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Original article

Analysis of four serum biomarkers in rheumatoid arthritis: association with extra articular manifestations in patients and arthralgia in relatives

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ABSTRACT

Objectives: To evaluate the frequency of four serum biomarkers in RA patients and their relatives and identify possible associations with clinical findings of the disease.
Methods: This was a transversal analytical study. Anti-cyclic citrullinated peptide (anti-CCP), anti-mutated citrullinated vimentin (anti-MCV) and IgA-rheumatoid factor (RF) were determined by ELISA and IgM-RF by latex agglutination in 210 RA patients, 198 relatives and 92 healthy controls from Southern Brazil. Clinical and demographic data were obtained through charts review and questionnaires.
Results: A higher positivity for all antibodies was observed in RA patients when compared to relatives and controls ($p < 0.0001$). IgA-RF was more frequent in relatives compared to controls (14.6% vs. 5.4%, $p = 0.03$, OR = 2.98; 95% CI = 1.11–7.98) whereas anti-CCP was the most common biomarker among RA patients (75.6%). Concomitant positivity for the four biomarkers was more common in patients (46.2%, $p < 0.0001$). Relatives and controls were mostly positive for just one biomarker (20.2%, $p < 0.0001$ and 15.2%, $p = 0.016$, respectively). No association was observed between the number of positive biomarkers and age of disease onset, functional class or tobacco exposure. In seronegative patients predominate absence of extra articular manifestations (EAMs) ($p = 0.01$; OR = 3.25; 95% CI = 1.16–10.66). Arthralgia was present in positive relatives, regardless the type of biomarker.
Conclusions: A higher number of biomarkers was present in RA patients with EAMs. Positivity of biomarkers was related to arthralgia in relatives. These findings reinforce the link between distinct biomarkers and the pathophysiologic mechanisms of AR.

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Análise de quatro marcadores sorológicos na artrite reumatoide: associação com manifestações extra-articulares no paciente e artralgia em familiares

Palavras-chave:
Artrite reumatoide
Biomarcadores
Fator reumatoide
Anti CCP
Anti vimentina citrulinada

RESUMO

Objetivos: Avaliar a frequência de quatro marcadores sorológicos em pacientes com AR e familiares e identificar possíveis associações com achados clínicos da doença.
Métodos: Estudo analítico transversal. Determinaram-se os níveis de anticorpos antipeptídeo citrulinado cíclico (anti-CCP), anticorpos antivimentina citrulinada-mutada (anti-MCV) e fator reumatoide (FR) IgA por Elisa e de FR-IgM por aglutinação em látex em 210 pacientes com AR, 198 parentes e 92 controles saudáveis do sul do Brasil. Coletaram-se dados clínicos e demográficos por meio da revisão de prontuários e questionários.
Resultados: Observou-se maior positividade para todos os anticorpos em pacientes com AR em comparação com os familiares e controles ($p < 0{,}0001$). O FR-IgA era mais frequente em familiares quando comparados com os controles (14,6% versus 5,4%, $p = 0{,}03$, OR = 2,98; IC95% = 1,11 a 7,98). O anti-CCP foi o biomarcador mais comum entre pacientes com AR (75,6%). A positividade concomitante para os quatro biomarcadores foi mais comum nos pacientes (46,2%, $p < 0{,}0001$). Familiares e controles eram positivos em sua maioria para apenas um biomarcador (20,2%, $p < 0{,}0001$ e 15,2%, $p = 0{,}016$, respectivamente). Não foi observada associação entre o número de biomarcadores positivos e a idade de início da doença, classe funcional ou exposição ao fumo. Em pacientes soronegativos, predominou a ausência de manifestações extra-articulares (MEA) ($p = 0{,}01$; OR = 3,25; IC95% = 1,16 a 10,66). A artralgia estava presente em familiares positivos, independentemente do tipo de biomarcador.
Conclusões: Uma maior quantidade de biomarcadores estava presente em pacientes com AR com MEA. A positividade dos biomarcadores estava relacionada com a artralgia em familiares. Esses achados reforçam a ligação entre os diferentes biomarcadores e os mecanismos fisiopatológicos da AR.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that occurs in 0.2–1% of the world population.[1] Its prevalence in first degree relatives of RA patients is of 2–4%, characterizing them as a risk group for RA.[2–5] In this context, investigation of serological markers in healthy relatives may be of value to identify earlier cases of RA[6,7] and to understand the pathophysiologic mechanisms underlying the disease process.

