |
| Arterial thrombosis | 7 |
| Thrombophlebitis | 2 |
| Stroke | 3 |
| Pregnancy loss | 4 |
| Thrombocytopenia | 3 |
| Ulcers | 1 |
| Pulmonary embolism | 1 |
| Budd-Chiari syndrome | 1 |
| Finger gangrene | 2 |
| Migraine | 1 |
| Hemolytic anemia | 1 |
| Cutaneous lesions | 4 |
| Purpura | 2 |
| Vasculitis | 3 |
| Dyspnea | 1 |
| Neurologic manifestations | 5 |
| Renal manifestation | 1 |
| Cardiac manifestations | 2 |

All sera were stored at −80°C until the use. The ethical committee of our hospital approved the study.

2.2 | Methods

2.2.1 | Anticardiolipin antibodies assays

The serum samples were evaluated for aCL-IgG, IgA, and IgM using a commercial enzyme-linked immunosorbent assay (ELISA; Orgentec Diagnostika®, Mainz, Germany). Results were expressed as arbitrary units with a cutoff for positivity of 10 U/ml for IgA and IgG and 7 U/ml for IgM following the manufacturer’s instructions.

2.2.2 | Anti-β2 glycoprotein I antibodies assays

The determination of α2GPI IgG, IgA, and IgM was carried out with a commercial ELISA (Orgentec Diagnostika®) using a purified human β2GPI. The results were expressed as arbitrary units with a cutoff for positivity of 8 U/ml following the manufacturer’s instructions.

2.2.3 | Rheumatoid factors and CCP-Ab assays

Serum samples were evaluated for IgG, IgA, and IgM rheumatoid factors (RF) by using an ELISA (Orgentec Diagnostika®) according
to the manufacturer’s instructions. CCP-Ab was detected by using an available second-generation ELISA (Euroimmun®, Lubeck, Germany). Optimal cut-off values were determined by plotting sensitivity against 1-specificity to receiver operating characteristic (ROC) curve as we have described in our previous studies.19,20

2.2.4 | Antinuclear antibodies assessment

Antinuclear antibodies screening was performed by the indirect immunofluorescence technique using human epithelial type 2 (HEp-2) as a substrate (Euroimmun®). Detection of ANA at a dilution superior to or equal to 1:80 was considered a positive result.

2.3 | Statistical analysis

Frequencies were compared using the chi-square test or Fisher’s exact test. A correlation study between β2GPI-IgM and CCP-Ab was done by calculating the Spearman’s correlation coefficient. A p-value of less than 0.05 was considered statistically significant.

3 | RESULTS

The characteristics of study sample are summarized in Table 2. We included 67 patients (43 females and 24 males) aged 29–80 years old (median age of 51 ± 14 years) and 90 HBD (56 females and 34 males, median age 37 ± 11 years). All patients were found negative for ANA. None of our patients had autoimmune diseases or hepatitis C or hepatitis B. Smoking status was not available.

All patients have β2GPI-IgA (100%), 17.9% have β2GPI-IgG and 19.4% have β2GPI-IgM. For aCL, 3.3% of patients have aCL-IgG and IgM and 7.8% have aCL-IgA (Table 3).

Seventeen patients and eight HBD had CCP-Ab and/or RF (25.4% vs. 8.9%, p = 0.005, CI 95% [14.95; 35.79], odds ratio = 3.5). The frequency of CCP-Ab was significantly higher in patients than in healthy subjects (14.9% vs. 3.3%, p = 0.009). Nine patients have only CCP-Ab (13.4%), while only three HBD (3.3%) have this antibodies without RF (p = 0.02). IgG isotype of RF was significantly higher in patients than in controls (7.5% vs. 0%, p = 0.02). One patient (1.5%) had both CCP-Ab and RF (Table 4). In male patients, CCP-Ab and/or RF were more frequent than in healthy male subjects (37.5% vs. 11.8%, p = 0.02). CCP-Ab were more frequent in male than in female patients (29.2% vs. 7%, p = 0.04; Table 5).

