Extended Autoantibody Panel in Turkish Patients with Early-Stage Systemic Sclerosis: Co-expressions and Their Influences on Clinical Phenotypes

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Abstract

Background/aim: To investigate the frequency and clinical relevance of an extended autoantibody (ab) profile in patients with SSc. Materials and Methods: In this cross-sectional study, serum from 100 consecutive patients was subjected to indirect immunofluorescence (HEp-20-10/primate liver mosaic) and Systemic Sclerosis Profile by EUROIMMUN (Lübeck, Germany) to evaluate ANA and autoantibodies against 13 different autoantibodies in patients with SSc less than three years. Results: 93 of 100 patients were positive for ANA by indirect immunofluorescence (IIF). The prevalence of Anti-Scl70 ab was 41%, anti-centromere (ACA) 27%, and anti-RNA polymerase (RNAPIII) 15%. Scl70 was more associated with diffuse subtype ($p<0.001$), ILD ($p<0.001$), and high mRSS ($p=0.002$); ACA with limited disease ($p<0.001$), less ILD ($p<0.001$), overlap ($p=0.017$) and low mRSS ($p=0.024$); RNAPIII with diffuse disease ($p=0.027$), ILD ($p=0.016$) and high mRSS ($p=0.001$). Fifty-three patients showed single positivity (26 anti-Scl70, 16 ACA, 6 anti-RNAPIII, 1 anti-Ku ab, 1 anti-PM/Scl100, 2 anti-PM/Scl75, 1 anti-Ro52), whereas 32 patients had multiple auto-antibody positivities. Among common SSc-specific autoantibodies, Scl70 and RNAPIII showed the highest co-occurrence (n=4). One patient was simultaneously positive for anti-RNAPIII ab and ACA, and one was positive for ACA and Scl70. The clinical features were not statistically different between single and multiple autoantibody-positivity for common SSc-specific autoantibodies (ACA, Scl70, and RNAP III), except for digital ulcer in multi-antibody positive ACA group ($p=0.019$). Conclusion: Based on our results, co-expression of auto-antibodies is not uncommon in SSc patients. Although SSc-specific auto-antibodies generally show known clinical features, the clinical presentation of the co-expression in specific and non-specific auto-antibody positivity continues to be important.

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Statements and Declarations: We hereby state that this manuscript is original, and no part of the manuscript has either been published before or is under consideration for publication elsewhere. This article does not contain any studies with animals performed by any of the authors. This study was approved by the Kocaeli University School of Medicine Ethics Committee, Kocaeli, Turkey, with study number KOU/GOKAEK 2017/347. Consent was obtained from all participants. Actelion company contributed financially to the study to be used during the purchase of the study kits. No other disclosures relevant to this article were reported. None of the authors have financial or non-financial conflicts of interest to disclose.

Abstract

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Materials and Methods: In this cross-sectional study, serum from 100 consecutive patients was subjected to indirect immunofluorescence (HEp-20-10/primate liver mosaic) and Systemic Sclerosis Profile by EUROIMMUN (Lübeck, Germany) to evaluate ANA and autoantibodies against 13 different autoantibodies in patients with SSc less than three years.

Results: 93 of 100 patients were positive for ANA by indirect immunofluorescence (IIF). Fifty-three patients showed single positivity (26 anti-Scl70, 16 ACA, six anti-RNAPIII, one anti-Ku ab, one anti-PM/Scl100, two anti-PM/Scl75, one anti-Ro52), whereas 32 patients had multiple auto-antibody positivities. Among common SSc-specific autoantibodies, Scl70 and RNAPIII showed the highest co-occurrence (n=4). One patient was simultaneously positive for anti-RNAPIII ab and ACA, and one was positive for ACA and Scl70. The clinical features were not statistically different between single and multiple autoantibody-positivity for common SSc-specific autoantibodies (ACA, Scl70, and RNAP III), except for digital ulcer in the multi-antibody positive ACA group (p=0.019).