Rheumatoid factor (RF) is a classical serological marker used for RA diagnosis, with IgM-RF being the most common isoform. In contrast, IgA-RF has been associated with erosive arthritis and seems to be a better indicator of disease severity than IgM-RF or IgG-RF.[8,9] Antibodies against citrullinated peptides (ACPAs), such as anti-cyclic citrullinated peptide (anti-CCP) and anti-mutated citrullinated vimentin (anti-MCV) are considered highly specific markers for RA.[10] Anti-CCP has both diagnostic and prognostic value and can be detected before clinical manifestations of the disease.[11] Recently, anti-MCV has also been proposed as a diagnostic marker for early arthritis, with the same specificity but higher sensitivity of anti-CCP.[12] In addition, anti-MCV has been detected in healthy relatives of RA patients[13] suggesting it as a novel prognostic marker for RA.

Positivity for more than one RA serological marker in the same patient may indicate worse prognosis or, in the case of relatives, early disease onset.[14,15] In fact, positivity for more than one autoantibody has been reported among unaffected relatives of RA patients, especially in families with multiple cases.[16,17]

To date, there are no reports considering the simultaneity of these biomarkers in RA patients and their relatives in the Brazilian population. Thus, in the present work we investigated the frequency of anti-CCP, anti-MCV, IgA-RF and IgM-RF in RA patients and their relatives, and tried to identify possible associations between the simultaneity of these biomarkers and clinical findings or diagnosis of RA.

Methods

This was a transversal and analytical study approved by the local Ethics Committee in Research. Informed consent was obtained from all subjects.

Subjects

Two-hundred and ten adult RA patients meeting the ACR classification criteria[18] from a single tertiary center were

Table 1 – Demographic and clinical features of RA patients.

	% (N)
Mean age (years)	51.1 ± 11.8
Female	84.8 (178/210)
Age at disease onset – (years; median)	45.0 ± 12.8
Disease duration (years; median)	7.0 ± 8.5
Eurodescendents (auto declared)	72.8 (131/180)
Afrodescendents (auto declared)	26.7 (48/180)
Tobacco exposure	46.0 (87/189)
Functional class	
Class I	47.4 (99/209)
Class II	37.7 (83/209)
Class III	10.5 (22/209)
Class IV	2.4 (5/209)
Extra articular manifestations	
Rheumatoid nodules	8.9 (17/190)
Secondary Sjögren syndrome	30.2 (49/162)
Pulmonary fibrosis	8.6 (15/175)

consecutively enrolled in the study from August 2007 to April 2009. Demographic and clinical features from RA patients are shown in Table 1.

A total of 198 relatives were investigated (61.1% female, 38.9% male; mean age 36.8 years; range 7–91 years, 94% first-degree), representing altogether 78 families (2.54 relatives/family). Additionally, sera from 92 healthy volunteers from the same geographical area, matched for gender and age with patients, were used as controls (82.6% female, 17.4% male; mean age 45.8 years; range 23–81 years). None of controls had familial RA cases.

Autoantibodies evaluation

Anti-CCP and IgA-RF were assessed by enzyme-linked immunosorbent assay (ELISA) from INOVA Diagnostics (San Diego, USA). Anti-MCV was determined using the ORGENTEC Diagnostika ELISA kit (Mainz, Germany). IgM-RF was measured using latex agglutination test (BioSystems S.A., Barcelona, Spain). Cut-off values were established according to the manufacturer's specifications.

Data collection

Demographic and clinical data from patients and relatives were obtained through medical record review and individual standard questionnaires applied by a rheumatologist at the time of enrollment (Tables 1 and 2). In RA patients the following extra articular manifestations (EAM) were taken into account: rheumatoid nodules, serositis, pericarditis, valvular disease, vasculitis, lung fibrosis, pneumonitis, Felty's syndrome and secondary Sjögren syndrome. Steinbrocker functional classification was applied in order to access the extent of physical disability in RA patients. Diagnosis of secondary Sjogren's syndrome followed the American European criteria.[19] Clinical follow-up of the positive relatives has been carried out for the median time of 7 years (range 2–8; IQR = 6–7 years).