In patients, mean titer of CCP-Ab was 4.6 ± 6.6 RU/ml. The mean titers of RF-IgG, IgA, and IgM were 18.8 ± 15.5 U/ml, 21.5 ± 55.6 U/ml, and 10.6 ± 32.7 U/ml.

In patients, no correlation was found between the levels of β2GPI-IgA and CCP-Ab (r = 0.082, p = 0.51). We also did not find a correlation between the level β2GPI-IgA and the level of the isotypes of RF (IgG, IgA, and IgM) in patients (r = 0.1, p = 0.37; r = 0.17, p = 0.17 and r = 0.07, p = 0.59 respectively).

4 | DISCUSSION

In this study, we evaluated the frequency of antibodies of RA in patients admitted for suspicion of APS. The inclusion criterion of our patients was positivity of β2GPI-IgA. Although the IgA isotype of β2GPI was not included in the classification criteria for the diagnosis of APS,3 some observations support the pathogenic role of these antibodies. It has been demonstrated a high binding activity of the IgA to domains IV and V of β2GPI which are associated with certain manifestations of APS.21,22 In an experimental model of APS, β2GPI-IgA has been demonstrated to induce increased thrombus formation and up-regulate tissue factor activity.5,22 Moreover, β2GPI-IgA positivity was reported to be associated with many clinical manifestations such as myocardial infarction, atherosclerosis, acute cerebral ischemia, thrombosis, and stroke.22 However, β2GPI-IgA can be observed in autoimmune diseases other than APS. In fact, we have previously demonstrated a significantly higher frequency of β2GPI-IgA in patients with celiac disease, primary biliary cholangitis, and RA than in a healthy population.9,9

In this study, the frequency of antibodies of RA (CCP-Ab or RF) was increased in patients compared with healthy subjects (25.4% vs. 8.9%, p = 0.005). Alessandri et al.23 evaluated the frequency of CCP-Ab in patients with APS and they found that out of 79 patients, only one had CCP-Ab. The frequency of CCP-Ab was therefore higher in our study than in that of Alessandri et al.23 (14.9% and 1.3% respectively). This discrepancy could be explained by the inclusion criteria in both studies. In fact, we included patients with β2GPI-IgA, while Alessandri et al.23 included patients with APS and for whom only the isotypes IgG and IgM have been done for both aCL and β2GPI.

In a previous study, we demonstrated a significantly higher frequency of β2GPI-IgA in patients with RA than in healthy subjects (26.7% vs. 7.8%, p = 0.0007).9 Thanks to the present study, we discovered that the vice versa is true, which means that autoantibodies of RA (CCP-Ab and/or RF) are significantly more frequent in patients with β2GPI-IgA than controls (25.4% vs. 8.9%, p = 0.005). Both APS and RA are autoimmune diseases and share common risk factors. Indeed, cigarette smoking, which is known to stimulate the anti-citrulline immunity,24 is a risk factor not only for RA25 but also for APS.24 Unfortunately, in Tunisia, the frequency

| TABLE 2 Characterisiticsof patients and the control group |
|-----------------------------------------------------------|
| Patients (n = 67)                                      | Control group (n = 90) | p     |
|-----------------------------------------------------------|
| Sex ratio (F/M)                                         | 1.8 (43/24)            | 1.6 (56/34) | 0.8 |
| Mean age                                                 | 51 ± 14 years          | 37 ± 11 years | <10^-6 |
| Age range                                                | 29–80 years            | 20–64 years | - |
| Abbreviations: F, female; M, male.                        |
| Bold values indicates the significant of p values.       |
of smoking is the highest in the world. Moreover, in our country, smoking is more frequent in men than in women. Interestingly, in the present study, the frequency of CCP-Ab was significantly higher in males than in females (29.2% vs. 7%, \( p = 0.04 \)). RF-IgA was also significantly more frequent in patients than in controls (7.5% vs. 0%, \( p = 0.02 \)). Cigarette smoking has been associated with RF-IgA in African Americans but not in Americans with European ancestry. Remarkably, our study is about Tunisian people who are of African ancestry.