Conclusion: Based on our results, co-expression of auto-antibodies is not uncommon in SSc patients. Although autoantibodies specific to SSc in early disease show generally known clinical features, it remains to be investigated how co-expression of specific and nonspecific autoantibody positivity will affect clinical presentation.

Keywords: Systemic sclerosis, Autoantibody, Scleroderma-specific antibodies, Immunoblot assay, Indirect immunofluorescence assay

Introduction

Systemic sclerosis (SSc) is a rare autoimmune disease characterized by progressive skin and internal organs fibrosis, vasculopathy, and autoantibody production [1]. Anti-nuclear antibodies (ANA) can be found in 90-95% of patients with SSc and SSc-specific autoantibodies in >80% [2]. Today, at least 10 SSc-specific autoantibodies, anticentromere, anti-Scl-70 (anti-topoisomerase I), anti-RNA polymerase III (anti-RNAPIII), anti-U3 ribonucleoprotein (anti-RNP), antiTh/To, anti-U11/U12 RNP, anti-PM/Scl, anti-Ku, antiRuvBL1/2, anti-U1 RNP antibodies (ab) have been reported in SSc patients [3, 4, 5].

The clinical course of SSc is not easily predictable because of the clinical and serologic heterogeneity. Nevertheless, from early to established SSc, autoantibodies are used as an indicator in diagnosis, predicting organ involvement, determining prognosis, and making treatment decisions [6, 7, 8]. Although recent classification criteria have confirmed their diagnostic utility and have long been used for prognostic stratification of patients, there is still a need to recognize the potential interaction between autoantibodies and their representation on clinical phenotypes of disease [9-11].

The most known and widely used autoantibodies targeting Scl-70, centromere proteins and RNAP-III have been reported to be mutually exclusive and strongly associated with certain clinical phenotypes [12]. However, there is also evidence of overlap between these auto-antibodies [3, 13]. Although the relationship between single positivity of some specific auto-antibodies and involvement of certain organs is well known today, the relationship between the positivity of compound auto-antibodies and their clinical significance in SSc patients with short disease duration has not been investigated in detail [14].

Therefore, we aimed to investigate the frequency and clinical relevance of autoantibodies in the early stage of SSc using an expanded panel of autoantibodies. We also aimed to follow these patients to investigate how the relationship between these autoantibodies and organ involvement progressed over time. Here we report our initial baseline data.

Methods

Patient Selection

One hundred patients with SSc were recruited consecutively from six tertiary centers in Turkey specializing in the care of patients with SSc. We included limited or diffuse cutaneous SSc patients [10] in the early stage of the disease with a disease duration of <3 years from the first non-Raynaud symptom [15], meeting

the 2013 American College of Rheumatology/European League Against Rheumatism (ACR/ EULAR) SSc classification criteria [16]. We included the SSc patients with overlap syndromes and the overlap syndromes were defined as cases meeting the classification criteria for one or more connective tissue diseases (CTDs) concurrent with SSc. Patients with other comorbidities that could lead to auto-antibody positivity were excluded.

Demographic characteristics and clinical and laboratory findings of the patients were recorded. Disease duration was calculated as the time between the onset of the first non-Raynaud symptom and the enrollment date. Variables included Raynaud's phenomenon, skin and musculoskeletal involvement, pulmonary arterial hypertension (PAH), interstitial lung disease (ILD), renal crisis (ever), gastrointestinal symptoms (dysphagia, reflux, early satiety, constipation, diarrhea) and if recorded any malignancy. The extent of the skin involvement was assessed using the modified Rodnan skin score (mRSS) [17]. Patients with a pulmonary artery pressure (PAP) above 45 mmHg on echocardiography underwent right heart catheterization because of the strong correlation between this estimated cut-off level and right heart catheterization [18]. Pulmonary hypertension was defined as a mean pulmonary arterial pressure of [?]20 mm Hg, and precapillary pulmonary hypertension was defined as pulmonary vascular resistance of [?]2 Wood units and a pulmonary capillary wedge pressure of [?]15 mm Hg on right-sided heart catheterization [19]. ILD was defined as the presence of any evidence of pulmonary fibrosis on lung imaging by high-resolution computed tomography scan. The renal crisis was described as an abrupt onset of severe hypertension (systolic blood pressure (BP) [?]180 mmHg and/or diastolic BP [?]100 mmHg) without an alternate etiology, with or without microangiopathic anemia or decline in renal function [20].

