Could the IgA isotype provide additional information in systemic sclerosis patients? A retrospective study entailing IgA isotyping in a Mediterranean systemic sclerosis cohort

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Abstract

Objective

Anti-CENP-B (ACA), anti-topoisomerase I (ATA) and anti-RNA polymerase III (RP3) autoantibodies are included in the 2013 SSc-ACR/EULAR classification criteria. The detection of additional autoantibodies is of interest when those are negative. Additionally, we wonder if the IgA isotype might play a role in SSc. The aims of the study were to assess the prevalence of ACA, ATA, RP3, and Ro52 autoantibodies of IgG and IgA isotype and to describe their association with clinical manifestations in a cohort of patients with SSc.

Methods

Samples from 97 patients with SSc fulfilling the 2013 ACR/EULAR classification criteria, and 50 blood donors were included and tested for IgA and IgG isotypes of ACA, ATA, RP3, and Ro52 by FEIA.

Results

The prevalence of IgG+IgA isotypes for the same specificity was 62.5%, 82.6%, 80.0%, 36.8%, for ACA, ATA, RP3 and Ro52, respectively. Isolated IgG was present in 35.4%, 13.0%, 20.0% and 42.1% of patients for ACA, ATA, RP3 and Ro52, respectively. Only six patients were isolated IgA for a unique specificity. Clinically, ILD tended to be associated with ATA-IgG and ATA-IgG+IgA, telangiectasias with ACA-IgG+IgA and arthritis with ACA-IgA. Indeed, digital ulcers were more frequent in ATA-IgG patients.

Conclusion

Most of the patients presented ACA, ATA, or RP3 autoantibodies of IgA isotype in addition to IgG. Regarding clinical relevance, Ro52-IgG+IgA and ACA-IgG had a tendency towards sineSSc phenotype, while ACA-IgG+IgA to lcSSc phenotype. Thus, if confirmed, the determination of ACA-IgA could provide a tool to stratify patients according to the cutaneous phenotype.

Key words

systemic sclerosis, IgA autoantibodies, interstitial lung disease

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Introduction

Systemic Sclerosis (SSc) is a rare and chronic systemic autoimmune rheumatic disease (SARD) characterised by the generation of autoantibodies, with typical vascular manifestations such as Raynaud's phenomenon (RP), pulmonary hypertension (PH), and scleroderma renal crisis (SRC). On the other hand, fibrosis is the result of an imbalance between antifibrotic and profibrotic factors, leading to disturbances in the skin and pulmonary interstitium (1). The incidence in women is 4-9 times higher than in men (2). Patients with SSc have been classified, according to the extent of skin involvement, into three types: limited cutaneous SSc (lcSSc), diffuse cutaneous SSc (dcSSc), and SSc sine-scleroderma (sineSSc) (3). Although RP is the most prevalent clinical symptom at the beginning of the disease, severe organ manifestations including digital ulcers, PH, interstitial lung disease (ILD), and SRC, have been traditionally associated with the cutaneous extension (4). The presence of PH is more prevalent in patients with lcSSc whereas ILD mainly affects patients with dcSSc. On the other hand, sineSSc patients, in which skin involvement is absent, have less severe disease than lcSSc patients (5).

Humoral immune response seems to play an important role in the pathogenesis of SSc. Anti-nuclear autoantibodies (ANA), detected by indirect immunofluorescence (IIF), are a hallmark of SSc and are found in almost all SSc patients (6, 7). Different types of SSc-specific autoantibodies have been described, being anti-centromere (ACA) IgG, anti-topoisomerase I or Scl70 (ATA) IgG and anti-RNA polymerase 3 (RP3) IgG the most prevalent ones, accounting for approximately 70-80% of patients (8). Moreover, these three IgG autoantibodies have been included in the 2013 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria (3). Additional autoantibodies specific for or associated with SSc are anti-Th/To, anti-U3-RNP/fibrillarin, anti-Ku, anti-PM-Scl, anti-NOR90, anti-PDGFR or anti-Ro52/TRIM21 (Ro52) (4, 9, 10). Some studies have shown that classification according to autoantibodies positivity is as strongly associated with the clinical manifestations as the categorisation into cutaneous subtypes (11, 12). In this sense, ATA has been associated with the dcSSc phenotype and the presence of ILD, with a worse prognosis. In contrast, ACA has been associated with the lcSSc phenotype and the presence of PH with a better prognosis although PH is one of the most prevalent causes of mortality in SSc patients. Additionally, RP3 has been associated with an increased risk of SRC and synchronous cancer and, in some studies, with a rapidly progressive ILD (5, 11).