In a previous study, we evaluated the frequency of autoantibodies of RA in patients with celiac disease. Our results are similar to those of the present study. That means not only CCP-Ab and RF were more frequent in celiac patients than in healthy subjects but also IgA was the predominant isotype of RF. So, we could imagine

| Anti-phospholipid antibodies | Patients (n = 67) % (n) | Control group (n = 90) % (n) | \( p \) | CI (95%) | Odds ratio |
|------------------------------|-------------------------|-----------------------------|-------|---------|------------|
| aCL (IgG and/or IgA and/or IgM) | 31.3 (21) | 5.5 (5) | <10\(^{-3}\) |
| aCL-IgG | 6 (4) | 2.2 (2) | 0.43 |
| aCL-IgA | 13.4 (9) | 2.2 (2) | 0.015 |
| aCL-IgM | 19.4 (13) | 4.4 (4) | 0.003 |
| aβ2GPI (IgG and/or IgA and/or IgM) | 100 (67) | 10 (9) | <10\(^{-6}\) |
| aβ2GPI-IgG | 17.9 (12) | 3.3 (3) | 0.002 |
| aβ2GPI-IgA | 100 (67) | 7.8 (7) | <10\(^{-6}\) |
| aβ2GPI-IgM | 19.4 (13) | 3.3 (3) | 0.001 |

Abbreviations: aβ2GPI, anti-β2 glycoprotein I antibodies; aCL, anti-cardiolipin antibodies.

Bold values indicates the significant of \( p \) values.

| TABLE 4 Frequency of CCP-Ab and RF in patients and in the control group |
|-----------------------------|-------------------------|-------|---------|-------|
| Autoantibodies | Patients (n = 67) % (n) | Control group (n = 90) % (n) | \( p \) | CI (95%) |
| CCP-Ab and/or RF | 25.4 (17) | 8.9 (8) | 0.005 | [14.95; 35.79] |
| CCP-Ab | 14.9 (10) | 3.3 (3) | 0.009 | [6.39; 23.46] |
| Isolated CCP-Ab | 13.4 (9) | 3.3 (3) | 0.02 | [5.27; 21.60] |
| RF (IgG and/or IgA and/or IgM) | 11.9 (8) | 5.5 (5) | 0.15 | - |
| RF-IgG | 6 (4) | 2.2 (2) | 0.23 | - |
| RF-IgA | 7.5 (5) | 0 | 0.02 | [1.17; 13.76] |
| RF-IgM | 6 (4) | 5.5 (5) | >0.99 | - |
| Isolated RF-IgA | 6 (4) | 0 | 0.06 | - |
| CCP-Ab and RF | 1.5 (1) | 0 | 0.85 | - |

Abbreviations: CCP-Ab, anti-cyclic citrullinated peptides; RF, rheumatoid factors.

Bold values indicates the significant of \( p \) values.

| TABLE 5 Frequency of CCP-Ab and RF according to sex |
|-----------------------------|-------------------------|-------------------------|-------|
| Autoantibodies | Females (Patients (n = 67) % (n)) | Control group (n = 90) % (n) | \( p \) | Males (Patients (n = 67) % (n)) | Control group (n = 90) % (n) | \( p \) |
| CCP-Ab and/or RF | 18.6 (8) | 7.1 (4) | 0.08 | 37.5 (9) | 11.8 (4) | 0.02 |
| CCP-Ab | 7 (3)\(^a\) | 1.8 (1) | 0.43 | 29.2 (7)\(^{ab}\) | 5.9 (2) | 0.04 |
| RF (IgG and/or IgA and/or IgM) | 11.6 (5) | 5.4 (3) | 0.44 | 12.5 (3) | 5.9 (2) | 0.67 |
| RF-IgG | 2.3 (1) | 1.8 (1) | >0.99 | 12.5 (3) | 2.9 (1) | 0.37 |
| RF-IgA | 9.3 (4) | 0 | 0.06 | 4.2 (1)\(^b\) | 0 | 0.83 |
| RF-IgM | 4.6 (2) | 5.4 (3) | >0.99 | 8.3 (2) | 5.9 (2) | >0.99 |

Abbreviations: CCP-Ab, anti-cyclic peptides antibodies; RF, rheumatoid factors.

\(^a\)Comparison of frequencies of CCP-Ab between male and female patients, \( p = 0.04 \).