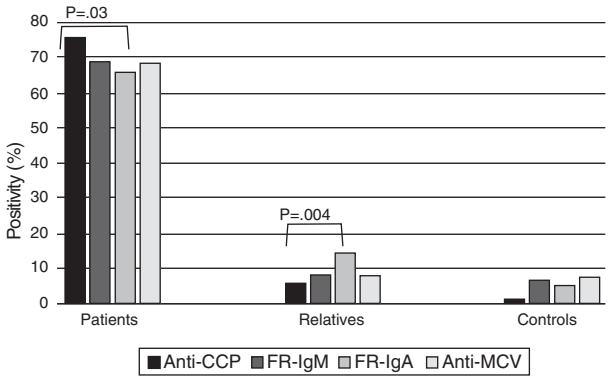

Fig. 1 – Positivity of biomarkers in the studied groups. Patients: anti-CCP+ vs. IgA-RF+: $p = 0.03$; OR = 1.62; 95% CI = 1.06–2.48. All other comparisons: p = ns. Relatives: anti-CCP+ vs. IgA-RF+: $p = 0.004$; OR = 0.34; 95% CI = 0.16–0.70. All other comparison: p = ns. Controls: all comparisons p = ns. Relatives IgA-RF+ vs. Controls IgA-RF+: $p = 0.03$; OR = 2.98; 95% CI = 1.11–7.98. All other comparisons: p = ns. ns, not significant.

Statistical analyses

Statistical analyses were performed using GraphPad Prism 4.0 (GraphPad Software Inc., La Jolla, USA) and Statistic 5.5 (StatSoft Inc., Tulsa, USA). Comparisons between autoantibodies levels were done applying nonparametric Kruskal–Wallis or Mann–Whitney tests. Chi-square and Fisher's exact tests were applied for the analyses of the positivity between groups. Spearman's test was used for correlation analysis. The significance level was set at 0.05.

Results

Prevalence of biomarkers in RA patients, relatives and controls

The prevalence of each biomarker in RA patients, their relatives and controls can be seen on Fig. 1, where it can be noted that the most prevalent biomarker in RA patients is anti CCP and the most common in relatives was IgA RF.

The median titer of each biomarker was studied in the 3 groups and results are showed in Fig. 2 where it is possible to see that relatives had an intermediate value between patients and controls.

In RA patients a significant correlation was observed among the titers of the four investigated biomarkers, but the correlation between anti-MCV and anti-CCP ($r = 0.73$), and IgM-RF and IgA-RF ($r = 0.71$) were both stronger compared to other associations (anti-MCV/IgM-RF, $r = 0.38$; anti-MCV/IgA-RF, $r = 0.41$; IgM-RF/anti-CCP, $r = 0.46$; IgA-RF/anti-CCP, $r = 0.47$).

The presence of the four biomarkers simultaneously positive in the RA group occurred in 46.2% while in the relatives it was found in 2% and in none of controls.

Table 2 – Association between demographic and clinical data and biomarkers in RA patients.