This study complied with the Declaration of Helsinki, and the study was approved by the Kocaeli University School of Medicine Ethics Committee, Kocaeli, Turkey, with study number KOU/GOKAEK 2017/347.

Autoantibody analysis

Sera from all patients were tested using a commercially available indirect immunofluorescence (Anti-nuclear antibody [ANA], Mosaic Hep-20-10/Liver; Euroimmun) assay and line immunoblot assay (Systemic Sclerosis [Nucleoli] Profile EuroLine [IgG]; Euroimmun) simultaneously in a single central laboratory. Serum aliquots were stored at -80 C until the time of testing. The assays were performed according to the manufacturer's instructions. For ANA, results above the dilution of 1:100 were considered positive. The Systemic Sclerosis [Nucleoli] Profile kit contained 13 recombinant antigens: those expressed in Escherichia coli (RNA polymerase III [RNAP III; subunits RP11 and RP155], fibrillarin, the 90-kd nucleolar protein NOR-90, and Th/To) or insect cells using the baculovirus system (CENP-A, CENP-B, PM/Scl-100, PM/ Scl-75, Ku, and tripartite motif-containing protein 21 [TRIM- 21]/Ro 52) plus PDGFR expressed in mammalian cells and native topo I (Scl-70) isolated from calf and rabbit thymus. Sera were analyzed at a dilution of 1:101, and autoantibodies were detected using alkaline phosphatase–labeled anti-human IgG. The EuroLine flatbed scanner was used to provide semi-quantitative results. Readings obtained with a signal intensity of 0-5, 6-10, 11-25, 26-50, and >50 were defined as negative, borderline, medium, strong, and very strong bands and were given equivalent scores of negative, (1+), 1+, 2+, and 3+, respectively.

Statistical analysis

When determining the sample size, the following criteria were applied to see how clinical characteristics differed between patients with and without positive autoantibodies (two independent groups): effect size=0.5, α=0.05, and power (1-β)=0.80. The total sample size required to meet these criteria was 128, and 100 patients were recruited. Descriptive statistics for clinical and demographic characteristics of the patients are presented as frequency and percentage (%) for categorical variables and mean with standard deviation (mean ± SD) or median with interquartile range (median [Q3–Q1]) according to the distribution of the continuous variables. The distribution normality was assessed visually and through the Shapiro–Wilk test. An independent sample T-test was used to analyze how specific autoantibodies affected clinical outcomes in positive and negative groups and in cases of single and multiple positivity. A chi-squared test was also performed for categorical variables.

In evaluating semi-quantitative results, we considered a score of [?] +1 for each autoantibody to be positive. In addition, in statistical analyses, we assumed CENPA and/or CENPB positivity as ACA positive and, similarly RNAP11 and/or RNA 155 positivity as RNAP III positive.

To reduce the dimensionality and increase the interpretability of the data while minimizing the information loss, we examined the data by principal components analysis (PCA) as performed in the R Library FactoMineR [21]. PCA is a method that successively maximizes variance by creating new unrelated variables. We used the intensity scores in PCA.

Statistical analyses of further demographic and phenotypic data were performed using SPSS, version 20.0 (IBM Inc., Chicago, IL, USA). Two-sided p values less than 0.05 were considered statistically significant ($p<0.05$).

Results

Characteristics of the study population and frequency of autoantibodies

Demographic, clinical, and serologic characteristics of the 100 SSc patients are presented in Table 1. The majority of the patients had lcSSc (63%), whereas 36 had diffuse involvement, and only one patient had sine scleroderma. Ninety-three out of 100 patients were positive for ANA by IIF. Anti-Scl-70 ab was the most frequent (41%) auto-antibody, followed by ACA with a rate of 27%. The anti - RNAPIII ab positivity was 15%. Except for common autoantibody (ACA, Scl-70, or RNAP III), anti - Ro52 was the most common SSc-specific autoantibody (22%). None of the patients were positive for either anti-Fibrillarin or Anti-PDGF abs.

