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

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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

1 | 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.[1] 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).[2] 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,[3] current evidence suggests that aβ2GPI-IgA may be involved in the pathophysiology of APS.[4-6] In fact, it has been

shown that isolated aβ2GPI-IgA positivity (without IgG or IgM aCL/aβ2GPI and LA) is associated with venous thrombosis and pregnancy morbidity.[4] However, aβ2GPI-IgA was demonstrated in autoimmune diseases other than APS.[7-9]

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.[10] 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.[11]

Common links between RA and APS have been reported. Glycosylation is implicated in the synthesis of highly pathogenic forms of antibodies to citrullinated proteins.[12] Moreover, dysregulation of glycosylation could also generate antibodies of APS.[1] Microparticles are considered mediators of cellular cross-talk inflammatory disease and high levels of microparticles have been found in both APS and RA.[13] Regarding cytokines, tumor necrosis factor (TNF) is of central importance in RA pathogenesis.[14] On the other hand, aβ2GPI induces monocyte release of TNF.[15] Another main link is the genetic predisposition, which is important in the development of antibodies of APS[16] and plays a role in RA risk, severity, and progression of this disease.[14,17] Common genes for the two diseases include partly HLA system (HLA-DR4).[14,16] In addition, an elegant recent study revealed that aβ2GPI-IgA is associated with the development and progression of coronary atherosclerosis in RA and predicts formation and persistence of high-risk mixed plaques.[18]

Since many similarities exist between APS and RA and we have previously demonstrated that aβ2GPI-IgA is frequent in patients with RA.[9] 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 aβ2GPI-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 aβ2GPI-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 aβ2GPI

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 aβ2GPI 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 aβ2GPI-IgA 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 aβ2GPI-IgA (100%), 17.9% have aβ2GPI-IgG and 19.4% have aβ2GPI-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 ($p = 0.02$). IgA 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 UI/ml.

In patients, no correlation was found between the levels of aβ2GPI-IgA and CCP-Ab ($r = 0.082$, $p = 0.51$). We also did not find a 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).

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 aβ2GPI-IgA. Although the IgA isotype of aβ2GPI 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, aβ2GPI-IgA has been demonstrated to induce increased thrombus formation and up-regulate tissue factor activity.[5,22] Moreover, aβ2GPI-IgA positivity was reported to be associated with many clinical manifestations such as myocardial infarction, atherosclerosis, acute cerebral ischemia, thrombosis, and stroke.[22] However, aβ2GPI-IgA can be observed in autoimmune diseases other than APS. In fact, we have previously demonstrated a significantly higher frequency of aβ2GPI-IgA in patients with celiac disease, primary biliary cholangitis, and RA than in a healthy population.[7–9]

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 aβ2GPI-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 aβ2GPI.

In a previous study, we demonstrated a significantly higher frequency of aβ2GPI-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 aβ2GPI-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 RA[25] but also for APS.[26] Unfortunately, in Tunisia, the frequency

TABLE 2 Characteristics of 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.

Anti-phospholipid antibodies	Patients (n = 67) % (n)	Control group (n = 90) % (n)	p
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**

TABLE 3 Frequency of antiphospholipid antibodies (aCL and aβ2GPI) in patients and control group

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%)	Odds ratio
CCP-Ab and/or RF	25.4 (17)	8.9 (8)	**0.005**	[14.95; 35.79]	3.5
CCP-Ab	14.9 (10)	3.3 (3)	**0.009**	[6.39; 23.46]	1.9
Isolated CCP-Ab	13.4 (9)	3.3 (3)	**0.02**	[5.27; 21.60]	4.5
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			Males		
	Patients (n = 43) % (n)	Control group (n = 56) % (n)	p	Patients (n = 24) % (n)	Control group (n = 34) % (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)[a,b]	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.

of smoking is the highest in the world.[27] Moreover, in our country, smoking is more frequent in men than in women.[27] 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.[25] 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.[28] 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

that our patients of the present study would develop celiac disease. Both in the present study and in our previous study on celiac disease,[28] 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.[29] Indeed, RF and especially the IgA isotype was the first appearing antibody in a study, which included 321 pre-symptomatic individuals.[29] CCP-Ab have also a high positive predictive value for the development of RA.[19] The combination of CCP-Ab and RF increases the prediction of future RA.[29,30] 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. In fact, it has been demonstrated that 34.9% of IgG-CCP-Ab negative patients with RA were IgA-CCP-Ab positive.[31] Moreover, it has been hypothesized that RA has mucosal origins.[32] Among mucosal sites, which exhibit autoantibody production, the gut plays a major role in the pathogenesis of RA. Especially, gut dysbiosis has been implicated in local CCP-Ab production.[32–35] In the present study, we screened for RA in patients suspected to have APS since a common link does exist between these two diseases. Interestingly, the role of gut microbiota has been described in the pathogenesis of RA[33,34] and also of APS.[36] An increase of several species characterizes the gut dysbiosis of early RA. Among these species, Prevotella could induce an anti-citrullinated immune response through a molecular mimicry mechanism.[35] Moreover, in APS, cross-reactive gut commensal antigens could induce aβ2GPI production.[37]

This study presents some limitations. First, the smoking status of patients and HBD was not specified in the medical records of the patients. Second, we do not have the lupus anticoagulant assay result for our patients. Lastly, the second sample for antiphospholipid antibodies assay was not available to confirm antiphospholipid antibody positivity 12 weeks after baseline screening.

In conclusion, in this study, we demonstrated a high frequency of autoantibodies of RA in patients with aβ2GPI-IgA suggesting that these patients could be at higher risk of developing RA. Following the clinical features and serological markers of these patients over the years could confirm this data.

CONFLICT OF INTEREST
None.

DATA AVAILABILITY STATEMENT
Data are not shared.

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