In clinical practice, specific-SSc autoantibodies positivity is based on the evaluation of autoantibodies of the IgG isotype. The determination of autoantibody isotypes besides IgG is restricted to a few autoimmune diseases, such as IgA in relation to coeliac disease, or IgM in the study of antiphospholipid syndrome or rheumatoid arthritis. However, in recent years, several studies have explored the role of the different isotypes of known autoantibodies in autoimmune diseases, such as SSc or rheumatoid arthritis (13-20). In SSc patients, only ACA-IgG and ATA-IgM had been associated with a higher degree of microangiopathy when defined by capillaroscopy (21). Additionally, both ACA-IgG and ACA-IgM had also been related to a definite SSc diagnosis. Moreover, higher levels of ACA-IgG were associated with disease progression to definite SSc within two years (22). In contrast, no relevant findings have been reported for the IgA isotype (21, 22).

On the other hand, the Ro52 autoantibodies are among the most frequent in SARD, including systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), neonatal lupus erythematosus (NLE), and subacute cutaneous lupus erythematosus (SCLE) despite not being specific for any disease. Furthermore, Ro52 has been associated with higher prevalence and worse prognosis of ILD in patients with SS, SSc, inflammatory myopathy, and mixed connective tissue disease (23, 24). In the Canadian Scleroderma Research Group

study including 963 patients with SSc, Ro52 autoantibodies (20.1%) were the second most common autoantibody after ACA (35.2%) followed by ATA and RP3 (15.6% and 18.6%, respectively) (25). Additionally, Ro52 autoantibodies were strongly associated with ILD (OR 1.53) and overlap syndrome (OR 1.75). Furthermore, Ro52 autoantibodies were detected in combination with ACA (23.4%), ATA (12.7%), and RP3 (16.0%) (26). In a small study of 62 patients with definite and suspected SARD diagnosis, isolated Ro52 has been hypothesised to be associated with a milder phenotype of undifferentiated connective tissue disease (27). Additionally, a large tri-nation cohort study with 1574 SSc patients, Ro52 monospecific positivity (n=103) was associated with ILD (OR 2.70) in comparison with Ro52 negative patients (n=1174). Of note, they were at significantly increased risk of death (25).

Improved biomarkers for early diagnosis and prognosis are still needed. Nowadays, cutaneous phenotype (lcSSc and dcSSc) and autoantibody profile could give some information to the clinicians but around 20% of patients are still negative for the autoantibodies included in the SSc classificatory criteria (SSc-cc negative autoantibodies group). Thus, only few patients are being diagnosed in very early stages and without a definite SSc diagnosis nor a clear cutaneous phenotype.

The aims of the present study were to assess the prevalence of ACA, ATA, RP3, and Ro52 autoantibodies of IgG and IgA isotype and to describe their association with clinical manifestations in a cohort of patients with SSc.

Materials and methods

Patients

Sera samples from 97 patients with SSc fulfilling the 2013 ACR/EULAR classification criteria were collected at the Department of Autoimmune Diseases of Hospital Clinic, Barcelona. All patients are included in the Spanish Scleroderma Registry (RESCLE). Clinical data including cutaneous phenotype, peripheral vascular and musculoskeletal manifestations, and gastrointestinal tract, lung, and renal involvement were reviewed from electronic health records. In addition, 50 serum samples from blood donors were analysed. Ethics board approval was obtained from our institution (Hospital Clinic, Barcelona, Spain, ref. HCB/2019/0852). Written informed consent was obtained from all patients and blood donors.