	Number of positive biomarkers N (%)					*p*
	0 (*n* = 28)	1 (*n* = 15)	2 (*n* = 28)	3 (*n* = 42)	4 (*n* = 97)	
Female gender	26/28 (92.8)	9/15 (60)	25/28 (89.3)	42/42 (100)	76/97 (78.4)	0.0006[a]
Age at disease onset (median)	18–75 (41.5; IQR 27.2–48.7)	24–69 (46.0; IQR 35.0–53.0)	19–69 (43.5; IQR 31.5–50.0)	18–83 (43.5; IQR 30–49.7)	16–71 (46; IQR 32.7–52.0)	0.50[c]
Age (mean)	48.2 ± 10.4	50.3 ± 12.9	50.1 ± 11.9	52.9 ± 13.79	51.7 ± 11.35	0.55[d]
Disease duration (median/years)	1–28 (7.0; IQR 3.0–12.8)	1–27 (4.0; IQR 2.0–7.0)	1–35 (7.5; IQR 3.2–14.2)	1–60 (7.0; IQR 3.0–11.7)	1–36 (6.5; IQR 3.2–12.0)	0.36[c]
Functional class						
Class I	15/28 (53.6)	8/15 (53.3)	13/28 (46.4)	22/42 (52.4)	41/96 (42.7)	0.75[a]
Class II	10/28 (35.7)	6/15 (40)	11/28 (39.3)	17/42 (40.5)	39/96 (40.6)	0.99[a]
Class III + IV	3/28 (10.7)	1/15 (6.7)	4/28 (14.3)	3/42 (7.1)	16/96 (16.7)	0.53[a]
Eurodescendent	18/24 (75)	10/13 (76.9)	17/26 (65.4)	28/38 (73.7)	58/79 (73.4)	0.91[a]
Afrodescendent	6/24 (25)	3/13 (23.1)	9/26 (34.6)	10/38 (26.3)	20/79 (25.3)	0.90[a]
Tobacco exposure	13/25 (52)	7/12 (58.3)	12/26 (46.1)	16/39 (41.02)	39/87 (44.8)	0.82[a]
Nodules	2/24 (8.33)	0/13 (0)	4/28 (14.28)	1/37 (2.7)	10/88 (11.36)	0.32[a]
Sjögren syndrome	1/19 (5.2)	3/11 (27.2)	4/24 (16.6)	16/33 (48.4)	25/75 (33.3)	0.009[a]
Lung fibrosis	1/22 (4.50)	1/11(9.0)	2/24 (8.3)	5/32 (15.6)	6/72 (8.3)	0.68[a]

IQR, interquartile range.

[a] Chi-square.

[b] Fisher test.

[c] Kruskal–Wallis test.

[d] Anova (one way).

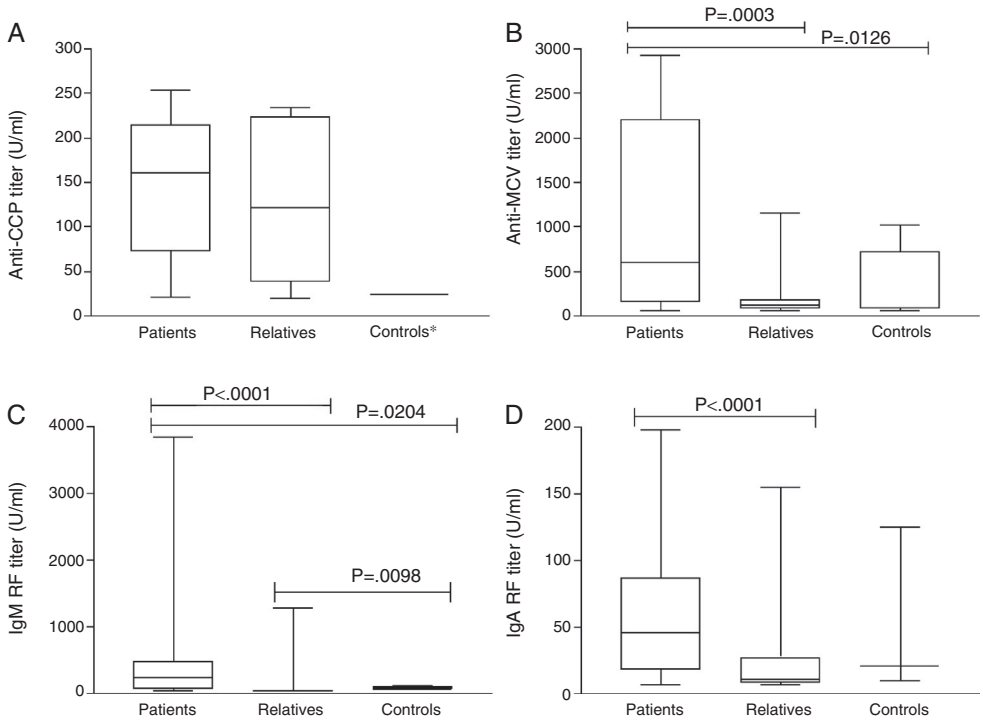

Fig. 2 – Anti-CCP, anti-MCV, IgM-RF and IgA-RF in patients, relatives and controls. *Analysis not applicable.

Association between biomarkers and clinical findings in RA patients and relatives

Table 2 shows demographic and clinical data of RA patients and their association with the number of positive biomarkers.

