

1-1-2018

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### Recommended Citation

YAYLA, MÜÇTEBA ENES; İLGEN, UFUK; and DÜZGÜN, NURŞEN (2018) "An analysis of the relationship between autoantibodies and clinical findings in patients with systemic sclerosis," *Turkish Journal of Medical Sciences*: Vol. 48: No. 1, Article 3. <https://doi.org/10.3906/sag-1708-67>  
Available at: <https://journals.tubitak.gov.tr/medical/vol48/iss1/3>

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## An analysis of the relationship between autoantibodies and clinical findings in patients with systemic sclerosis

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Received: 12.08.2017 • Accepted/Published Online: 19.11.2017 • Final Version: 23.02.2018

**Background/aim:** We aimed to investigate the prevalence of anti-RNA polymerase (RNAP) III and other autoantibodies in a group of Turkish patients with systemic sclerosis (SSc) and their relation with clinical features.

**Materials and methods:** The prevalence of anti-RNAP III and other autoantibodies was analyzed in 93 patients with SSc and control groups including 86 patients with systemic lupus erythematosus (SLE) and 65 healthy subjects, respectively. Their relationship with diseases findings was assessed in a cross-sectional manner.

**Results:** Prevalences of anti-RNAP III were 2/93 (2.2%) in SSc, 1/86 (1.2%) in SLE, and 1/65 (1.5%) in the healthy group and there was no difference among groups ( $P > 0.999$ ). Anti-Sm was significantly more common in SLE patients ( $P < 0.001$ ), whereas antitopoisomerase I and anticentromere protein B were significantly more common in SSc patients ( $P < 0.001$ ). There was a significant association between antitopoisomerase I positivity and interstitial lung disease ( $P < 0.001$ ), and interestingly there was also a significant association between anti-SS-A 52 positivity and the presence of digital ulcers in patients with SSc.

**Conclusion:** Our data show that anti-RNAP III in SSc patients was low in frequency in a Turkish population.

**Key words:** Systemic sclerosis, anti-RNA polymerase III, ANA staining pattern, interstitial lung disease, digital ulcer

### 1. Introduction

Systemic sclerosis (SSc) is a chronic connective tissue disease characterized by vascular damage, inflammation resulting in fibrosis in the skin and internal organs, and the presence of autoantibodies (1,2). SSc is mainly classified into diffuse and limited cutaneous forms (3). The correlation between autoantibodies and clinical findings in SSc has been well established (4). Antinuclear antibodies (ANAs) are present in 80%–95% of patients with SSc (2,5). Autoantibodies such as antitopoisomerase I antibody (ATA), anticentromere antibody (ACA), and anti-RNA polymerase III antibody (anti-RNAP III) are helpful for diagnosis and classification of SSc (5–9). ATA is classically associated with the diffuse form (dcSSc) and ACA is typically associated with the limited form (lcSSc). ATA is also associated with pulmonary fibrosis and renal crisis. Anti-RNAP III is reported to be associated with dcSSc, renal crisis, and worse prognosis (10).

In this study, we aimed to investigate the prevalence of anti-RNAP III and other autoantibodies in a group of patients with SSc and their relation with clinical features.

This is also the first study investigating the prevalence of anti-RNAP III and its relation with clinical features in SSc patients in a Turkish population.

### 2. Materials and methods

SSc patients followed in the Department of Rheumatology of Ankara University Medical School referring between October 2014 and June 2015 were included in the study. Inclusion criteria for the patient group were diagnosis of SSc according to the classification criteria defined by the American College of Rheumatology (11) and being over 18 years of age. Patients were classified as having diffuse or limited cutaneous SSc according to LeRoy's classification (3). Clinical data including sex, age, age at diagnosis, duration of disease, vascular symptoms, and visceral organ involvements were recorded. In patients with SSc, age at the first symptom except for Raynaud's phenomenon was accepted as the disease onset age. Presence of Raynaud's phenomenon, digital ulcers, arthritis, and extent of cutaneous sclerosis was based on history and physical examination. Pulmonary arterial pressure (PAP) was

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detected by echocardiography and elevated systolic PAP was defined as  $\geq 40$  mmHg. Lung involvement was defined as typical bilateral subpleural fine reticular to advanced fibrotic changes on high-resolution computed tomography with or without symptoms or functional test abnormality, gastrointestinal involvement as dysphagia and/or motility disorder without alternative etiology, and renal crisis as acute deterioration in kidney function with hypertension plus compatible renal biopsy findings. As controls, we studied the sera of 65 healthy blood donors and 86 patients with systemic lupus erythematosus (SLE). Written informed consent was obtained from each patient and control. The study was in compliance with the principles outlined in the declaration of Helsinki and was approved by the local ethics committee.