Detection of autoantibodies

Serum samples were tested for the presence of IgA and IgG isotypes of ACA, ATA, RP3, and Ro52 by fluorescence enzyme-linked immunoassay (FEIA) using the EliA™ platform (Thermo Fisher Scientific, Sweden). Notably, the IgA isotype was detected using a prototype assay with the EliA™ system. Tests were run in a Phadia 250 system (Thermo Fisher Scientific, Sweden). In accordance with the established standard protocols, a calibration curve for each isotype was included to calculate the defined units of autoantibodies measured with commercially available assays or the concentration of autoantibodies measured with prototype assays, which have no defined units. IgG and IgA positive and negative controls were included in each tests round.

The cut-off value for the IgG isotype (ACA, ATA, RP3, and Ro52 autoantibodies) used in this study was 7 U/mL, according to the manufacturer's instructions. The cut-off value for the IgA isotypes (prototype reagents; ACA, ATA, RP3, and Ro52 autoantibodies) was calculated using the results obtained from the blood donor samples (n=50). A concentration of 1.65 μg/L was chosen as the cut-off for all biomarkers (giving ≥98% specificity for blood donors). For both IgG and IgA, results were considered negative below the cut-off, low positive between 1 and 5 times the cut-off, and high positive above 5 times the cut-off.

Statistical analysis

IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, N.Y., USA) and GraphPad Prism for Windows, version 8.3.0 (GraphPad Software, La Jolla, California, USA) were used for statistical analyses. Results with p-values <0.05 were considered statistically significant. Continuous data were described by the mean and standard deviation or median and 25th-75th interquartile range (IQR), according to their distribution. Categorical variables were expressed as absolute numbers and percentages. Differences in proportions were analysed using the Fisher's exact test. Differences in means of continuous variables were analysed using the parametric Student t test or the Mann-Whitney non-parametric U-test when the variable distribution was not normal. Associations between categorical variables were determined using the Fisher's exact test. The relative measure of an effect was expressed as the odds ratio (OR) and the 95% confidence interval (CI) when considering autoantibody positivity as the exposure. The multiple comparisons results were interpreted correcting with a False Discovery Rate value of 0.05.

Results

The patient cohort included 97 patients, 92 of whom were women (94.8%). The mean age of the patients at diagnosis was 49.5 years. The mean disease duration since diagnosis was 7 years and 12.7 years since the first non-Raynaud symptom related to SSc (Table I). Fifty-five patients (56.7%) presented a lcSSc phenotype, 15 (15.5%) a dcSSc, and 27 (27.8%) a sineSSc phenotype (Fig. 1). SSc criteria autoantibodies (IgG isotype) distribution in cutaneous phenotypes is shown in Figure 1.

Prevalence of autoantibodies included in classification criteria (IgG isotype)

ACA (48.5%) was the most prevalent autoantibody in our cohort, whereas ATA and RP3 were present in 22.7% and 5.2% of the patients, respectively. Only one patient was double positive for ATA (197.4 U/mL) and RP3 (15.2 U/mL). Twenty-four (24.7%) patients were negative for the three autoantibodies included in classification criteria (Fig. 2) but all of them were ANA positive by IIF on HEp-2 cells.

Clinical characteristics

The demographic and clinical characteristics of the whole cohort according to the presence of autoantibodies

Table I. Demographic characteristics, cutaneous phenotype, and clinical manifestations of the patients with systemic sclerosis according to SSc-criteria autoantibodies.