\(^b\)Comparison between frequencies of CCP-Ab and RF-IgA in male patients, \( p = 0.04 \).

Bold values indicates the significant of \( p \) values.
that our patients of the present study would develop celiac disease. Both in the present study and in our previous study on celiac disease, patients with RF-IgA had no symptoms of RA. However, they could develop RA in the future. In fact, RF-IgA can appear 15 years before the symptoms of RA. Indeed, RF and especially the IgA isotype was the first appearing antibody in a study, which included 321 pre-symptomatic individuals. CCP-Ab have also a high positive predictive value for the development of RA. The combination of CCP-Ab and RF increases the prediction of future RA. In the present study, 14.9% of our patients had CCP-Ab and 11.9% had RF. However, when we combined both tests, the frequency of autoantibodies of RA increased to 25.4% in the whole group and to 37.5% in the male group.

In our laboratory, only the IgG isotype of CCP-Ab is done, so we hypothesize that if sera of our patients could be tested in the IgA isotype of CCP-Ab, so the global frequency of CCP-Ab could be higher than 14.9% in the whole group and more than 29.2% in males. IgA isotype of CCP-Ab, so the global frequency of CCP-Ab could be in the male group.

In conclusion, in this study, we demonstrated a high frequency of antiphospholipid antibodies in primary biliary cirrhosis. J Clin Lab Anal. 2015;29(1):32-36.

Melayah S, Changuel M, Mankaï A, Ghedira I. IgA is the predominant isotype of anti-j2 glycoprotein I antibodies in celiac disease. Pathol Biol (Paris). 2012;60(5):291-295.

Mankaï A, Manoubi W, Ghozzi M, Melayah S, Sakly W, Ghedira I. High frequency of antiphospholipid antibodies in primary biliary cirrhosis. J Clin Lab Anal. 2015;29(1):32-36.

Jang S, Kwon EJ, Lee JJ. Rheumatoid arthritis: pathogenic role of diverse immune cells. Int J Mol Sci. 2022;23(2):905.

Shapiro SC. Biomarkers in rheumatoid arthritis. Curr. 2021;13(5):e15063.

Coutant F. Pathogenic effects of anti-citrullinated protein antibodies in rheumatoid arthritis – role for glycosylation. Joint Bone Spine. 2019;86(5):562-567.

Distler JH, Huber LC, Gay S, Distler O, Pisetsky DS. Microparticles as mediators of cellular cross-talk in inflammatory disease. Autoimmunity. 2006;39(8):683-690.

Firestein GS, McInnes IB. Immunopathogenesis of rheumatoid arthritis. Immunity. 2017;46(2):183-196.

Sorice M, Longo A, Capozzi A, et al. Anti-beta2-glycoprotein I antibodies induce monocyte release of tumor necrosis factor alpha and tissue factor by signal transduction pathways involving lipid rafts. Arthritis Rheum. 2007;56(8):2687-2697.

Juliano A, Galeazzi M, Sebastiani GD. Antiphospholipid syndrome’s genetic and epigenetic aspects. Autoimmun Rev. 2019;18(9):102352.

Viatte S, Barton A. Genetics of rheumatoid arthritis susceptibility, severity, and treatment response. Semin Immunopathol. 2017;39(4):395-408.

Karpouzas GA, Ormseth SR, Hernandez E, Bui VL, Budoff MJ. Beta-2-glycoprotein-I IgA antibodies predict coronary plaque progression in rheumatoid arthritis. Semin Arthritis Rheum. 2021;51(1):20-27.

Sghiri R, Bouajina E, Barguoui D, et al. Value of anti-mutated citrullinated vimentin antibodies in diagnosing rheumatoid arthritis. Rheumatol Int. 2008;29(1):59-62.

Sghiri R, Bouajina E, Zaglauh H, et al. Diagnostic performances of anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis. Rheumatol Int. 2007;27(12):1125-1130.

