Anti-SSA Ro52 and anti-Ro60 autoantibodies: association with clinical phenotypes

C. Mazeda[1-3], N. Oliveira[4], C. Abreu[5], V. Fraga[5], I. Maduro[6], A. Saraiva[6], L. Inês[6,7], C. Ferreira[8], A.M. Correia[8], R. Nicolau[9], F. Farinha[10], I. Villanueva[11], D. Jesus[7,12], P. Abreu[13], A. Neto[14], J. Silva Dinis[15], A. Barcelos[1-3,16]

[1]Dept. of Rheumatology, Centro Hospitalar Baixo Vouga, Aveiro; [2]Egas Moniz Health Alliance AcademicClinical Center, Aveiro; [3]EpiDoc Unit, Nova Medical School, NOVA University Lisbon, Lisbon; [4]Dept. of Mathematics, Universidade de Aveiro, Aveiro; [5]Dept. of Rheumatology, Hospital Garcia de Orta, Almada; [6]Dept. of Rheumatology, Centro Hospitalar e Universitário de Coimbra, Coimbra; [7]Faculty of Health Sciences, Universidade da Beira Interior, Covilhã; [8]Dept. of Rheumatology, Hospital de Braga, Braga; [9]Dept. of Rheumatology, Centro Hospitalar e Universitário São João, Porto; [10]Dept. of Rheumatology, and [11]Dept. of Clinical Pathology, Hospital Distrital de Santarém, Santarém; [12]Dept. of Rheumatology, Centro Hospitalar de Leiria, Leiria; [13]Dept. of Rheumatology, ULS Castelo Branco, Castelo Branco; [14]Dept. of Rheumatology, Hospital Dr. Nélio Mendonça, Funchal; [15]Dept. of Rheumatology, Centro Hospitalar Universitário Lisboa Central, Lisbon; [16]Comprehensive Health Research Center, Universidade NOVA de Lisboa, Lisbon, Portugal.

Abstract

Objective

Anti-SSA autoantibodies can be differentiated according to their antigenic target proteins as anti-Ro60 (60 kDa) or anti-Ro52 (52 kDa). Anti-SSA(Ro60) antibodies are clearly associated with connective tissue diseases (CTD), but the clinical significance of anti-SSA(Ro52) antibodies remains unclear. The aim of the present study was to analyse the disease phenotype of patients with anti-Ro52 and/or anti-Ro60 antibodies.

Methods

A multicentre, cross-sectional study was carried out of positive anti-Ro52 and/or Ro60 antibodies patients followed at 10 Rheumatology centres from January 2018 until December 2021. Patients were categorised into 3 groups: group 1 (Ro52+/Ro60-); group 2 (Ro52-/Ro60+); group 3 (Ro52+/Ro60+). Antinuclear antibodies were evaluated by indirect immunofluorescence assay and further screened for anti-extractable nuclear antigen (ENA) antibodies. Demographicsand clinical data were compared between the 3 groups, by patients' medical chart review. Univariate analysis was performed and subsequently logistic regression was used to identify intergroup differences and calculate the odds ratio with a 95% confidence interval (95% CI).

Results

We included 776 patients [female: 83.1%; median age: 59 (46-71) years]. Groups 1, 2, and 3 comprised 31.1%, 32.6%, and 36.3% of the patients, respectively. Anti-Ro52 antibody alone was more frequently associated with non-rheumatic diseases, older age, and men ($p<0.05$). Among patients with CTD, the diagnosis of systemic lupus erythematosus is 3 and 2 times more prevalent in groups 2 and 3, respectively, than in group 1 [OR 2.8 (95% CI 1.60, 4.97), $p<0.001$; OR 2.2 (95% CI 1.28, 3.86), $p<0.01$]. In group 2, the diagnosis of undifferentiated CTD is more frequent than in the other groups. Group 1 was more frequently associated with inflammatory myositis than group 2 [OR 0.09 (95% CI 0.01, 0.33), $p<0.001$] or group 3 [OR 0.08 (95% CI 0.01, 0.29), $p<0.001$]. Group 1 was also more frequently associated with arthritis ($p<0.01$), interstitial lung disease ($p<0.01$), and myositis ($p<0.01$).