	SSc cohort (n=97)	IgG ACA positive (n=47)	IgG ATA positive (n=22)	IgG RP3 positive (n=5)	Negative SSc-criteria autoantibodies (n=24)	*p*-value
Demographic characteristics						
Age at SSc diagnosis, (years)	49.5 ± 15.9	54.1 ± 15.9	46.2 ± 12.0	52.2 ± 12.1	43.1 ± 17.5	0.052
Female	92 (94.8%)	47 (100%)	20 (90.9%)	5 (100%)	21 (87.5%)	0.059
Disease duration, since first non-Raynaud symptom, (years)	12.7 ± 12.3	13.0 ± 12.9	14.5 ± 12.5	5.8 ± 6.6	11.4 ± 11.9	0.518
Disease duration since diagnosis, (years)	7.0 ± 10.0	6.7 ± 10.5	9.1 ± 10.4	4.4 ± 7.2	5.8 ± 9.2	0.711
Follow-up time, (years)	6.5 ± 1.5	6.6 ± 1.6	6.0 ± 1.3	7.2 ± 1.3	6.5 ± 1.6	0.165
Anti-nuclear autoantibodies	97 (100%)	47 (100%)	22 (100%)	5 (100%)	24 (100%)	1.000
Compatible IIF pattern (28)	71/73 (97.3%)	46 (97.9%)	21 (95.5%)	5 (100%)	---	0.786
Clinical manifestations						
Raynaud's phenomenon	94 (96.9%)	47 (100%)	22 (100%)	5 (100%)	21 (87.5%)	**0.042**
Digital ulcers	18 (18.6%)	7 (14.9%)	8 (36.4%)	0	3 (12.5%)	0.129
Telangiectasias	47 (48.5%)	28 (59.6%)	12 (54.5%)	1 (20.0%)	6 (25.0%)	**0.025**
Calcinosis	9 (9.3%)	5 (10.6%)	2 (9.1%)	1 (20.0%)	1 (4.2%)	0.495
Arthritis	10 (10.4%) (n=96)	4 (8.7%) (n=46)	1 (4.5%)	1 (20.0%)	4 (16.7%)	0.870
Myositis	4 (4.2%) (n=96)	0 (n=46)	0	0	4 (16.7%)	**0.017**
Oesophageal involvement	62 (65.3%) (n=95)	32 (68.1%)	14 (66.7%) (n=21)	3 (75.0%) (n=4)	14 (58.3%)	0.870
Interstitial lung disease (ILD)	43 (44.3%)	13 (27.7%)	18 (81.8%)	3 (60.0%)	10 (41.7%)	**<0.001**
Severe ILD (FVC <70%)	17 (17.5%)	6 (12.8%) 46.2% ILD	5 (22.7%) 27.8% ILD	1 (20.0%) 33.3% ILD	5 (20.8%) 50.0% ILD	0.536
Pulmonary hypertension	16 (24.2%) (n=66)	7 (21.9%) (n=32)	2 (14.3%) (n=14)	1 (20.0%)	6 (37.5%) (n=16)	0.462
Scleroderma renal crisis	1 (1.0%)	0	1 (4.5%)	0	0	0.268
Neoplasia	16 (16.7%) (n=96)	9 (19.6%) (n=46)	3 (13.6%)	2 (40.0%)	3 (12.5%)	0.728

Results of quantitative variables are expressed as mean ± standard deviation and those of qualitative variables as number and percentage. Information in parenthesis indicates number of column total patients for whom clinical data is available.

included in the classification criteria (ACA-IgG, ATA-IgG, and RP3-IgG) are summarised in Table I. There were no differences in the age at SSc diagnosis, sex or SSc disease duration between SSc-criteria groups. The lcSSc subtype (56.7%) was the most prevalent skin phenotype in our cohort. The dcSSc subtype (15.5%) was uniformly present in the ATA-IgG, RP3-IgG, and SSc-cc negative autoantibodies group but it was absent in the ACA-IgG group ($p<0.001$). On the other hand, sineSSc subtype (27.8%) was mostly represented in ACA-IgG and SSc-cc negative autoantibodies group but without statistically significant difference (Table I). Regarding clinical manifestations, telangiectasias were significantly more prevalent in the ACA-IgG and ATA-IgG groups than in the RP3-IgG and SSc-cc negative autoantibodies group ($p=0.025$). In contrast, myositis was only present in SSc-cc negative autoantibodies group ($p=0.017$) being three of these four patients positive for anti-Ku and the other one positive for anti-U3-RNP/fibrillarin autoantibodies. All of them were negative for Ro52 autoantibodies. Moreover, ILD was more prevalent in patients with ATA-IgG than in other groups ($p<0.001$).