The majority of the patients exhibited single or multiple positivities for analyzed autoantibodies. The distribution of the autoantibody positivities of the patients is shown in Figure 1. Of 100 patients, 53 were single positivity for any autoantibodies and 48 of which were common SSc-specific autoantibodies (26 with anti-Scl-70 ab, 16 with ACA, 6 with anti-RNAPIII ab), and 5 were uncommon autoantibodies (1 with anti-Ku ab, 1 with ant -PM/Scl100 ab, 2 with anti-PM/Scl75 ab and 1 with anti-Ro52 ab). There were 32 patients with multiple antibody positivity. Amongst the common SSc-specific autoantibodies, anti-Scl70 and anti-RNAPIII antibodies showed the highest co-occurrence and were simultaneously positive in 4 patients. One patient was positive for anti-RNAPIII ab and ACA, and another for ACA and anti-Scl70 ab simultaneously. All patients with anti-Ro52 ab except one were also positive for the other antibodies. Eight patients (8%) were positive for uncommon scleroderma-specific autoantibodies with single or multiple staining. No specific autoantibodies were detected in 15 patients (15%), and ANA was positive in 11 of them.

Associations of autoantibodies with clinical features

The association between the common SSc-specific autoantibodies (ACA, Scl70, and RNAP III) and clinical features was evaluated by comparing the antibody-positive patients (regardless of being single or multiple positive) with the rest of the study population (Table 2). Overlap syndrome was more common in patients with ACA, and only 1 (3.7%) patient with ACA had ILD. Anti-RNAPIII ab was associated with a common disease subtype, ILD, and the highest mRSS among the three groups. Among all SSc patients, two out of 3 patients with malignancy were anti-RNAP antibody positive.

When we compared the clinical features of the patients in terms of single and multiple antibody positivity for each of the common autoantibodies specific to SSc (ACA, Scl70, and RNAP III) the clinical features were not different between the subgroups (Table 3). However, the digital ulcers were more frequent in the multiple antibody-positive ACA group compared to single positives.

Clinical features of the patients who were positive only for uncommon SSc-specific autoantibodies are shown in Table 4. All the patients except one with anti-Ku (dcSSc) and another with PM/Scl100 (sine scleroderma) had limited lcSSc. Only one patient with Ro52 had overlap syndrome (Sjogren's syndrome). None of these patients had either ILD, PAH, or malignancy.

When we compared the Ro52 positive and negative patients, we found that DUs and ACA positivity were more common in Ro52 positive patients compared to negative ones (27.3% vs. 9%, p=0.035, and 45.5% vs. 21.8%, p=0.027, respectively). Ro52-positive patients also showed more NOR90 positivity simultaneously (9.1% vs. 0, p=0.047). There was no difference between Ro52 positive and negative patients regarding ILD, PAH, GIS involvement, disease duration, or other auto-antibody positivities.

Principle component analysis

Based on the principal component analysis (PCA), 63% of the variability in the data was explained by the first three principal components. The staining intensity scores of the autoantibodies were used to select the main determinants of each component. Th/To, PM/Scl75 and PM/Scl100 were determinants of the first dimension, ACA (CENP A and B) second, and RNAP-III (RP11 and RP155) third dimension (Figure 2).

Discussion

In this study, we investigated an extended autoantibody profile and its association with the clinical manifestations in a group of patients with early-stage SSc. Consistent with the general knowledge, well-known common SSc-specific autoantibodies (Scl-70, ACA, RNAPIII) were more frequent among all tested autoantibodies and exhibited the expected clinical features. However, our results revealed that a substantial proportion of patients were positive for more than one auto-antibody, including common SSc-specific autoantibodies known as mutually exclusive. Another result that should be considered is that in patients negative for common SSc-specific autoantibodies (8%), an extended test profile showed the presence of another autoantibody.