Conclusion

Anti-Ro52+ antibody alone is frequently found in patients with non-rheumatic diseases. In addition, anti-Ro52+ antibody is also prevalent in patients with CTD and associated with clinical phenotypes that are different from anti-Ro60+ antibody.

Key words

anti-Ro52, anti-Ro60, Sjögren's syndrome, systemic lupus erythematosus, idiopathic inflammatory myositis

Carolina Mazeda, MD
Natacha Oliveira
Catarina Abreu, MD
Vanessa Fraga, MD
Isabel Maduro, MD
André Saraiva, MD
Luís Inês, PhD
Carla Ferreira, MD
Ana Margarida Correia, MD
Rafaela Nicolau, MD
Filipa Farinha, MD
Ingrid Villanueva, MD
Diogo Jesus, MD
Pedro Abreu, MD
Agna Neto, MD
Joana Silva Dinis, MD
Anabela Barcelos, MD

Please address correspondence to:
Carolina Mazeda
Rheumatology Department,
Centro Hospitalar do Baixo Vouga,
Rua Artur Navarra,
3814-501 Aveiro, Portugal.
E-mail: carolina_m43@hotmail.com

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ORCID iD:
C. Mazeda: 0000-0002-9200-5022
L. Inês: 0000-0003-3172-3570
F. Farinha: 0000-0002-1517-4383
J. Dinis: 0000-0002-4829-1551
A. Barcelos: 0000-0002-4116-8964

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Introduction

Anti-SSA/Ro antibodies are one of the most frequently detected autoantibodies in sera patients and contain two main isoforms with molecular weights of 60 kDa and 52 kDa (1, 2). Historically, anti-SSA/Ro antibodies were described as a cytoplasmic ribonucleoprotein complex consisting of two different proteins, however, current evidence shows that they are located in different cellular compartments coded by distinct cDNAs and can be differentiated according to their antigenic target proteins, anti-Ro60 (60 kDa) or anti-Ro52 (52 kDa) (3). The presence of anti-Ro52 or anti-Ro60 antibodies seems to be linked with distinct biochemical and immunological associations. The Ro52 antigen has been recognised as the Tripartite motif-containing protein 21 (TRIM21), which is a member of the TRIM protein family. It acts in the ubiquitination process and can be positively regulated and translocated to the nucleus in a pro-inflammatory environment and regulate the production of type 1 interferon and cytokines (4, 5) Ro-60 is a protein that is involved in binding to RNAs that acts in the quality control process of these through the identification and dealing with defective or misfolded RNA molecules (6). Anti-Ro60 antibodies are clearly associated with connective tissue diseases (CTD), namely systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS) (7, 8), but the clinical significance of anti-Ro52 antibody remains unclear. Ro52 reactivity has been reported in several CTD and organ-specific autoimmune disorders, such as autoimmune hepatitis and primary biliary cholangitis (7-9), but also in non-autoimmune conditions such as infections and neoplastic diseases (10). Furthermore, in previous studies, the anti-Ro52 antibody has been associated with intestinal lung disease (ILD) in the spectrum of various rheumatologic diseases and also seems to indicate a more aggressive ILD in idiopathic inflammatory myositis (IIM) and systemic sclerosis (SSc) (11-14). Thus, these antibodies appear to exhibit a limited structural and functional homology between them, and despite their high prevalence, the information available on their individual clinical significance is limited. The aim of our study was to analyse the disease phenotypes of patients with anti-Ro52 and/or anti-Ro60 antibodies.

Material and methods

Study design and patients

This was a multicentre cross-sectional study, including patients followed at ten Rheumatology centres from January 2018 until December 2021. We included all adult patients (≥18-year-old) who had at least one Rheumatology appointment and presenting at least once with positive anti-Ro52 and/or Ro60 antibodies. Patients were categorized into three groups according to their positivity for anti-Ro52 and/or anti-Ro60 antibodies: group 1 (Ro52+/Ro60-); group 2 (Ro52-/Ro60+) and group 3 (Ro52+/Ro60+) (Fig. 1).