Prevalence of IgA isotype of ACA, ATA and RP3 autoantibodies

Regarding IgA autoantibodies, 55 out of 97 (56.7%) patients were IgA positive and most of them (53/55; 96.4%) were double positive, IgG and IgA, for the same specificity, being 30 ACA (groups 1 to 7) (Fig. 2), 19 ATA (groups 11 to 20) (Fig. 2) and 4 RP3 (groups 24 to 26) (Fig. 2). In addition, only two patients were single positive for IgA isotype, one for ACA (lcSSc) and another for ATA (lcSSc) (groups 10 and 23, respectively) (Fig. 2). Therefore, the SSc-cc negative autoantibodies group (n=24) was reduced by two pa-

tients considering IgA isotype (n=22; groups 27 to 30) (Fig. 2).

Prevalence of Ro52 autoantibodies

The prevalence of Ro52 autoantibodies in our cohort was 19.6% (19/97 patients). In contrast to criteria autoantibodies, only 7 patients (36.8%) were double positive, IgG and IgA (groups 3, 4 and 27) (Fig. 2), whereas 8 (42.1%) (groups 7, 9, 15, 16, 26, 28 and 29) (Fig. 2) were isolated IgG and 4 (21.1) (groups 13, 14 and 18) (Fig. 2) isolated IgA.

There were no significant differences in the prevalence of Ro52 (IgG and/or IgA) among the four different groups (14.6%, 26.1%, 25.0% and 22.7% in ACA, ATA, RP3, and SSc-cc negative autoantibodies group, respectively; p=0.661).

Autoantibody combinations

Regarding ACA autoantibodies, when IgG and IgA isotypes were present (n=30; groups 1 to 7) (Fig. 2), IgG titres were high and IgA titres were low in 73.3% of patients. In relation to ATA, when both isotypes were present (n=19; groups 11 to 20) (Fig. 2), IgG and IgA titres were high in 68.4% of patients. Whereas for RP3, we cannot observe a clear trend with 2 patients double positive at high titres for both isotypes, one patient low positive for both isotypes and one patient low for IgG and high for IgA (n=4; groups 24 to 26) (Fig. 2). Finally, all Ro52 double positive patients had high IgG and low IgA titres.

When only patients with isolated IgG or IgA positivity were analysed, 20 out of 28 (71.4%) of those IgG positive were at high titres while 12 out of 14 (85.7%) isolated IgA positive patients were positive at low IgA titres.

Among the patients who are positive for several targets (ACA, ATA and/or RP3), 8 patients were double positive for IgA autoantibodies (Groups 5, 6, 13, 14, 17, 19), whereas only 1 patient was double positive for 2 IgG autoantibodies (high titres of ATA and low titres of RP3; group 20). Positivity to more than one autoantibody (including Ro52 autoantibodies as well as SSc-criteria autoantibodies) was more frequent in ATA (9/23; 39.1%. Groups 13 to 20) patients than in those with ACA (10/48; 20.8%. Groups 3 to 7 and 9) (Fig. 2).

Regarding Ro52, there were no differences in Ro52 prevalence (19.8% *vs.* 20.8%) between positive patients for the SSc autoantibodies included in the classification criteria (groups 1 to 9, 11 to 22 and 24 to 26) and SSc-cc negative autoantibodies group (groups 10, 23 and 27 to 30), respectively (Fig. 2).

Association with clinical manifestations

Although some tendencies were observed, after correcting for multiple comparisons, any association reached statistical significance.

Considering the cutaneous phenotype, ACA-IgG+IgA tended to lcSSc and ATA-IgG to dcSSc phenotype. ACA-IgG and Ro52-IgG+IgA had a tendency towards sineSSc (non-adjusted p-values: 0.029, 0.004, 0.005 and 0.017, respectively).

Regarding other clinical manifestations, ATA-IgG and ATA-IgG+IgA positivity tended to be more prevalent in patients with ILD (non-adjusted p-value: 0.084 and 0.001). ACA-IgG+IgA had a tendency towards telangiectasias and ACA-IgA towards arthritis (non-adjusted p-value: 0.027 and 0.083). Moreover, ATA-IgG positive patients had a higher prevalence of digital ulcers.

Three out of 5 (60.0%) Ro52 monospecific patients (groups 27, 28 and 29) had ILD whereas 35 out of 78 (44.9%) Ro52 negative patients had ILD (Fig. 2). These differences were not statistically significant (OR: 1.84; p=0.656).