The prevalence of anti-Scl70, ACA, and anti-RNAPIII abs in our patients was 41%, 27%, and 15%, respectively. These results were close and consistent with those previously reported in the literature [22–25]. However, the frequency of autoantibodies may vary by ethnicity, and data on SSc-specific autoantibodies from Turkey are limited to the frequencies of anti-Scl70 and anti-centromere antibodies [26]. In addition, in only one study, the frequency of RNAPIII antibodies was reported as 2.2%, which was lower than our results [27]. The difference in results may be because the previous study was conducted in patients with extensive SSc and long disease duration or because the two studies' antibody analysis methods were different.

Our results revealed that a substantial proportion of patients were positive for more than one auto-antibody, including common SSc-specific auto-antibodies. Although common SSc-specific autoantibodies (ACA, topo I, and RNAP III) are thought to be mutually exclusive and do not change from one to another during the disease, there is evidence that they may occur together [3, 23, 28]. With the recent advent of multiplexed immunoassays, the notion that these autoantibodies are mutually exclusive is slowly disappearing [29]. Consistent with these views, we detected the co-expression of common SSc-specific autoantibodies in some patients: anti-Scl70 ab and anti-RNAPIII Ab positivity in 4, ACA and anti-RNAP III ab in 1 and anti-Scl70 ab and ACA in 1[30-31]. Regardless of being single or multiple positive, comparison of common SSc-specific antibody-positive patients with the rest of the study population showed expected clinical associations with these abs (ACA with lcSSc, anti-Scl ab with ILD, and anti-RNAPIII ab with dcSSc and malignancy, etc.). When we compared the single and multiple positivities for each of common SSc-specific abs in terms of clinical involvements, there were no significant differences between subgroups, except for the higher occurrence of DUs in patients who were also positive to ACA. There are uncertainties about the clinical features of multiple antibody-positive SSc patients in the studies reported to date. Unexpectedly, in 7 dcSSc patients with anti-RNAPIII ab and ACA, reported by Satoh et al., none had significant organ involvement, such as renal crisis during the disease course [32]. Similarly, in our study, none of the patients with multiple ab positivity (1 with anti-Scl70 ab and ACA, and 2 with anti-RNAPIII ab and ACA positivity) had severe internal organ involvement; interestingly, all had lcSSc. It was difficult to comment on the clinical significance of these multiple autoantibody positivities because the number of patients with uncommon SSc-specific antibodies needed to be higher to make detailed comparisons.

Ro52 was positive in 22% of the patients in our study. It is mainly expressed in Sjogren's syndrome and reported in varying frequencies in SSc. Results from two large cohorts of the German network for systemic

Scleroderma and the Canadian Scleroderma Research Group demonstrated that anti-Ro52 was the second most common autoantibody in patients with SSc [33, 12]. It was our study's third most common auto-antibody following anti-Scl 70 and ACA antibodies. Anti-Ro52-positive SSc patients were more likely to be older, to have ILD, and to have overlap syndrome compared with anti-Ro52-negative patients [34]. Unlike these results, we found anti-Ro52 was more prevalent in ACA-ab-positive patients and more associated with DUs. Regarding the aforementioned clinical conditions, it seems essential to re-evaluate the patients during the follow-up period of the disease.

Although anti-RNAPIII (18%) positivity was not uncommon in our study patients, none of them had SRC. In a large SSc cohort of 1325 patients, more than 90% of the patients developed SRC within five years of SSc onset [11]. The occurrence estimates of SRC were 6.5%, 7.1%, and 7.6% at 5, 10, and 15 years, respectively. SRC prevalence is lower in North America than in Europe [35]. SRC is considered less frequent in the Turkish SSc population, with a reported frequency of %3 in a cross-sectional study from Turkey [22]. Therefore, the absence of SRC in our study can be partly explained by genetic and geographical differences and the short disease duration of the patients in our study population.