For analysis of autoantibodies, ANA was detected by indirect immunofluorescence with Hep-2 cells at a screening dilution of 1:100. Serum anti-RNAP III was measured using a commercial ELISA kit (Quanta Lite RNA Pol III, Inova Diagnostics, San Francisco, CA, USA). Presence of autoantibodies (ATA, anticentromere protein B [anti-CENP B], anti-PM/Scl, anti-Sm, anti-SS-A 52, anti-SS-A 60, and anti-SS-B) was assessed using a commercial test (IMTEC ANA Line Immune Assays Maxx, Human Diagnostics, Wiesbaden, Germany).

All calculations were performed with IBM SPSS for Windows version 21 software. The data were analyzed using the chi-square test for comparison between groups. Odds ratios (ORs) with 95% confidence intervals were calculated where appropriate.  $P < 0.05$  was considered statistically significant.

### 3. Results

Clinical, laboratory, and demographic data are presented in Table 1. Prevalences of anti-RNAP III positivity were 2/93 (2.2%) in SSc, 1/86 (1.2%) in SLE, and 1/65 (1.5%) in the healthy control group ( $P > 0.999$ ).

Serum samples of 83 (89%) SSc patients and all SLE patients were available for analysis of presence of ANA.

The ANA staining pattern was studied for 80 (86%) SSc patients and all SLE patients. Autoantibody specificities were studied for 82 (88%) SSc patients and all SLE patients. ANA was not evaluated in the healthy control group. SSc and SLE groups were compared in terms of ANA staining pattern and autoantibody specificities. The data are represented in Table 2. Homogeneous (OR = 6.14 [2.21–17],  $P < 0.001$ ) and cytoplasmic (OR = 3.61 [1.57–8.27],  $P = 0.002$ ) staining patterns were detected significantly in favor of SLE and centromeric staining property was significantly in favor of SSc (OR = 0.02 [0.006–0.12],  $P < 0.001$ ). Anti-Sm was significantly more common in SLE patients (16.3 [3.73–71.8],  $P < 0.001$ ). ATA (OR = 0.32 [0.004–0.24],  $P < 0.001$ ) and anti-CENP B were significantly more common in SSc patients (OR = 0.04 [0.009–0.18],  $P < 0.001$ ).

Fourteen (15.1%) SSc patients had dcSSc and 79 (84.9%) had lcSSc. Rates of interstitial lung disease (ILD) were 92.9% and 36.7% in the dcSSc and lcSSc groups, respectively (OR = 22.41 [2.78–180.2],  $P < 0.001$ ). Data regarding clinical features of dcSSc and lcSSc groups are represented in Table 3.

dcSSc and lcSSc patients were compared in terms of ANA positivity, ANA staining patterns, and autoantibody specificities (Table 4). Centromeric staining property was significantly in favor of lcSSc (OR = 0.16 [0.03–0.8],  $P = 0.017$ ) whereas speckled and nucleolar staining properties were significantly in favor of dcSSc (OR = 5.6 [1.4–22.3],  $P = 0.008$  and OR = 8.51 [2.28–31.78],  $P = 0.001$ , respectively). ATA was significantly more common in dcSSc patients (OR = 17.57 [4.14–74.34],  $P < 0.001$ ) and anti-CENP B in lcSSc patients ( $P < 0.001$ ).

The relationships between clinical features and specific autoantibodies (antitopoisomerase I, anti-CENP B, SS-A 60/52, SS-B) in SSc patients were evaluated. The relationship between ATA positivity and the presence of ILD was significant (OR = 6.09 [1.96–18.95],  $P = 0.001$ ). Patients with positive anti-SS-A 52 had higher digital ulcer rates (OR = 4.21 [1.22–14.49],  $P = 0.017$ ).

**Table 1.** Clinical, laboratory, and demographic data of SSc, SLE, and healthy control groups.

	SSc n = 93	SLE n = 86	Healthy control n = 65	
Age (mean $\pm$ SD, years)	50.4 $\pm$ 13.4	46.5 $\pm$ 12	48 $\pm$ 11.5	P = 0.121
Sex (female / male) (%)	83 / 10 (89.2 / 10.8)	80 / 6 (93 / 7)	58 / 7 (89.2 / 10.8)	P = 0.627
Anti-RNAP III (n, %)	2 (2.2)	1 (1.2)	1 (1.5)	P > 0.999

SSc = Systemic sclerosis; SLE = systemic lupus erythematosus; n = number; SD = standard deviation; anti-RNAP III = anti-RNA polymerase III.