Study clinical and laboratory variables

Demographic, laboratory, and clinical data for all participants were collected by reviewing their clinical charts at time of inclusion and registered in an anonymised study database. The clinical diagnosis of each patient was established by the attending rheumatologist. Study variables also included: gender; age; race; anaemia (defined as <11.5 and <13 g/dL for females and males, respectively); leukopenia (<11.1x10^9/L); lymphopenia (<1.0×10^9); thrombocytopenia (<100x10^9/L); hypergammaglobulinaemia (gammaglobulin >15 g/L, by nephelometry); renal insufficiency (glomerular filtration rate <60 mL/min/1.73m^2); hycomplementaemia C3 (<90 mg/dL); hypocomplementaemia C4 (<12 mg/dL). In patients with CTD, data regarding organ involvement (skin, mucosae, vascular (microvascular dysfunction), haematological, joints/muscles, lung, heart, liver, gastrointestinal, kidney, peripheral, and central nervous systems) were collected, defined based on specific symptoms, signs, laboratory, radiologic, and/or histopathologic results.

Autoantibody variables

Antinuclear autoantibodies (ANA) were determined at each hospital's immunology laboratory with an indirect

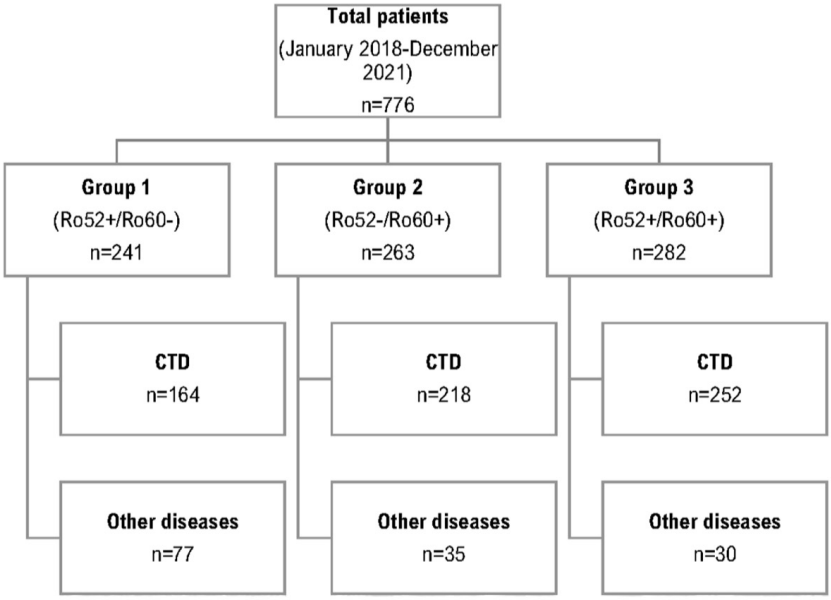

Fig. 1. Study flow chart of patient selection.
CTD: connective tissue diseases; n: number.

immunofluorescence test (IIFT) on human epithelial cells (HEp-2) and the positivity threshold was set at a titre of 1/160. The anti-extractable nuclear antigens (ENA) were detected using an ELISA technique or by line immunoassay (LIA) (Euroimmun, Lubeck, Germany). Anti-Ro52 and anti-Ro60 antibodies were defined as positive/detected if the line density reading exceeded 10 units, as per the manufacturer's recommendations. The presence of other anti-ENA antibodies specificity or other autoantibodies [anti-cyclic citrullinated peptide (anti-CCP), rheumatoid factor (RF), anticardiolipin antibodies IgG or IgM, anti-beta-2-glycoprotein-I antibodies (anti-β2GP1) IgG or IgM, lupus anticoagulant (LA)] were detected by the usual methods used at local centres. For each autoantibody variable, positivity was defined as at least one positive determination.