Anti-PM/Scl antibodies were positive in 10% of the patients in our study. They have been reported to be associated with inflammatory myositis and calcinosis [36]. In a multinational cohort of SSc, myositis was seen only in subjects positive for both anti-PM/Scl75 and anti-PM/Scl100 antibodies [37]. From this perspective, the three patients with both anti-PM/Scl75 and anti-PM/Scl100 antibodies appeared at higher risk for myositis in our study. However, none had myositis or calcinosis at baseline clinical evaluation at enrollment. Despite all these results, continuous monitoring of serum creatine kinase levels in anti-PM/Scl ab positive patients may be helpful in the further diagnosis of myositis.

In order to look at multiple auto-antibody positivity from a different perspective, we tried to reduce the dimensionality and increase the interpretability of the data by the principal component analysis using the staining intensities of auto-antibodies. Three significant dimensions (Th/To - PM/Scl 100 - PM/Scl 75, ACA, and anti-RNAPIII positive groups) were produced by PCA, and anti-Scl70 ab was ignored. However, it was numerically higher in the number of patients since its intensity was low. Therefore, we refrained from interpreting the clinical relevance according to the results of PCA. However, we included our results to compare with the PCA analyses of other studies in the literature (38).

One of the limitations of our study was the small sample size. The most important reason for this was to include patients who met the 2013 ACR/EULAR classification criteria and had as short a disease duration as possible. This situation prevented reaching statistically significant results in some subgroup analyses. Another limitation of our study was that the study kits did not contain all SSc-specific auto-antibodies, such as anti-U1RNP and anti-U11/U12 RNP. Another issue that could be responsible for the lack of clinical involvement at study enrollment was the short disease duration.

In conclusion, our results revealed that among SSc-specific autoantibodies, anti-Scl70, ACA, and anti-RNAPIII are more common in patients with early SSc, and co-expression of autoantibodies is not infrequent. Testing a broad panel of autoantibodies yielded diagnostic support in 8% of the patients who were negative for common SSc-specific antibodies. Although autoantibodies specific to SSc in early disease show generally known clinical features, it remains to be investigated how co-expression of specific and nonspecific autoantibody positivity will affect clinical presentation.

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Data availability All data have been included in the manuscript.

Declarations

Conflict of interest None of the authors has financial or non-financial conflicts of interest to disclose.

Ethical approval This study was approved by the Kocaeli University School of Medicine Ethics Committee, Kocaeli, Turkey, with study number KOU/GOKAEK 2017/347

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Table 1 . Demographic, clinical, and laboratory characteristics of the SSc patients

		N (%) or mean±SD	N (%) or mean±SD
Female	Female	87	(87%)
Age, years	Age, years	48.9	±12.2
Disease duration, years	Disease duration, years	2,1	±1.4
mRSS	mRSS	10.8	±10.4
Disease classification	Disease classification		
	Diffuse	36	(36%)
	Limited	63	(63%)
	Sine scleroderma	1	(1%)
PAH	PAH	3	(3%)
ILD	ILD	33	(33%)
GIS	GIS	60	(60%)
DU	DU	14	(14%)
SRC	SRC	0	
Myositis	Myositis	0	
Malignancy	Malignancy	3	(3%)
Overlap	Overlap	23	(23%)
Anti-nuclear antibody profile	Anti-nuclear antibody profile	93	(93%)
Staining ANA pattern	Staining ANA pattern		
	Speckled	65	(65%)
	Nucleolar	13	(13%)
	Centromere	29	(29%)
	Homogeneous	3	(3%)
	Reticular	3	(3%)
Staining SSc profile pattern	Staining SSc profile pattern		
	Scl 70	41	(41%)
	ACA	27	(27%)
	CENPA	27	(27%)

		N (%) or mean±SD	N (%) or mean±SD
	CENPB	26	(26%)
	RNAPIII	15	(15%)
	RNAP11	9	(9%)
	RNAP155	13	(13%)
	NOR90	2	(2%)
	Th/To	1	(1%)
	PM/Scl 75	8	(8%)
	PM/Scl 100	5	(5%)
	Ku	6	(6%)
	Ro52	22	(22%)
	Fibrillarin	0	
	Anti-PDGF	0	

mRSS, modified Rodnan skin score; PAH, pulmonary arterial hypertension; ILD, interstitial lung disease; GIS, gastrointestinal system; DU, digital ulcer; SRC, scleroderma renal crisis; ANA, antinuclear antibody; ACA, anticentromere; RNAPIII, RNA polymerase III