We did not find statistically significant clinical association according to the presence or absence of Ro52 autoantibodies in positive patients for autoantibodies included in the SSc classificatory criteria (groups 1 to 9, 11 to 22 and 24 to 26). However, Ro52 presence tended to be more frequent in dcSSc patients (28.6% in Ro52 positive *vs.* 8.5% in Ro52 negative; p=0.062) while neoplasia tended to be more frequent in Ro52 negative patients (22.0% in Ro52 negative *vs.* 0% in Ro52 positive; non adjusted p-value=0.059).

Interestingly, when comparing the patients with low titres of autoantibodies (groups 9, 10, 22, 23, 26 and 29) with negative patients for ACA, ATA, RP3 and Ro52 (group 30), the prevalence of ILD was higher in the low titre group (4/6; 66%) than in negative patients (5/17; 29.4%) but not statistically significant (p=0.162) (Fig. 2).

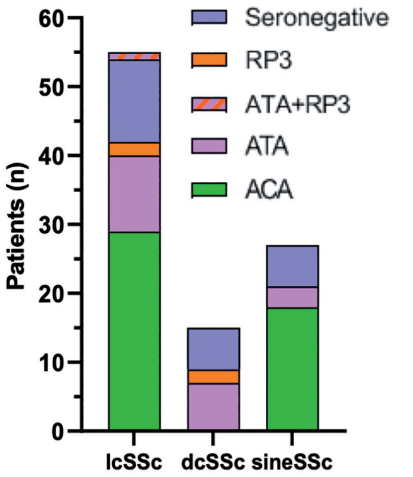

Fig. 1. SSc criteria autoantibody (ACA, ATA and RP3 from IgG isotype) distribution in the three different cutaneous phenotypes.
ACA: anti-centromere; ATA: anti-topoisomerase-1; dcSSc: diffuse cutaneous phenotype; lcSSc: limited cutaneous phenotype; sineSSc: sine scleroderma phenotype; RP3: anti-RNA polymerase III.

Discussion

This study described the prevalence of IgG and IgA isotypes of SSc criteria autoantibodies and their association with clinical manifestations in a Spanish cohort of SSc patients. Moreover, Ro52 autoantibodies were also analysed. The main finding of this study is that most of the SSc patients presented with ACA, ATA, or RP3 autoantibodies of IgA isotype in addition to IgG.

According to SSc-criteria autoantibodies, in the sample analysed within our cohort, the prevalence of ACA was higher and the prevalence of ATA was lower than those from the EUSTAR registry (29), which included 8432 patients with SSc (ACA 48.5% *vs.* 37.0%; p=0.002 and ATA 23.7% *vs.* 39.0%; p=0.026, respectively). In contrast, RP3 prevalence in our cohort was similar to that of the EUSTAR registry cohort (5.2% *vs.* 4.0%; p=0.593). As is known, most of the SSc patients are monospecific for the autoantibodies