Statistical analysis

All analyses were performed in the software R (v. 4.1.2) and Rstudio IDE (v. 2022.07.1+554) Descriptive statistics were presented as median and interquartile range (IQR) for continuous variables and absolute and relative frequencies for categorical variables. Statistical tests were applied to compare the three groups [group 1 (Ro52+/Ro60-); group 2 (Ro52-/Ro60+) and group 3 (Ro52+/Ro60+)]. Univariate analysis was performed using Kruskal-Wallis, chi-square, and Fisher's exact tests, as appropriate. p-values ≤ 0.05 were considered statistically significant. Subsequently, significant variables were subjected to multiple comparisons (chi-square, Fisher's exact, and Dunn's tests), under Bonferroni correction (significance level: $p \leq 0.0167/1.67\%$), to detect differences between individual serology-based groups. Logistic regressions were used to calculate related odds ratios (OR) with a 95% confidence interval (CI). Cases with missing information were excluded from the respective inferential analysis.

Ethical considerations

This study was approved by the Centro Hospitalar Baixo Vouga Ethics Committee (no: 44-05-2022/CES). The ethics committee approved the waiver of informed consent due to the retrospective nature of the study. This work's databases and all research process steps were fully anonymised.

Results

We included 776 patients with positive anti-Ro52 and/or anti-Ro60 antibodies. Among these patients, 645 (83.1%) were female with a median age of 59 (46–71) years. The most frequent CTD diagnosis was SS (52.1%), followed by SLE (22.1%), undifferentiated CTD (UCTD) (10.6%), rheumatoid arthritis (RA) (8%), IIM (3%) and mixed connective tissue disease (MCTD) (2.5%). Serum anti-Ro52 antibodies were present in 241 (31.1%), anti-Ro60 in 253 (32.6%) and both antibodies in 282 (36.34%) of patients. Other autoantibodies were detected in participants' sera, particularly anti-La, RF, anti-dsDNA, and anti-RNP. LA was also positive in 8.4% of the patients (Table I).

Patients in these three groups were different with respect to demographical, clinical, and immunological data (Tables I and II). In the group with positive anti-Ro52 antibody (group 1), patients were older [64 (52-76) *vs*. 56 (44-67) in group 2 *vs*. 57 (44-69) in group 3, $p<0.001$] and had a lower proportion of females (76.8% *vs*. 84.6% in group 2 *vs*. 87.2 in group 3, $p=0.005$). Regarding disease diagnosis, when anti-Ro52 antibody was present alone, it was more frequently associated with non-rheumatic diseases, with neoplasia and infectious diseases being the most frequent pathologies (28.6% and 14.3%, respectively). In this group 1, the probability of diagnosing CTD is 2.9 and 3.9 times lower compared to groups 2 and 3, respectively [OR 2.92 (95% CI 1.88, 4.62), $p \leq 0.001$; OR 3.94 (95% CI 1.50, 6.36), $p \leq 0.001$].

Distinctive differences in associations with CTD and other autoantibodies became evident when comparing the three groups. The diagnosis of SLE was 3 and 2 times more prevalent in groups 2 and 3, respectively, than in group 1 [OR 2.8 (95% CI 1.60, 4.97), $p<0.001$; OR 2.2 (95% CI 1.28, 3.86), $p=0.007$]. Also, in these groups, 2 and 3, the proportion of individuals with anti-dsDNA antibodies was four times higher compared to group 1 ($p<0.0001$). It is noteworthy that isolated anti-Ro60 antibody patients showed a slightly higher prevalence of anti-LA ($p=0.008$) and anticardiolipin antibodies ($p=0.024$), but not anti-β2GP1 antibodies ($p=0.736$).

Regarding SS, it was equally prevalent in group 1 and group 3, but the probability of this condition in these groups was significantly higher than in group 2 [OR 0.53 (95% CI 0.35, 0.79), $p=0.003$; OR 2.15 (95% CI 1.49,

Table I. Characteristics of the study population according to the groups of positivity anti-SSA(Ro) antibodies.