Table 2 . The association of the common SSc specific autoantibodies and clinical features

	Scl 70	Scl 70	Scl 70	ACA	ACA	ACA	RNAPIII	RNAPIII	RN
	positive n=41	negative n=59	*p*	positive n=27	negative n=73	*p*	positive n=15	negative n=85	*p*
lcSSc	15 (36.6%)	48 (81.4%)	<0.001	27 (100%)	36 (49.3%)	<0.001	5 (33.3%)	58 (68.2%)	0.0
Female	38 (92.7%)	49 (83.1%)	0.229	26 (96.3)	61 (83.6%)	0.177	11 (73.3%)	76 (89.4%)	0.1
ILD	24 (58.5)	9 (15.3%)	<0.001	1 (3.7%)	32 (43.8%)	<0.001	9 (60%)	24 (28.2%)	0.0
PAH	1 (2.4%)	2 (3.4%)	1.000	0	3 (4.1%)	0.561	2 (13.3%)	1 (1.2%)	0.0
Malignancy	0	3 (5.1%)	0.267	0	3 (4.1%)	0.561	2 (13.3%)	1 (1.2%)	0.0
DU	4 (9.8%)	9 (15.3%)	0.551	4 (14.8%)	9 (12.3%)	0.743	2 (13.3%)	11 (12.9%)	1.0
GIS	24 (58.5%)	36 (61%)	0.803	15 (55.6%)	45 (61.6%)	0.581	11 (73.3%)	49 (57.6%)	0.3
Overlap	5 (12.2%)	19 (32.2%)	0.021	11 (40.7)	13 (17.8%)	0.017	1 (6.7%)	23 (27.1%)	0.1
Age	46.9±12.7	51±11.8	0.210	50.4±10.3	48.4±12.9	0.602	55.3±8.5	47.7±12.5	0.0
Disease duration	2.03±1.5	2.14±1.3	0.471	2±1.4	2.13±1.4	0.770	2.6±1.6	2±1.3	0.1
mRSS	14.3±11.5	8.6±9	0.002	6.5±5.2	12.5±11.4	0.024	20.4±13.8	9.2±8.6	0.0

ACA, anti centromere antibody; RNAPIII, RNA polymerase III; lcSSc, limited cutaneous systemic sclerosis; mRSS, modified Rodnan skin score; PAH, pulmonary arterial hypertension; ILD, interstitial lung disease; GIS, gastrointestinal system; DU, digital ulcer; SRC, scleroderma renal crisis; ANA, antinuclear antibody

Table 3 . Clinical features of single and multiple antibody positive patient subgroups within each common SSc specific autoantibody groups

	Scl 70 (N=41)	Scl 70 (N=41)	Scl 70 (N=41)	ACA (N=27)	ACA (N=27)	ACA (N=27)	RNAPIII (N=15)	RNAPIII (N=15)	RN (N
	Single n=28	Multiple n=13	*p*	Single n=16	Multiple n=11	*p*	Single n=6	Multiple n=9	*p*
lcSSc	9 (32.1%)	6 (46.2%)	0.386	16 (100%)	11 (100%)	NA	2 (33.3%)	3 (33.3%)	1.0
Female	27 (96.4%)	11 (84.6%)	0.176	15 (93.8%)	11 (100%)	1.000	4 (66.7%)	7 (77.8)	0.6
ILD	17 (60.7%)	7 (53.8%)	0.678	0	1 (9.1%)	0.407	4 (66.7%)	5 (55.6)	0.6
PAH	1 (3.6%)	0	0.490	0	0	NA	1 (16.7%)	1 (11.1%)	0.7
Malignancy	0	0	NA	0	0	NA	2 (33.3%)	0	0.1
DU	2 (7.1%)	2 (15.1%)	0.579	0	4 (36.4%)	0.019	1 (16.7%)	1 (11.1%)	0.7
GIS	15 (53.6%)	9 (69.2%)	0.499	8 (50%)	7 (63.6%)	0.696	4 (66.7%)	7 (77.8%)	0.6
Overlap	4 (14.3%)	1 (7.7%)	1.000	7 (43.8%)	4 (36.4%)	0.508	0	1 (11.1%)	1.0
Age	47.9±12.9	44.8±12.6	0.792	51.5±10.2	48.9±10.7	1.000	60.2±9.9	52.1±5.9	0.0
Disease duration	2±1.5	2.1±1.5	0.810	1.9±1.1	2.1±1.8	0.809	3±1.3	2.3±1.8	0.3
mRSS	13.9±9.3	15.2±15.5	0.690	6.6±5.4	6.3±5.1	0.942	14.5±12.6	24.3±13.9	0.1