**Table 2.** ANA staining patterns and autoantibody specificities in SSc and SLE groups.\*

	SSc	SLE	OR (95% CI)	
ANA positivity	77 (92.8)	81 (94.2)	1.26 (0.37–4.3)	P = 0.709
ANA staining pattern				
Homogeneous	5 (6.2)	25 (29.1)	6.14 (2.21–17)	P < 0.001
Speckled	35 (43.8)	71 (82.6)	6.08 (2.98–12.3)	P = 0.05
Cytoplasmic	9 (11.2)	27 (31.4)	3.61 (1.57–8.27)	P = 0.002
Granular	1 (1.2)	2 (2.3)	1.88 (0.16–21.1)	P > 0.999
Nucleolar	23 (28.8)	14 (16.3)	0.48 (0.22–1.02)	P = 0.06
Centromeric	37 (46.2)	2 (2.3)	0.02 (0.006–0.12)	P < 0.001
Autoantibody specificities				
Anti-Sm	2 (2.4)	25 (29.1)	16.3 (3.73–71.8)	P < 0.001
ATA	22 (26.8)	1 (1.2)	0.32 (0.004–0.24)	P < 0.001
Anti-histone	0	9 (10.5)	N/A	P = 0.003
Anti-CENP B	30 (36.6)	2 (2.3)	0.04 (0.009–0.18)	P < 0.001
Anti-PM/SCL	1 (1.2)	0	N/A	P = 0.304

ANA = Antinuclear antibody; SSc = systemic sclerosis; SLE = systemic lupus erythematosus; ATA = antitopoisomerase I antibody; OR = odds ratio; CI = confidence interval; N/A = not applicable.

\*Serum samples of 83 (89%) SSc patients and all SLE patients were available for analysis of ANA. The ANA staining pattern was studied in 80 (86%) SSc patients and in all SLE patients. Autoantibody specificities were studied in 82 (88%) SSc patients and in all SLE patients. All data are represented as number (percentages).

**Table 3.** Clinical features of SSc patients.

	dcSSc n = 14 (15.1%)	lcSSc n = 79 (84.9%)	OR (95% CI)	
Sex, female †	11 (78.6)	72 (91.1)		P = 0.171
Age ± SD, years	52.9 ± 13.2	49.9 ± 13.5		P = 0.451
Disease onset ± SD, years	45.1 ± 15.8	44.2 ± 13.4		P = 0.269
Disease duration ± SD, years	8 ± 6.46	6.35 ± 7.3		P = 0.153
Raynaud's phenomenon †	11 (78.6)	75 (94.9)	0.19 (0.03–0.99)	P = 0.067
Digital ulcer †	7 (50)	37 (73.4)	2.76 (0.94–1.49)	P = 0.113
Digital amputation †	1 (7.1)	6 (7.6)	0.93 (0.10–8.42)	P > 0.999
Gastrointestinal involvement †	2 (14.3)	9 (11.4)	1.29 (0.24–6.75)	P = 0.757
Interstitial lung disease †	13 (92.9)	29 (36.7)	22.41(2.78–180)	P < 0.001
Elevated PAP †‡	2 (20)	11 (19.3)	1.04 (0.19–5.62)	P > 0.999

SSc = Systemic sclerosis; dcSSc = diffuse cutaneous SSc; lcSSc = limited cutaneous SSc; n = number; PAP = pulmonary arterial pressure, years = years; SD = standard deviation; OR = odds ratio; CI = confidence interval.

† Data are represented as numbers (percentages).

‡Four (29%) dcSSc patients and 22 (28%) lcSSc patients lacked echocardiographic PAP measurements.

**Table 4.** ANA staining pattern and autoantibody specificities in dcSSc and lcSSc.\*

	dcSSc (n, %)	lcSSc (n, %)	OR (95% CI)	
ANA positivity	13 (100)	64 (91.4)	N/A	P = 0.583
ANA staining pattern				
Homogeneous	1 (7.7)	4 (6)	1.31 (0.13–12.78)	P > 0.999
Speckled	10 (76.9)	25 (37.3)	5.6 (1.4–22.3)	P = 0.008
Cytoplasmic	1 (7.7)	8 (11.9)	0.61 (0.07–5.38)	P > 0.999
Granular	0 (0)	1 (1.5)	N/A	P > 0.999
Nucleolar	9 (69.2)	14 (20.9)	8.51 (2.28–31.78)	P = 0.001
Centromeric	2 (15.4)	35 (52.2)	0.16 (0.03–0.8)	P = 0.017
Autoantibody specificities				
Anti-CENP B	0 (0)	30 (43.5)	N/A	P = 0.003
ATA	11 (84.6)	11 (15.9)	17.57 (4.14–74.34)	P = 0.001
Anti-RNAP III	1 (7.1)	1 (1.3)	6 (0.35–102.01)	P = 0.28

n = Number; ANA = antinuclear antibody; dcSSc = diffuse cutaneous systemic sclerosis; lcSSc = limited cutaneous systemic sclerosis; CENP B = centromere protein B; ATA = antitopoisomerase I antibody; RNAP III = RNA polymerase III; N/A = not applicable.