	Group 1 (n=241) Ro52+/Ro60-	Group 2 (n=253) Ro52-/Ro60+	Group 3 (n=282) Ro52+/Ro60+	*p*
Age, median (IQR)	64 (52-76)	56 (44-67)	57 (44-69)	**<0.001**
Female, n (%)	185 (76.8)	214 (84.6)	246 (87.2)	**0.005**
Other anti-ENA, n (%)				
Anti-La	24 (10)	50 (19.8)	114 (40.4)	**<0.001**
Anti-RNP	11 (4.6)	23 (9.1)	17 (6.0)	0.115
Anti-Scl70	3 (1.2)	4 (1.6)	6 (2.1)	0.146
Anti-Jo1	7 (3)	1 (0.4)	3 (1.1)	0.070
Anti-Sm	1 (0.4)	7 (2.8)	6 (2.13)	0.098
Anti-Pl7/Pl12	4 (1.7)	0 (0)	1 (0.4)	**0.044**
Anti-dsDNA, n (%)	11 (4.6)	39 (15.4)	37 (13.1)	**<0.001**
Anti-centromere, n (%)	12 (5)	3 (1.2)	6 (2.1)	**0.026**
Lupus anticoagulant, n (%)	10 (4.2)	32 (12.7)	23 (8.2)	**0.008**
Anti-cardiolipin, n (%)	8 (3.3)	23 (8.2)	10 (3.9)	**0.024**
Anti-2 glycoprotein 1, n (%)	6 (2.5)	10 (3.9)	10 (3.6)	0.736
Rheumatoid factor, n (%)	46 (19.1)	44 (17.4)	81 (28.7)	**0.001**
Anti-CCP, n (%)	11 (4.6)	15 (5.9)	19 (6.7)	0.327
Non-rheumatic disease, n (%)	77 (32)	35 (13.8)	30 (10.6)	**<0.001**
Infections	11 (14.3)	2 (5.7)	1 (3.3)	0.192
Neoplasms	22 (28.6)	3 (8.6)	6 (20.0)	0.057
Interstitial lung disease	5 (6.5)	4 (11.4)	0	0.168
Other diseases	46 (59.7)	25 (71.4)	22 (73.3)	-
Immune-mediated rheumatologic disease, n (%)	164 (68.1)	218 (86.2)	252 (89.4)	**<0.001**
Sjögren syndrome	92 (56.1)	88 (40.3)	150 (59.5)	**<0.001**
Systemic lupus erythematosus	20 (12.2)	61 (28)	59 (23.4)	**0.001**
Systemic sclerosis	11 (6.7)	7 (3.2)	8 (3.2)	0.150
Inflammatory myositis	15 (9.2)	2 (0.9)	2 (0.8)	**<0.001**
Rheumatoid arthritis	18 (11)	17 (7.8)	16 (6.4)	0.234
Undifferentiated connective tissue disease	11 (6.7)	35 (16.1)	21 (8.3)	**0.004**
Mixed connective tissue disease	6 (3.7)	6 (2.8)	4 (1.6)	0.406
Other diseases*	9 (5.5)	8 (3.7)	10 (4.0)	-

*Included patients diagnosed with psoriatic arthritis (n=8), anti-phospholipid syndrome (n=5), osteoporosis (n=4), primary biliary cholangitis (n=3), polymyalgia rheumatica (n=3), ankylosing spondylitis (n=2) and nodal osteoarthrosis of the hand (n=2).
n: number; IQR: interquartile range; ENA: extractable nuclear antigens; anti-dsDNA: anti-double stranded DNA; anti-CCP: anti-cyclic citrullinated peptide.

Table II. Associations between connective tissue diseases and presence of anti-Ro52 and/or anti-Ro60 antibodies.