ACA, anti centromere antibody; RNAPIII, RNA polymerase III; lcSSc, limited cutaneous systemic sclerosis; mRSS, modified Rodnan skin score; PAH, pulmonary arterial hypertension; ILD, interstitial lung disease; GIS, gastrointestinal system; DU, digital ulcer; SRC, scleroderma renal crisis; ANA, antinuclear antibody

Table 4. Clinical features of the patients who were positive only for uncommon scleroderma-specific autoantibodies

	Staining intensity	Staining intensity	Staining intensity	Staining intensity	Staining intensity	Stai
Patient number	NOR90	Th/To	PM/Scl 75	PM/Scl 100	Ku	Ro5
1	Neg	Neg	+	Neg	Neg	Neg
2	Neg	Neg	Neg	+	Neg	Neg
3	Neg	Neg	+++	+++	Neg	Neg
4	Neg	Neg	Neg	Neg	+++	Neg
5	Neg	Neg	Neg	Neg	+	++
6	+	Neg	Neg	Neg	Neg	+
7	Neg	Neg	Neg	+	Neg	Neg
8	Neg	Neg	Neg	Neg	Neg	++

Neg, negative; Border, borderline; +, medium staining; ++, strong staining; +++ very strong staining. PAH, pulmonary arterial hypertension; ILD, interstitial lung disease; GIS, gastrointestinal system; DU, digital ulcer; SRC, scleroderma renal crisis; ANA, antinuclear antibody; mRSS, modified Rodnan skin score

Figure 1. Distribution of the autoantibody positivities of the patients.

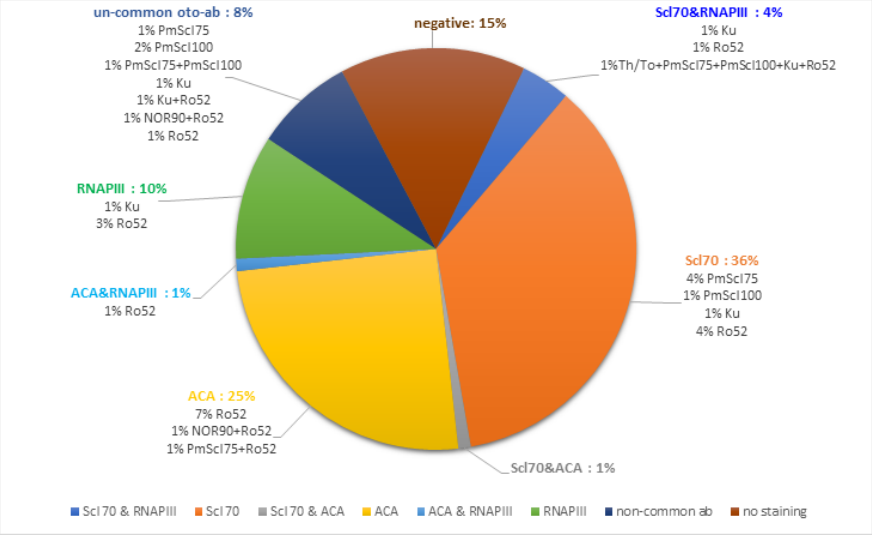

Figure 2 . Eigenvectors of the first three principal components (A, B, C) and their contribution weights for each of the dimensions (D).