EAM were detected in 34.8% (69/198) of the patients. Patients that were negative for all biomarkers had a lower frequency of EAM (5.8% vs. 17.8%, $p = 0.01$; OR = 3.25; 95% CI = 1.16–10.66). An increase in the concomitant positivity for 3 biomarkers was observed in patients with EAM compared to patients without it (Table 3). In the one by one analysis of the different EAM, only the p value for secondary Sjögren syndrome remained significant ($p = 0.01$; OR = 2.73; 95% CI = 1.24–6.08).

Table 4 shows association between demographic, clinical and serological data and biomarkers positivity in relatives. Among the relatives 11.7% (22/197) had arthralgia, and a significant frequency of arthralgia was observed in positive relatives, regardless of the type of biomarker. Each biomarker presented significant association with the others autoantibodies tested (Table 4).

Follow-up of positive relatives

The relatives were followed prospectively for a median time of 7 years (range 2–8; IQR = 6–7 years) with biannual evaluations. At moment, three positive relatives (two women and one man, mean age 43.3 years) had the diagnosis for RA confirmed; all of them with high biomarker titers. Among those relatives, one was positive for all 4 biomarkers and his sibling (with RA) had high titers of anti-CCP. In addition, seven unaffected relatives with high autoantibody titers and some symptoms of the disease are being followed. Two of them are positive for 4 biomarkers concomitantly, two for 2 biomarkers and three for one biomarker. None of the other positive relatives for tested biomarkers had diagnosis criterions for RA.

No RA cases were detected among relatives negatives for autoantibodies.

Discussion

This is a pioneer study in which four serological biomarkers were evaluated concomitantly in patients with RA and their relatives in a Southern Brazilian population. Previous studies have showed an increased positivity for autoantibodies in unaffected family members when compared to healthy control subjects[5,17,20,21] and the present results corroborate these findings.

Although IgA-RF specificity for RA is not as high as anti-CCP, IgA-RF can be detected several years before the symptoms of the disease, suggesting a primary role of IgA-RF in the pathogenesis of RA.[14] This is a very interesting observation since in the present study IgA-RF was the biomarker with higher positivity among relatives. In addition, several relatives were positive for RF and anti-MCV, but the three relatives that had confirmed RA were all positive for anti-CCP, reinforcing the high specificity (99%) and positive predictive value (99%) for this autoantibody. Supporting this observation, anti-CCP was the only biomarker with no significantly different titers when patients and relatives were compared (Fig. 2A).

Regarding the number of positive biomarkers, a previous study[22] showed that most RA patients were positive for the 4 biomarkers simultaneously while relatives usually had just one antibody, as confirmed in this study. One explanation for

Table 3 – Extra articular manifestations according to the number of positive biomarkers.

Number of positive biomarkers	With EAM (*n* = 69) N (%)	Without EAM (*n* = 129) N (%)	*p*
4 (*n* = 90)	34 (49.3)	56 (43.4)	ns
3 (*n* = 39)	19 (27.5)	20 (15.5)	0.05
2 (*n* = 28)	9 (13.1)	19 (14.7)	ns
1 (*n* = 14)	3 (4.3)	11 (8.5)	ns
No biomarker (*n* = 27)	4 (5.8)	23 (17.8)	0.01

EAM, extra articular manifestation; ns, not significant.
Note: 3 biomarkers with EAM vs. 3 biomarkers without EAM; $p = 0.059$; OR = 1.26; 95% CI = 0.70–2.27. No biomarker with EAM vs. No biomarker without EAM; $p = 0.0179$; OR = 3.25; 95% IC = 1.16–10.66. Fisher test.

Table 4 – Association between demographic, clinical and serological data and biomarkers positivity in the RA patients relatives.