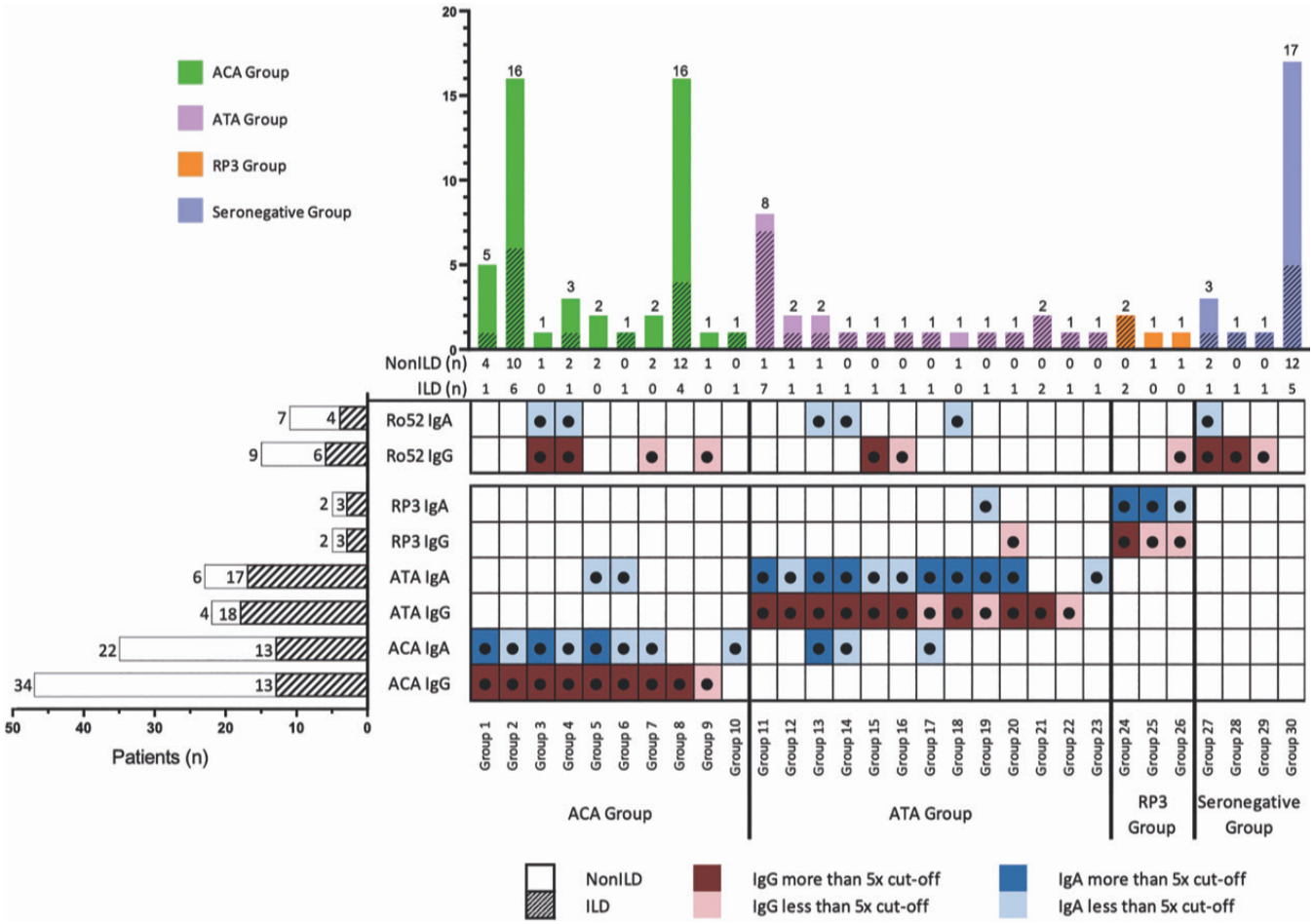

Fig. 2. UpSet plot. Combination of autoantibodies in SSc cohort. Bars on the left indicate number of patients with and without ILD with positive antibody. Columns represent number of patients with and without ILD presenting the same autoantibody profile. Green columns indicate ACA group, purple columns ATA group, orange columns RP3 group, and blue columns SSc-cc negative autoantibodies group. Autoantibody positive signals are divided into high (more than 5x cut-off) and low titres (less than 5x cut-off).
ACA: anti-centromere; ATA: anti-topoisomerase-1; ILD: interstitial lung disease, RP3: anti-RNA polymerase III.

included in the 2013 SSc classification criteria (10). According to previous studies describing double positivity for autoantibodies targeting different antigens in only a few SSc patients (8), we only found one double positive patient with high ATA-IgG and low RP3-IgG, and these results were confirmed by other methods.

The performed study showed that IgA isotype autoantibodies were present in many patients with SSc. The high prevalence of ACA-IgA and ATA-IgA in the presence of the IgG isotype, as previously described in other studies (30-33) was confirmed in our study. Most patients presented double positivity for the IgG and IgA isotypes for the same specificity, while the number of patients uniquely positive for IgA positivity was very low. This fact undermines the opportunity to minimise the seronegative gap of SSc criteria with IgA isotyping but may still provide more accurate information about the clinical association and prognosis.