Serrano M, Martinez-Flores JA, Norman GL, Naranjo L, Morales JM, Serrano A. The IgA isotype of anti-j2 glycoprotein I antibodies recognizes epitopes in domains 3, 4, and 5 that are located in a lateral zone of the molecule (L-shaped). Front Immunol. 2019;10:1031.
22. Murthy V, Willis R, Romay-Penabad Z, et al. Value of isolated IgA anti-β2-glycoprotein I positivity in the diagnosis of the antiphospholipid syndrome. *Arthritis Rheum*. 2013;65(12):3186-3193.

23. Alessandri C, Agmon-Levin N, Conti F, et al. Anti-mutated citrullinated vimentin antibodies in antiphospholipid syndrome: diagnostic value and relationship with clinical features. *Immunol Res*. 2017;65(2):524-531.

24. Perricone C, Versini M, Ben-Ami D, et al. Smoke and autoimmunity: the fire behind the disease. *Autoimmun Rev*. 2016;15(4):354-374.

25. Mikuls TR, Hughes LB, Westfall AO, et al. Cigarette smoking, disease severity and autoantibody expression in African Americans with recent-onset rheumatoid arthritis. *Ann Rheum Dis*. 2008;67(11):1529-1534.

26. Binder SR, Litwin CM. Anti-phospholipid antibodies and smoking: an overview. *Clin Rev Allergy Immunol*. 2017;53(1):1-13.

27. Fakhfakh R, Hsairi M, Maalej M, Achour N, Nacef T. Tobacco use in Tunisia: behaviour and awareness. *Bull World Health Organ*. 2002;80:350-356.

28. Ghozzi M, Melayah S, Adaily N, Ghedira I. Frequency of serological markers of rheumatoid arthritis in adult patients with active celiac disease. *J Clin Lab Anal*. 2022;36(3):e24249.

29. Brink M, Hansson M, Mathsson-Alm L, et al. Rheumatoid factor isotypes in relation to antibodies against citrullinated proteins and carbamylated proteins before the onset of rheumatoid arthritis. *Arthritis Res Ther*. 2016;18:43.

30. Greenblatt HK, Kim HA, Bettnar LF, Deane KD. Preclinical rheumatoid arthritis and rheumatoid arthritis prevention. *Curr Opin Rheumatol*. 2020;32(3):289-296.

31. Sokolova MV, Hagen M, Bang H, Schett G, Rech J, Steffen U. RETRO study group. IgA anti-citrullinated protein antibodies (IgA ACPA) are associated with flares during DMARD tapering in rheumatoid arthritis. *Rheumatology (Oxford)*. 2021;61:2124-2131.

32. Holers VM, Demoruelle MK, Kuhn KA, et al. Rheumatoid arthritis and the mucosal origins hypothesis: protection turns to destruction. *Nat Rev Rheumatol*. 2018;14(9):542-557.

33. Horta-Baas G, Romero-Figueroa MDS, Montiel-Jarquin AJ, Pizano-Zárate ML, García-Mena J, Ramirez-Durán N. Intestinal dysbiosis and rheumatoid arthritis: a link between gut microbiota and the pathogenesis of rheumatoid arthritis. *J Immunol Res*. 2017;2017:4835189.

34. Zaiss MM, Joyce Wu HJ, Mauro D, Schett G, Ciccia F. The gut-joint axis in rheumatoid arthritis. *Nat Rev Rheumatol*. 2021;17(4):224-237.

35. Lucchino B, Spinelli FR, Iannuccelli C, Guzzo MP, Conti F, Di Franco M. Mucosa-environment interactions in the pathogenesis of rheumatoid arthritis. *Cell*. 2019;8(7):700.

36. Ruff WE, Vieira SM, Kriegel MA. The role of the gut microbiota in the pathogenesis of antiphospholipid syndrome. *Curr Rheumatol Rep*. 2015;17(1):472.

37. Ruff WE, Kriegel MA. Autoimmune host-microbiota interactions at barrier sites and beyond. *Trends Mol Med*. 2015;21(4):233-244.

How to cite this article: Melayah S, Ghozzi M, Mankaï A, Mechi F, Ghedira I. Frequency of serological markers of rheumatoid arthritis in patients with IgA anti-β2 glycoprotein I antibodies. *J Clin Lab Anal*. 2022;36:e24537. doi: 10.1002/jcla.24537