	Ro52-Ro60+ *vs*. Ro52+Ro60- OR (CI 95%), *p*	Ro52+Ro60+ *vs*. Ro52+Ro60- OR (CI 95%), *p*	Ro52+Ro60+ *vs*. Ro52-Ro60+ OR (CI 95%), *p*
Sjögren syndrome	**0.53 (0.35, 0.79), *p*=0.003**	1.14 (0.77, 1.70), *p*=0.587	**2.15 (1.49, 3.12), *p*<0.001**
Systemic lupus erythematosus	**2.80 (1.60, 4.97), *p*<0.001**	**2.2 (1.28, 3.86), *p*=0.007**	0.78 (0.51, 1.18), *p*=0.282
Inflammatory myositis	**0.09 (0.01, 0.33), *p*<0.001**	**0.08 (0.01, 0.29), *p*<0.001**	0.473 (0.26, 0.83), *p*=0.145
Undifferentiated connective tissue disease	**2.66 (1.35, 5.65), *p*=0.009**	1.26 (0.60-2.78), *p*=0.68	**0.47 (0.26,0.83), *p*=0.014**

OR: odds ratio; CI: confidence interval.

3.12), $p<0.001$]. Group 3 exhibited a statistically significant prevalence of anti-La comparing to the other groups ($p<0.001$).

The presence of isolated anti-Ro52 (group 1) was more frequently associated with IMM than in group 2 [OR 0.09 (95% CI 0.01, 0.33), $p\leq0.001$] or group 3 [OR 0.08 (95% CI 0.01, 0.29), $p\leq0.001$]. In this group, a significantly higher proportion of anti-PL7 and PL12 antibodies was observed ($p=0.04$), however the three groups did not differ in the prevalence of other specific myositis antibodies, namely anti-Jo1 and anti-Mi2.

UCTD was more prevalent in group 2 than in the other groups, being approximately two times more frequent in this group than in group 1 [OR 2.66 (95% CI 1.35, 5.65), $p=0.009$]. Regarding the other conditions, namely RA, SSc, and MCTD, no differences were observed among the three groups.

Among patients with CTD, we assessed the presence of specific organ involvement in each group, as represented in Table III. When evaluating the associations of specific clinical manifestations from different systems and organs with anti-Ro52 and/or anti-Ro60 antibodies reactivity, we found that double positivity (group 3) more frequently associated with mucocutaneous involvement, specifically sicca symptoms ($p=0.014$), malar rash ($p<0.001$) and oral ulcers

Table III. System and organ involvements according to the groups of anti-Ro positivity in patients with connective tissue diseses.

Organ involvement, n (%)	Group 1 (n=164)	Group 2 (n=218)	Group 3 (n=252)
Mucocutaneous	87 (53.1)*	81 (37.2)	141 (56.0)***
Musculoskeletal	80 (48.8)*	65 (29.8)	100 (39.7)
Haematological	42 (25.6)	79 (36.2)	74 (29.4)
Pulmonary	24 (14.6)*,**	10 (4.7)	19 (7.5)
Cardiovascular	2 (1.2)	4 (1.8)	2 (0.8)
Renal	6 (3.7)	13 (6.0)	17 (6.8)
Neurological	1 (0.6)	2 (0.9)	7 (2.8)

n: number. *p<0.05 for the comparison between solo anti-Ro52 *vs*. solo anti-Ro60;
**p<0.05 for the comparison between solo anti-Ro52 *vs*. combined anti-Ro52 and anti-Ro60;
* p<0.05 for the comparison between solo anti-*Ro60 vs*. combined anti-Ro52 and anti-Ro60.

(p=0.004). On the other hand, isolated Ro52 positivity (group 1) was more frequently associated with arthritis (p=0.006), ILD (p=0.002), and myositis (p=0.009).

Discussion

In our cohort, the evaluation of positive patients for anti-Ro52 antibodies revealed different clinical and laboratory characteristics associated with the presence or absence of anti-Ro60 autoantibodies. The co-existence of both antibodies was associated with higher frequency of primary SS and associated with mucocutaneous involvement, including sicca symptoms, malar rash, and oral ulcers. Isolated anti-Ro52 antibodies were clinically associated with arthritis, ILD, and myositis. Patients with isolated anti-Ro60 antibodies were more frequently diagnosed with SLE. Consequently, these associations could assist in identifying distinct patient subgroups.