\*Serum samples of 83 (89%) SSc patients were available for analysis of ANA. ANA staining pattern was studied in 80 (86%) and autoantibody specificities were studied in 82 (88%) SSc patients.

**Table 5.** Frequency of SSc-specific autoantibodies in different racial groups (24–27).

	Turkish	Mexican (24)	Caucasian (25)	Japanese (26,27)	African American (25,27)
Anti-CENP or ACA (%)	36.6	29	32	16	4–11
ATA (%)	26.8	28	13	25–28	24–26
Anti-PM/SCL(%)	1.2	9	2–4	0	0–3
Anti-RNAP III (%)	2.2	1.4	8	5	13–14

SSc = Systemic sclerosis; anti-CENP = anticentromere protein; ACA = anticentromere antibody; ATA = antitopoisomerase I antibody; anti-PM/SCL = antipolymyositis/scleroderma; anti-RNAP III = anti-RNA polymerase III.

#### 4. Discussion

Prevalence of anti-RNAP III in SSc patients varies in previously published studies. In a cohort study from Pittsburgh, USA, prevalence was 25% (12). In studies conducted in Europe it is found that prevalence decreases from north to south, being 22% in Sweden, 12% in England, 8% in Italy, and 5% in Poland. In a multicenter study performed in France, prevalence of anti-RNAP III was found to be 9.4%. Studies conducted in Asian countries observed a further decrease in the prevalence, at

6% in Japan and 3.4% in South Korea (6,12–17). We found only two anti-RNAP III-positive cases among 93 SSc patients (2.2%). There are several reasons for the variation in prevalence. First, anti-RNAP III prevalence varies depending on the method used. In a study conducted in France, different results were obtained in evaluations made using two different ELISA kits (13,18,19). Second, patient selection also affects the prevalence of anti-RNAP III. For example, in a study conducted by Parker et al., SSc patients were selected according to their ANA staining properties

and a high prevalence (15.4%) was reported (20). Another study was conducted among patients diagnosed with dcSSc and the prevalence was detected to be 67% (10). Determination of different prevalences among the above-mentioned studies, conducted in many different countries, raises concerns about race and ethnicity (19). In our study, prevalence of anti-RNAP III was 2.2% in SSc cases, 1.2% in SLE cases, and 1.5% in healthy subjects and there was no significant difference between groups. The reason for this may be the relatively low number of patients, the cross-sectional manner of the study, and patient selection. Prospective cohort studies will be more informative for true incidences and disease phenotype–autoantibody associations in patients with SSc.

Previous studies have shown the associations of anti-RNAP III with higher modified Rodnan skin scores (mRSS), renal crisis, tendon friction rubs, and dcSSc (5,6–9). None of our SSc patients had a history of renal crisis and as a limitation we did not evaluate tendon friction rubs or mRSS. We had two anti-RNAP III-positive SSc patients, one with dcSSc and the other with lcSSc, and further statistical analysis was not possible.

Vascular phenomena (Raynaud's and digital ulcers) frequency and high pulmonary arterial pressure were found to be significantly less common in ANA-negative SSc patients than ANA-positive SSc patients (21). In the same study, there was no significant relationship between ANA positivity and ILD and there was a significant relationship

between negative ANA and gastrointestinal involvement. In our study, we detected no significant relationship between ANA positivity with abnormal capillaroscopy, Raynaud's phenomenon, digital ulcers, gastrointestinal involvement, and ILD. Despite the known association between high ACA titers and pulmonary hypertension (22), we found no relationship between positive anti-CENP B and high systolic PAP.

Association between positive anti-SS-A 52 and the presence of digital ulcers was not reported before in patients with SSc, although anti-SS-A 52 was previously reported to be associated with pulmonary fibrosis in patients with mixed connective tissue disorder (23). This issue requires a more detailed research.

Previous studies investigated the frequency of SSc-specific autoantibodies in different ethnic groups (24–27). These studies have demonstrated that there are differences in the distribution of autoantibodies. We compared the results of these studies with our own data in Table 5.

In conclusion, the prevalence of anti-RNAP III differs in different populations and is relatively low in Turkish patients with SSc.

### Acknowledgments

The authors thank Zeynep Gençtürk, Semahat Özartam, and Professor Hüseyin Tutkak for assistance in laboratory and statistical work. This study was supported by a grant from Ankara Tiplilar Vakfi.

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