	IgM-RF+ (*n* = 16)	IgM-RF− (*n* = 182)	*p*
Euro-descendent	14/16	140/182	0.53[a]
Afrodescendent	2/16	42/182	0.53[a]
Tobacco exposure	3/16	54/182	0.56[a]
Female	13/16	108/182	0.11[a]
Mean age (years)	40.3 ± 12.3	36.5 ± 15.6	0.34[c]
Arthralgia	6/16	16/178	0.004[a]
Anti-CCP+	5/16	6/182	0.0006[a]
IgA-RF+	6/16	23/182	0.01[a]
Anti-MCV+	5/16	11/182	0.004[a]
	IgA-RF+ (*n* = 29)	**IgA-RF− (*n* = 169)**	***p***
Euro-descendent	24/29	130/169	0.63[a]
Afrodescendent	5/29	39/169	0.63[a]
Tobacco exposure	9/29	48/169	0.77[b]
Female	17/29	104/169	0.76[b]
Mean age (years)	40.6 ± 15.4	36.1 ± 15.3	0.15[c]
Arthralgia	6/29	16/165	0.08[b]
Anti-CCP+	4/29	7/169	0.059[a]
IgM-RF+	6/29	10/169	0.007[b]
Anti-MCV+	7/29	9/169	0.0006[b]
	Anti-CCP+ (*n* = 11)	**Anti-CCP− (*n* = 187)**	***p***
Euro-descendent	9/11	145/187	1.00[a]
Afrodescendent	2/11	42/187	1.00[a]
Tobacco exposure	3/11	54/187	1.00[a]
Female	9/11	112/187	0.37[a]
Mean age (years)	39.3 ± 15.9	36.6 ± 15.4	0.57[c]
Arthralgia	4/11	18/183	0.02[a]
IgM-RF+	5/11	11/187	0.0006[a]
IgA-RF+	4/11	25/187	0.05[a]
Anti-MCV+	5/11	11/187	0.0006[a]
	Anti-MCV+ (*n* = 16)	**Anti-MCV− (*n* = 182)**	***p***
Euro-descendent	12/16	142/182	0.75[a]
Afrodescendent	4/16	40/182	0.75[a]
Tobacco exposure	4/16	53/182	1.00[a]
Female	14/16	107/182	0.03[a]
Mean age (years)	33.5 ± 16.9	37.1 ± 15.2	0.37[c]
Arthralgia	6/16	22/178	0.0006[a]
Anti-CCP+	5/16	6/182	<0.0001[b]
IgM-RF+	5/16	11/182	0.004[a]
IgA-RF+	7/16	22/182	0.002[b]

[a] Fisher test.
[b] Chi-square.
[c] Unpaired t test.

this outcome may be the epitope spreading phenomenon that causes increasing antibody levels just prior to the flare of clinical disease.[23]

Some studies have suggested that anti-MCV has a comparable value to anti-CCP for RA diagnosis, with even higher sensitivity.[24,25] This was not the case in the present study when the prevalence, sensitivity and specificity of anti-MCV were lower than those of anti-CCP.

A strong correlation between RA biomarkers has been previously disclosed. Poulsom and Charles[26] showed a good correlation between anti-MCV and anti-CCP and between anti-MCV and IgM-RF, but not between anti-CCP and IgM-RF. We found significant correlation between all markers, especially between anti CCP and anti MCV and between RF IgA and IgM. This pattern was previously described in first RA degree relatives[27] and may be associated with the presence of common epitopes.

Our casuistic presented a high prevalence of EAMs (34.8%) that may be explained by genetic and environmental factors that influence the immune reactions leading to their development.[28] We observed positivity for a higher number of biomarkers among patients with EAMs, in special secondary SS.

The present results for relatives of RA patients demonstrated that serological screening is feasible and useful tool for the screening of those individuals at risk, as emphasized by other authors.[17,21,22] The high prevalence of arthralgia in relatives positive to any of the four biomarkers highlights this hypothesis, since some of these relatives (three) have already evolved to full blown RA. All the relatives who developed RA presented high biomarkers titers; one was positive for all four biomarkers and had one extra sibling positive for anti-CCP. Among the other two, one was positive for both anti-CCP and IgM-RF, while the other was only positive for anti-CCP. The majority of relatives with autoantibodies who did not develop the disease so far were positive for just one biomarker. However this does not rule out the great risk for them to develop the disease. It is possible that they may posteriorly seroconvert to two, three or even more biomarkers as shown in the patients with established RA.

Finally, the number of biomarkers was higher in RA patients with EAMs but it was not linked to duration of the disease and/or the functional class. On the other hand, seronegative patients presented less EAMs. High frequency of arthralgia was observed in positive relatives, regardless of the biomarker type. Future studies may highlight the fascinating link between the presence of distinct biomarkers and the pathophysiologic mechanisms of AR.

Conflicts of interest

The authors declare no conflicts of interest.

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