We observed that autoreactivity for both Ro52 and Ro60 antibodies was the most frequently detected, as previously reported (5, 15), and most patients were diagnosed with CTD. An association between anti-Ro52 antibodies and specific clinical features, namely ILD, and survival in patients with CTD was reported (7). The co-existence of both antibodies associated with primary SS was also reported by Robbins *et al*. (15) and, as expected, this group of patients was more frequently positive for anti-SSB. On the other hand, patients with isolated anti-Ro60 antibodies were more frequently diagnosed with SLE (3, 15, 16). Supporting the significant role of this specificity as a prominent autoantigen in SLE, it has been demonstrated that mice lacking this protein develop a lupus-like syndrome and it has been reported that anti-Ro60 antibodies frequently initiate the human autoimmune response in SLE (17, 18). In these patients, it was also frequent an association with LA and anticardiolipin antibodies. This correlation has been scarcely explored in the previous literature. Similar to our study, Robbins *et al*. found a positive association with these antibodies,15 however a contrasting negative relationship was reported in a single study (19). This occurrence could be attributed to a specific patient phenotype observed in SLE and antiphospholipid antibodies (15, 20). The prevalence of CTD in the isolated anti-Ro52 group (68.1%) is consistent with other cohorts (31.3% to 73.5%) (3, 10, 15, 21). Anti-Ro52 antibodies are considered myositis-associated autoantibodies and are often detected alongside antibodies to aminoacyl-tRNA synthetase. Multiple studies have demonstrated their diagnostic value in patients with IIM, distinguishing patients with antisynthetase syndrome (ASS) who experience more severe ILD, and a less favourable prognosis compared to ASS patients without anti-Ro52 antibodies (22). Regarding SS, some studies show that SS patients with isolated Ro-52 had a greater degree of sicca symptoms (23), and others describe a more severe disease with higher disease activity (ESSDAI) and increased incidence of cryoglobulinaemia (24). Our study was not specifically designed to predict the severity of pathologies. However, an important strength of our research lies in identifying distinct laboratory profiles associated with anti-Ro52 and anti-Ro60 antibodies, independent of specific diagnosis of CTD. This aspect has been minimally explored in previous studies. Furthermore, understanding the patients' autoantibody profile can aid in predicting and improving the screening for potential clinical syndromes that may arise. Several studies suggest that the isolated presence of anti-Ro52 antibody may be associated with the occurrence of ILD in different conditions beyond IIM (22), such as SS and SSc, constituting an independent risk factor for this manifestation (7, 25-29). Isolated anti-Ro52 antibodies were frequently associated with non-CTD diagnoses, such as neoplastic and infectious diseases (3, 10, 15).

An important limitation of our study was the retrospective and cross-sectional nature of the analysis. Some autoantibodies exhibit significant variability in their reactivities over time, potentially altering the categorisation of anti-Ro antibodies (30). Thus, conducting a longitudinal study will allow monitoring of the appearance of new organ involvements or laboratory data in each CTD, as well as changes in the serological status of patients. Additionally, longitudinal analysis of patients with isolated anti-Ro52 antibodies could be instrumental in determining whether this serological profile represents mere epiphenomena resulting from a dysregulated immune response or serves as a predictive biomarker for autoimmune diseases. Other limitations to consider were the fact that the study was conducted across multiple centres and different physicians, and CTD diagnoses were based on the clinical judgment of the rheumatologist and did not consider classification criteria. Although this limitation is inherent to multicentre studies, all considered patients were evaluated by an experienced rheumatologist.

Considering the results of our study, we believe it is important to detect anti-Ro52 and anti-Ro60 antibodies individually. These antibodies are linked to various clinical features of diseases, providing valuable insights into identi-

fying a subset of patients predisposed to specific manifestations. Performing further studies, particularly longitudinal studies, will be vital to understand whether the identification of these autoantibodies and recognition of the key associated characteristics can assist us in improving the management of CTDs and implementing therapies at an earlier stage.

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