Regarding clinical relevance, the most significant finding was the tendency for ACA-IgG positivity in patients with the sineSSc phenotype, while ACA-IgG+IgA was more frequent in the lcSSc phenotype. Moreover, Ro52-IgG+IgA had also a tendency towards the presence of sineSSc. However, ACA and Ro52 were independent (p=0.124). Although these tendencies must be confirmed in other studies, the determination of ACA-IgA could provide an extra tool to stratify patients according to the cutaneous phenotype. However, in this sense, we found that ACA-IgG+IgA was more prevalent in patients with telangiectasias, and isolated ACA-IgA in patients with arthritis.

As reported previously (10), Ro52 appeared concomitant with ACA, ATA, or RP3 autoantibodies in 20% of patients with SSc-criteria in our cohort. The prevalence of Ro52 was similar among the groups with ACA, ATA, RP3, or SSc-cc negative autoantibodies patients. Monospecific Ro52 (without SSc-criteria autoantibodies) has been associated with ILD (OR 2.70) (25). However, in our study, ILD prevalence was similar regardless of the status of Ro52 (monospecific versus Ro52 negative). In contrast to other SSc cohorts (25,26), Ro52 prevalence in our study (19.6%) was lower than ACA (48.5%) and ATA (22.7%) prevalence. As is common knowledge, the different autoantibodies detection methods, including those to detect anti-Ro52, have different sensitivity (34,35) and this fact could account for these dif-

ferences. Interestingly, we found that positivity to Ro52-IgG+IgA as well as ACA-IgG tended to be more frequent in sineSSc patients. Conversely, we did not find differences in the proportion of Ro52-IgG+IgA between patients with isolated ACA-IgG and those negative for ACA-IgG (p=0.124). Therefore, these findings could indicate that ACA-IgG and Ro52-IgG+IgA may have an independent role in the sineSSc phenotype, which may characterise different sineSSc clinical phenotypes.

Patients with low titres (1-5x cut-off) of any of the evaluated autoantibodies, when compared with negative patients, had a higher prevalence of ILD (66% *vs*. 29%; p=0.162). Although there was a small number of patients and differences were not statistically significant, this result may point out that clinicians have to be aware that even low titre autoantibodies could be relevant, and these patients could have an increased risk to develop ILD.

A limitation of our study is the low number of patients with SSc and, subsequently, the low number of patients within each group (ACA, ATA, RP3 and lcSSc, dcSSc, sineSSc). Due to the high prevalence of double IgG+IgA positive patients in ACA and even more in ATA patients, it was difficult to decipher the role of the IgA isotype, and some tendencies resulting from association analyses should be considered carefully and confirmed in other studies. However, our findings suggest a role for Ro52 in addition to ACA in the sineSSc phenotype that could be related to the absence of ACA-IgA or the presence of the Ro52-IgA isotype. Another important limitation is that autoantibodies had only been assessed using one technique, but most of the results were in accordance with IIF, and the discordant results were within the equivocal range. Additionally, only the three autoantibodies included in the classification criteria were assessed. However, there are other autoantibodies detected in patients with SSc, such as U3-RNP/fibrillarin, Th/To, PM-Scl, Ku, NOR90, that were not tested because its low prevalence, and the corresponding need of larger cohorts for a thorough analysis. As strength, our cohort could be representative of the Mediterranean or southern European SSc population. The differences with the EUSTAR registry may also be due to geographical divergence.

The high IgG and IgA positivity prevalence makes us consider the sequential appearance of these autoantibodies since the very first symptoms of the disease, or even before, and if they are associated with one or other SSc clinical phenotype. However, as our study is retrospective and only includes well-established SSc diagnosed patients resulting in a high prevalence of autoantibodies. For these reasons, these questions should be addressed in subsequent prospective studies including longitudinal sampling of patients since first SSc symptoms for confirmation. These prospective studies will highlight the diagnosis power of IgA isotyping. Thus, larger studies are needed to confirm the observed tendencies between autoantibodies positivity and clinical findings, and a prospective study, including early SSc or recently diagnosed "definite SSc" patients, may be mandatory.

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